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### META-ANALYSIS

- 1 Empathy assessment in healthcare students is highly heterogeneous: A systematic review and meta-analysis (2012-2016)  
*Fragkos KC, Sotiropoulos I, Frangos CC*



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## Empathy assessment in healthcare students is highly heterogeneous: A systematic review and meta-analysis (2012-2016)

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### Abstract

#### BACKGROUND

Clinical empathy leads to improved patient satisfaction and better clinical outcomes. Currently, there are multiple empathy scales with minimal or no efforts to produce an integrated definition of clinical empathy which can be assessed sufficiently by only a few scales. Moreover, there is an unclear overall reliability of these empathy scales, hence limiting comparative evaluation.

#### AIM

To examine which empathy scales have been used in healthcare students and to estimate their overall internal consistency.

#### METHODS

A systematic review was performed with inclusion criteria any empirical study with quantitative data examining empathy of healthcare students toward patients between 2012 and 2016. A random effects model was used to produce a pooled estimate of the Cronbach's alphas. The Hakstian-Whalen transformation was used for analyses based on the Rodriguez-Maeda method. Heterogeneity was quantified using the  $I^2$  statistic and further investigated with subgroup analysis and meta-regression. Publication bias was assessed using funnel plots, Egger's test, Begg's test, and the trim and fill analysis.

#### RESULTS

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Thirteen scales have been used to assess clinical empathy in healthcare students from forty nine studies with total sample size 49384 students. The most frequently used scale is the Jefferson Scale of Physician Empathy followed by Davis' Interpersonal Reactivity Index. The overall reliability was 0.805 (95%CI 0.786-0.823), which is acceptable, but there was heterogeneity and publication bias. Some heterogeneity was explained by the different countries of the studies under investigation and student types but most heterogeneity remained unexplained.

## CONCLUSION

The results indicate that scales have satisfactory internal consistency but there are a multitude of scales, definitions and empathy components. Future research should focus on standardizing scales and creating consensus statements regarding the definition of empathy and use of appropriate scales.

**Key words:** Empathy; Scale; Reliability; Systematic review; Meta-analysis; Heterogeneity

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**Core tip:** No consensus is available about which tools are more reliable to assess clinical empathy in healthcare students. The present study is the first to assess the reliability of clinical empathy scales with meta-analysis. The most frequently used scale is the Jefferson Scale of Physician Empathy followed by Davis' Interpersonal Reactivity Index. The results indicate that scales have satisfactory internal consistency but there are a multitude of scales, definitions and empathy components, with the presence of heterogeneity and publication bias. Future research should focus on standardizing scales and creating consensus statements regarding empathy definitions and use of appropriate scales.

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## INTRODUCTION

At its core, empathy is a particular form of interpersonal understanding and stands for a basic sensitivity to the mindedness of other persons<sup>[1]</sup>. Empathy is commonly distinguished between cognitive empathy and affective empathy<sup>[2]</sup>. Cognitive empathy denotes the ability to ascribe mental states to others, such as beliefs, intentions, or emotions; and affective empathy essentially involves affect on the part of the empathizer<sup>[3]</sup>. Its archetypal form is empathy with a person in need (*e.g.*, someone who is in pain, sad, or in an upsetting situation)<sup>[3]</sup>. An agreed definition of empathy remains an issue of controversy. A recent systematic review identified 109 suitable articles that could provide a definition of clinical empathy and they first identified three elements to its definition: thinking, feeling and acting. Each definition incorporates one or more of these elements<sup>[4]</sup>. Components of thinking that emerged from the systematic review were: cognition, perspective-taking, imagination/imagining, apprehension, understanding, seeing, perceiving, processing, comprehend, appreciation of, knowledge, recognize, identification, controlled, intellectually sense, role-taking, grasp, and identify with. Components of feeling were: Compassion, feeling, emotion, concern, joining with patient's feelings, to enter into or join with feelings, socio-emotional, care, emotional participation, affective, vicarious emotional response, generation of similar feelings, sharing of emotions, sense, emotional contagion, sympathize, match/ experience someone's emotional state, emotive, and specific feeling words: *e.g.*, angry, enjoy, care, sad. And finally, components of acting were: Communication, conveying, behavioural, express, listen, interrupt, and eye contact<sup>[4]</sup>. However the authors didn't arrive at a complete succinct definition.

Affective approaches to empathy are defined as having a matching reaction to the



emotions of another individual. More recently, integrated approaches comprising of both types of empathy have emerged and appear to be more pragmatic<sup>[5]</sup>. Empathy should not be confused with compassion which requires emotion and action on the part of respondents, based on love, vulnerability, and reciprocity, and is actualized by disempowering one's self and empowering the subject<sup>[6]</sup>. Similar concepts are sympathy, which is a subjective response based on pity towards another, humanism, caring, altruism, respect and integrity<sup>[7]</sup>. Empathy also has a strong neurobiological basis, although this is not discussed in the present study<sup>[8-11]</sup>.

Assessing empathy is one of the most important aspects of investigating empathy with over 10 scales currently in use<sup>[4,12-14]</sup>. Differences between empathy measures depend on the conceptualization of empathy, factor structures and solutions and the target audience. For example, the Jefferson Scale of Physician Empathy traditionally is a 20-item scale and has three or four subscales depending on the sample under analysis: Perspective taking, compassionate care or standing in the patient's shoes. Davis' Interpersonal Reactivity Index consists of four subscales, Fantasy and Perspective Taking, which mirror the emotional aspect of empathy; and Empathetic Concern and Personal Distress, which reflect the cognitive dimension<sup>[15]</sup>.

In general, it is considered that clinical empathy leads to improved patient satisfaction, greater adherence to therapy, better clinical outcomes, greater quality of service delivery perception and lower malpractice liability<sup>[16,17]</sup>, while for clinicians and society it promotes the values of humanism<sup>[18]</sup>, reduces professional burnout and increases diagnostic accuracy, and increases public trust to the healthcare system<sup>[19,20]</sup>. Empathy has been found to decline over the course of clinical education, with clinical contact, distress, mistreatment by superiors, vulnerability, lack of social support, high workload, unsuitable learning environments, unsatisfactory role models and short patient admissions contributing towards this<sup>[19]</sup>. A demographic that plays a role in empathy is gender with females having been reported to usually have higher scores in excess of about 1-10 units in the Jefferson Scale of Physician Empathy<sup>[21-33]</sup>.

Nevertheless, there are unresolved issues. First, there is a multitude of empathy scales with minimal or no efforts to produce an integrated definition of clinical empathy which can be assessed sufficiently by only a few scales. Next, there are 38 systematic reviews in the topic of empathy<sup>[4,5,12-14,16,19,34-64]</sup>, but only six of them are meta-analyses<sup>[5,41,44,50,54,62]</sup>. The lack of quantitative synthesis does not allow for aggregation of research into meta-studies and umbrella reviews that are better in summarizing evidence and guiding policy and practice. Our hypotheses are that multiple empathy scales are currently in use and their results are heterogeneous. Hence, the present study's objectives are to answer which empathy scales have been used in the last years (2012-2016) to assess and measure empathy in healthcare students, what their reliability is and which factors contribute to their heterogeneity.

## MATERIALS AND METHODS

### *Study eligibility criteria*

Inclusion criteria were any empirical study (full paper) with quantitative data examining empathy of healthcare students towards patients. There was a restriction to English language cross-sectional studies published between 2012 and 2016. Papers had to provide sufficient data to produce an effect measure for the meta-analysis. Empathy was only considered from healthcare students towards patients. Studies were excluded when they had other designs (case studies, pre-post experimental designs, empathy intervention studies), didn't report quantitative data, were non-English, and investigated other types of empathy: empathy from professionals, non-healthcare students, teachers (teacher empathy), perceived empathy of patients from their healthcare professionals, empathy among adolescents and high school students, empathy towards HIV patients, cross-cultural empathy, multicultural empathy, and empathy towards particular medical conditions.

### *Search strategy and terms*

PRISMA guidelines for systematic reviews and meta-analyses were followed<sup>[65]</sup>. Electronic database searches were conducted in Google Scholar, PubMed/Medline, Scopus, CINAHL, EMBASE and ERIC. The keywords for searching were: empathy, caring, humanism, cognitive, emotional, healthcare, medical students, nursing students, allied healthcare professionals students, dental students, reliability, validity, psychometric properties, validity, definition, compassion, care, Cronbach's alpha, reliability, and consistency. The date of search was 1 January 2017 and included articles from 1 January 2012 up until 31 December 2016. The bibliographies from all included manuscripts and hand searching of relevant healthcare education journals

(Academic Medicine, Advances in Health Sciences Education, International Journal of Medical Education, Medical Education Online, Medical Teacher, Medical Education, Teaching and Learning in Medicine, Perspectives on Medical Education, BMC Medical Education) were further literature search strategies.

### Study selection, data extraction and quality assessment

The abstracts of the resulting studies were evaluated in terms of inclusion criteria. The full text was consulted when reading the abstract was not adequate to warrant inclusion. We extracted the following data from the included studies: Country, sample size, age, gender distribution, field, study design, aim, main results, scale, Cronbach's alpha and number of items. The quality of studies (risk of bias) was assessed with the criteria suggested by Reilly *et al*<sup>[66]</sup>.

### Statistical analysis

Cronbach's alpha (or alpha coefficient) is a measure of internal consistency of a test or scale and is expressed between 0 and 1. It essentially examines whether the scale under investigation measures what it claims to measure, by computing the inter-correlations of the items. When Cronbach's alpha ranges from 0.70 to 0.95, a scale is considered to have acceptable internal consistency. Heterogeneous constructs, apparent lack of correlation between items, and too few scale items can lead to a low value of alpha<sup>[67,68]</sup>.

Quantitative analysis was performed with Review Manager 5.3 and R 3.5.1. A random effects model was used to produce a pooled estimate of the Cronbach's alphas. The Rodriguez-Maeda method is adopted to normalise the alpha coefficient by transforming it to the Hakstian-Whalen  $T = (1 - \alpha)^{1/3}$ , which is a variable with standard normal distribution<sup>[69,70]</sup>. All analyses are performed using the transformed values and the results, such as the summary effect and its confidence interval, would then be converted back to alpha coefficients for presentation.

Heterogeneity between studies was quantified using the  $I^2$  statistic ( $P \leq 0.10$  for significance)<sup>[71]</sup>, with values over 50 % indicating considerable heterogeneity<sup>[72-76]</sup>. Sources of heterogeneity were explored performing subgroup analysis and meta-regression. Publication bias was assessed using funnel plots, Egger's and Begg's tests, and the trim and fill analysis<sup>[77-79]</sup>. A funnel plot is a scatter plot of the effect estimates from individual studies against a measurement of the study's sample size or precision<sup>[72]</sup>. Resemblance of a symmetrical inverted funnel supports that findings are due to sampling variation alone; thus, absence of bias<sup>[79]</sup>. In the present study, the funnel plot depicts the Hakstian-Whalen  $T$  against the study's sample size, since the standard error of the effect size is dependent of the effect size<sup>[70,80]</sup>.

## RESULTS

### Studies and quality assessment

The flow chart of study selection is shown in **Figure 1**. The initial search revealed 2491 studies which after applying the inclusion criteria were reduced to 49 studies. In total, 14 scales of empathy were used. The total number of participants was 49384 students, mean age was 23.5 years and average male percentage was 44.6%. Sample sizes ranged from 44 to 5343. The distribution of student types are: dentistry (2 studies), healthcare (medicine, nursing, physiotherapy, occupational therapy, paramedics, midwifery, nutrition and dietetics) (1 study), medicine (31 studies), nursing (7 studies), osteopathic medical students (2 studies), paramedics (1 study), pharmacy and nursing (2 studies), pharmacy (1 study), physician assistant students (1 study), and speech and hearing sciences (1 study). Overall, medicine and dentistry students were 43028, nursing students were 3242 and allied professions students were 3114. Included studies' information is shown in **Table 1**. The overall quality of studies was satisfactory indicating low sampling bias and investigator bias with sufficient sample size in almost each study (**Figure 2**).

### Meta-analysis of Cronbach's alpha

The overall meta-analytic mean of Cronbach's alpha is 0.805 (95%CI 0.786-0.823) (**Figure 3A**). The distribution of the alpha coefficients is shown in **Figure 3B**. There is heterogeneity ( $I^2 = 98\%$ ) and publication bias is possibly present, as shown with partial asymmetry in the funnel plot (**Figure 3C**). However, Egger's and Begg's tests are not significant ( $P = 0.076$  and  $P = 0.648$ , respectively). The average alpha coefficients per scale are shown in **Table 2**. Trim and fill funnel analysis, indicated that most likely 11 studies more are unpublished due to publication bias and this is significant ( $P < 0.05$ ). The scale with lowest Cronbach's alpha is the Balanced Emotional Empathy Scale (0.720) and the one with the highest Cronbach's alpha is the

Table 1 Scales and studies in the meta-analysis

Study	Country	n	mean Age	Male %	Field	Reliability	Items	Aims	Main results
Balanced emotional empathy scale									
Dehning <i>et al</i> <sup>[82]</sup>	Ethiopia	237	21.4	87.3	Medical Students	0.72	30	To examine the differences in empathy between first year and final year medical students in Jimma University, Ethiopia	Male students had statistically significant lower empathy scores
Caring ability inventory									
Ma <i>et al</i> <sup>[91]</sup>	China	598	20.9	6.4	Nursing Students	0.77	37	To investigate baccalaureate nursing students' caring ability in the context of China and to explore the role of clinical practice learning in the development of students' caring skills	Students in the clinical stage of training scored lower than students in the pre-clinical stage.
Caring behaviour inventory tool									
Labrague <i>et al</i> <sup>[111]</sup>	Greece, Philippines, India, Nigeria	586	22.3	10.1	Nursing Students	0.92	42	To identify the correlation between instructors' and students' caring behaviours and to explore the impact of instructors' caring on students' perceptions of their own caring behaviours	The highest self-reported subscale in the Caring Behaviour Inventory was assurance (mean = 4.796), and the lowest self-rated subscale was connectedness (mean = 4.541)
Loke <i>et al</i> <sup>[92]</sup>	Singapore	657	20.3	13.2	Nursing Students	0.922	42	To evaluate the impact of Singapore's pre-registration nursing programmes on students' concept of caring	Results indicated a statistically significant reduction in the overall level of caring behaviour in first to final year students
Davis' interpersonal reactivity index									
Neumann <i>et al</i> <sup>[112] (1)</sup>	Germany	44	22.8	54.5	Medical Students	0.721	28	To investigate the psychometric properties of two empathy scales	Reliability was satisfactory and comparable to international adaptations



van Ryn <i>et al</i> <sup>[113]</sup> (1)	USA	4732		50.1	Medical Students	0.825	14	To examine individual predictors of first semester medical students' attitudes toward the value of physician empathy in clinical encounters	In univariate and multivariate analyses, Discomfort with uncertainty, close-mindedness, dispositional empathy, elitism, medical authoritarianism, egalitarianism, self-concept and well-being predicted students' empathy
Costa <i>et al</i> <sup>[95]</sup> (1)	Portugal, Brazil, UK, New Zealand, Ireland	3069		38.5	Medical Students	0.776	24	To examine psychometric properties (reliability, factor structure) of two empathy scales and compare them	The Interpersonal Reactivity Index and Jefferson Scale of Physician Empathy are only weakly related, suggesting that they may measure different constructs (maximum correlation 0.313)
Emotional intelligence assessment scale - empathy									
Senyuva <i>et al</i> <sup>[97]</sup>	Turkey	471	20.65	16.6	Nursing Students	0.87	6	To analyse the correlation of self-compassion and emotional intelligence of nursing students	There was a correlation between self-compassion and emotional intelligence ( $r = 0.400$ , $P < 0.05$ ) and that emotional intelligence has positive contributions to the features of nurses with developed self-compassion
Jefferson scale of physician empathy									

Kimmelman <i>et al</i> <sup>[114]</sup>	USA	415	26	54	Osteopathic medical students	0.83	20	To determine differences according to year of schooling in mean levels of empathy among osteopathic medical students	There were no statistically significant differences by year of schooling in respondents' gender, ethnicity, or specialty orientation and no statistically significant differences by year of schooling in the mean empathy scores
Mandel and Schweinle <sup>[115]</sup>	USA	328	24	17.4	Physician Assistant Students	0.8	20	To investigate empathy trends among physician assistant students through their education and included gender differences and specialty job interest	62% had lower median empathy scores toward the end of their didactic training than at the time of matriculation ( $P = 0.0001$ ). Female students were significantly more empathetic at the time of matriculation than men ( $P = 0.0003$ ), while both genders appeared to lose empathy in a parallel fashion during didactic training ( $P = 0.76$ ). There was no association between empathy scores and prospective job category interest
Neumann <i>et al</i> <sup>[112]</sup> (2)	Germany	44	22.8	54.5	Medical Students	0.803	20	To investigate the psychometric properties of two empathy scales	Reliability was satisfactory and comparable to international adaptations

Paro <i>et al</i> <sup>[90]</sup>	Brazil	299		61.7	Medical Students	0.84	20	To adapt the Jefferson Scale of Empathy to the Brazilian culture and to test its reliability and validity among Brazilian medical students	Principal component analysis confirmed the construct validity of the scale for three main factors: Compassionate Care (first factor), Ability to Stand in the Patient's Shoes (second factor), and Perspective Taking (third factor). Gender differences with respect to empathy were not significant
Calabrese <i>et al</i> <sup>[116]</sup>	USA	373	26.1	52.8	Osteopathic medical students	0.84	20	To investigate correlations between empathy and interprofessional collaboration in osteopathic medical students and to examine differences in empathy and interprofessional collaboration scores by sex, class year, and specialty interest	Significant correlation was found between scores on the empathy and attitudes scales ( $r = 0.42$ , $P < 0.01$ ). Women scored higher than men on the empathy scale (117.1 <i>vs</i> 111.9). No statistically significant difference on the scores of the 2 scales was observed among students who planned to pursue "people-oriented" specialties compared with those interested in "technology/procedure-oriented" specialties as well as in different years if education



Costa <i>et al</i> <sup>[89]</sup>	Portugal	77		31.2	Medical Students	0.77	20	To model empathy longitudinally during medical school at three time points: at the entrance, final of pre-clinical phase and at the beginning of clinical training	Empathy scores at all times were higher for females than for males, but only significantly at the end of the preclinical phase. The model had satisfactory fit student's empathy did not decline over time. Empathy scores were significantly and positively related with Openness to Experience and Agreeableness at admission
Gonçalves-Pereira <i>et al</i> <sup>[117]</sup>	Portugal	202		32.7	Medical Students	0.75	20	To examine the relationship of empathy with professionalism	There was a weak association between empathy and person-orientation
Hsiao <i>et al</i> <sup>[118]</sup>	Taiwan	613	23.3	89.1	Nursing Students	0.93	20	To examine the psychometric properties of a Chinese version of the Jefferson Scale of Empathy-Health Profession Students among Taiwanese undergraduate nursing students	The content validity index of 0.89. Factor analysis yielded three components of perspective taking, compassionate care and standing in the patient's shoes, explaining 57.14% of total variance. Women scored higher on empathy than men
Kiersma <i>et al</i> <sup>[96]</sup> (1)	USA	216	20.5	24.1	Pharmacy and nursing students	0.855	20	To validate an empathy scale to measure empathy in pharmacy and nursing students.	The Kiersma-Chen Empathy Scale scores on the empathy scale were positively associated with Jefferson scale scores ( $P < 0.001$ ). Factor analysis showed a poor fit for the Kiersma-Chen Empathy Scale

Preusche and Wagner-Menghin[81]	Austria	516		47.8	Medical Students	0.823	20	To adapt the Jefferson Scale of Physician Empathy into a German version, examine its psychometric properties, to compare the level of attitude towards empathy with other adaptations	Item-total score correlations were all positive. Reliability was high; a 6-7 wk test-retest correlation for a subsample was 0.45. Factor analysis revealed a four-factor solution
Shariat and Habibi <sup>[119]</sup>	Iran	1187	22.6	36.1	Medical Students	0.79	20	To examine empathy in Iranian medical students and the psychometric properties of Jefferson Scale of empathy	Female students had higher scores of empathy and empathy decreased with higher years of education. The scale had acceptable internal consistency and test re-test reliability with a three-structure solution emerging from factor analysis
Wen <i>et al.</i> <sup>[33]</sup>	China	753		36.8	Medical Students	0.83	20	To examine empathy among medical students in China	The three factors solution accounted for 48% of the variance. The mean empathy score was 109.60. The empathy score of medical students had significant differences between male and females ( $P < 0.05$ ) and academic year ( $P < 0.05$ )

Williams <i>et al</i> <sup>[88]</sup>	Australia	330		34.8	Paramedics	0.75	20	To investigate psychometric properties of Jefferson Scale of Physician Empathy in paramedic students	The 2-factor solution, "compassionate care" and "perspective taking", accounted for 44.2% of the total variance. The 17-item two-factor model produced good model fit and good reliability estimates. Three of the original items did not fit the model.
Khademalhosseini <i>et al</i> <sup>[120]</sup>	Iran	260	20.9	46.2	Medical Students	0.76	20	To measure the empathy score among medical students	Empathy scores decreased with increase in the students' age ( $P = 0.001$ ) year of study ( $P = 0.030$ ). Mean empathy score in basic science level (65.5) was higher than clinical level empathy (55.5). Female students had higher mean empathy score (65.53) than male students (59.02).
Leombruni <i>et al</i> <sup>[121]</sup>	Italy	257	20.6	44.4	Medical Students	0.76	20	To examine psychometric and confirm factor structure of the Italian version of the Jefferson Empathy Scale in Italian medical students	The empathy scale showed an acceptable internal consistency ( $r = 0.76$ ) and test-retest reliability ( $r = 0.72$ ). Confirmatory factor analysis found that the 3-factor structure has acceptable data fit. Female medical students showed a higher mean empathy score than did males.



Mostafa <i>et al</i> <sup>[28]</sup>	Bangladesh	348	29.9	Medical Students	0.88	20	To measure and examine empathy among a sample of undergraduate medical students of Bangladesh	Mean empathy score was 110.41. There were significant associations between gender and empathy scores. The level of empathy in medical students gradually increases after clinical training in medical college. Non-significant difference were noted between empathy scores and specialty preferences.
van Ryn <i>et al</i> <sup>[113]</sup> (2)	USA	4732	50.1	Medical Students	0.88	20	(see details above)	(see details above)
Williams <i>et al</i> <sup>[122]</sup>	Australia	1111	18.4	Healthcare students (medicine, nursing, physiotherapy, occupational therapy, paramedics, midwifery, nutrition and dietetics)	0.78	20	To examine self-reported empathy levels of students enrolled in different health disciplines from two large Australian universities.	The mean female empathy score was significantly higher than the mean male score. Paramedic students had significantly lower empathy scores than all other participants except nursing students ( $P < 0.0001$ )

Youssef <i>et al.</i> <sup>[84]</sup> (1)	Trinidad and Tobago	667	22.2	35	Medical Students	0.77	20	To explore the empathy profile of students across five years of medical training and to examine whether the Jefferson Scale for Physician Empathy correlated with a measure of cognitive empathy, the Reading the Mind in the Eyes Test and a measure of affective empathy, the Toronto Empathy Questionnaire	There was a significant correlation between the Jefferson Scale of Physician Empathy and the Toronto Empathy Questionnaire ( $\rho = 0.48$ ). There was a decline in medical student empathy scores over time. There was weak little correlation between scores from the Reading the Mind in the Eyes Test and the Jefferson Scale of Physician Empathy. Female students demonstrated significantly higher scores on all three measures.
Hojat and Gonnella <sup>[123]</sup>	USA	2637	23.4	49	Medical Students	0.8	20	To provide typical descriptive statistics, score distributions and percentile ranks of the Jefferson Scale of Empathy-Medical Student version	The score distributions of the Jefferson Scale of Empathy tended to be moderately skewed and platykurtic. Women obtained a significantly higher mean score ( $116.2 \pm 9.7$ ) than men ( $112.3 \pm 10.8$ ) on the Jefferson Scale of Empathy ( $P < 0.01$ ). The tentative cut-off score to identify low scorers was $\leq 95$ for men and $\leq 100$ for women.

Jeon and Cho <sup>[103]</sup>	South Korea	447		18.1	Pharmacy students	0.713	20	To validate an empathy scale and to investigate the empathy levels of pharmacy students in South Korea	The 3-factor model of the empathy scale was confirmed by confirmatory factor analysis and the convergent validity was also supported by its correlations with the interpersonal reactivity index subscales.
Montanari <i>et al</i> <sup>[104]</sup>	Italy	797	22.63	26	Nursing Students	0.78	20	To test the psychometric properties of the Jefferson Scale of Empathy-Health Professional Student's version and to describe their empathic engagement	Fit for a three-factor solution for 14 items: compassionate care/emotional engagement, perspective-taking, and standing in the patient's shoes. Confirmatory factor analysis on the second half of the sample showed good fit indexes for the 14-item solution and the 20 item solution of the scale, with the exception of one item
Park <i>et al</i> <sup>[87]</sup>	South Korea	5343	26.4	61.5	Medical Students	0.83	20	To evaluate empathy in Korean medical students throughout the country and to make suggestions to improve empathy	Females and post-baccalaureate students had higher scores. Students from higher grade levels had lower scores than those from the lower grade levels
Park <i>et al</i> <sup>[99]</sup>	South Korea	2692	24.7	62	Medical Students	0.715	20	To examine the relationship between stress, social support, and empathy among medical students	Empathy and social support were positively correlated, and empathy and stress negatively correlated. In the regression model, stress and social support predicted empathy

Petek Ster and Selic <sup>[102]</sup>	Slovenia	845	22.5	31.4	Medical Students	0.781	20	To re-validate the Jefferson Scale of Empathy (Student version) and its factor structure prior further research on empathy in medical students.	Females achieved higher empathy scores. The three-factor structure of empathy was confirmed. A higher proportion of explained variation was observed with Perspective Taking and Standing in the Patient's Shoes, and better internal consistency was noted in a reduced-item scale (16-18 items).
Williams <i>et al</i> <sup>[94]</sup>	Malaysia	204	20	44.3	Medical Students	0.7	20	To examine empathy scores in undergraduate medical students	The mean empathy score for first year students was significantly higher than second year students ( $P < 0.05$ ). No significant difference relating to gender
Aggarwal <i>et al</i> <sup>[86]</sup>	India	978	21.6	31.6	Dentistry Students	0.677	20	To measure the self-reported empathy levels among dental undergraduate and postgraduate students and to review factors that could affect empathy	There were significant differences in empathy scores by gender and age ( $P < 0.01$ )
Costa <i>et al</i> <sup>[95]</sup> (2)	Portugal, Brazil, UK, New Zealand, Ireland	3069	38.5		Medical Students	0.69	20	(see details above)	(see details above)
Ferreira-Valente <i>et al</i> <sup>[101]</sup>	Spain	1104	20.7	32	Medical Students	0.78	20	To examine the psychometric properties of a Spanish empathy scale	The Spanish scale had acceptable to good sensitivity, convergent validity and reliability. The confirmatory factor analysis supported the three-factor solution and the second order latent factor model

Jordan and Foster <sup>[98]</sup>	USA	163			Medical Students	0.8	20	Examination of the interpersonal theory of clinical, personality, and social psychology to examine the construct of empathy and theorize about likely interpersonal correlates	All factors of empathy were related to interpersonal warmth. Perspective taking and compassionate care were associated with submissiveness. Walking in the patient's shoes was correlated with social support and less loneliness
Mahoney <i>et al</i> <sup>[124]</sup>	Australia	281	26	42	Medical Students	0.815	20	To examine student and doctor empathy, and possible associations between empathy and the structure of clinical learning	Empathy decreased during the course of each year, but no differences between years of clinical education.
Sng <i>et al</i> <sup>[93]</sup>	Singapore	881		46.3	Medical Students	0.83	20	To investigate psychometric properties of Jefferson Scale of Physician Empathy	Empathy declined between preclinical and clinical years. Female and medical specialty interest respondents had higher scores but factor analysis suggested that the three factor model did not fit adequately
Spasenoska <i>et al</i> <sup>[100]</sup>	Malaysia	193	19.3	43	Medical Students	0.68	20	To investigate psychometric properties of Jefferson Scale of Physician Empathy	The scale was best interpreted as a two factor solution of perspective taking and compassionate care
Stansfield <i>et al</i> <sup>[125]</sup>	USA	4797	23.7	50.5	Medical Students	0.86	20	Analysis of factor structure of empathy and relations to other factors	Components of empathy change over time during undergraduate medical education (towards the end of education, it is a four factor structure of feelings, importance, ease, and metacognitive effort

Kiersma-Chen empathy scale

Kiersma <i>et al</i> <sup>[96]</sup> (2)	USA	216	20.5	24.1	Pharmacy and nursing students	0.86	15	(see details above)	(see details above)
Narcissism, aloofness, confidence, empathy (NACE) scale - empathy									
Pitt <i>et al</i> <sup>[85]</sup>	Australia	133	27	14	Nursing Students	0.79	24	To describe the personal qualities of newly enrolled Bachelor of Nursing students; to determine if these a change according to age, gender, and time	Females were significantly more conscientious, community orientated and involved while males had significantly higher narcissism and aloofness scores and lower empathy
Patient-Practitioner orientation scale									
Dockens <i>et al</i> <sup>[126]</sup>	USA	93	22.6	6.5	Speech and hearing sciences students	0.78	18	To determine preferences to patient-centeredness in pre-service speech and hearing students in the field of speech and hearing sciences	Across exposure levels, students exhibited high preference to patient centeredness with a mean empathy score of 4.13. A paired sample t-test revealed a significant difference ( $P < 0.0001$ ) between the caring and sharing subscales of the empathy scale. No significant differences across levels of exposure for sharing subscale and caring subscale
Professionalism assessment scale - empathy									
Klemenc-Ketis and Vrecko <sup>[127]</sup>	Slovenia	122	22.1	22.1	Medical Students	0.84	10	To develop and validate a scale for the assessment of professionalism in medical students based on students' perceptions of and attitudes towards professionalism in medicine	The scale was developed with 22 items. The Cronbach's alpha of the scale was 0.88. Factor analysis revealed three factors: empathy and humanism, professional relationships and development and responsibility
Pro-Social personality battery (Other-Oriented empathy)									



Eley <i>et al</i> <sup>[128]</sup>	USA	145	24	40.7	Medical Students	0.77	18	To examine personality trait profiles of rural longitudinal integrated clerkships students	Rural longitudinal integrated clerkships students who intended and matched to family medicine showed the highest levels of Reward Dependence (warm sociability) and Other-Oriented Empathy compared to any other specialty
Toronto composite empathy scale									
Tsiantou <i>et al</i> <sup>[83]</sup>	Greece	460	20.7	34.8	Dentistry Students	0.75	52	To examine empathy among dentistry students in Greece and validate the Toronto Composite Empathy Scale	The scale has good discriminant and convergent validities. Test-retest reliabilities ranged from 0.478 to 0.779. Rotated factor analysis indicated that items loaded on two cognitive and three emotional factors. Females had significantly higher empathy scores
Toronto Empathy Questionnaire									
Youssef <i>et al</i> <sup>[84]</sup> (2)	Trinidad and Tobago	662	22.2	35	Medical Students	0.85	16	(see details above)	(see details above)

Caring Behaviour Inventory tool (0.921).

### **Analysis of heterogeneity, sensitivity analysis and cumulative meta-analysis**

Subgroup analyses were performed with regards to scale (Figure 4A) and country (Figure 4B), and subject of study (Figure 4C). With respect to country, heterogeneity reduces to non-significant in Portugal, Australia, Italy, Malaysia, Iran, and Germany (Figure 4B). Regarding subject of study, heterogeneity is non-significant only in osteopathic medical students and pharmacy and nursing students (Figure 4C). Finally, a meta-regression of mean age and male percentage as predictors of reliability is not significant for both parameters (Table 3).

Sensitivity analysis did not identify any study which influenced the meta-analytic outcome significantly (Figure 5A). Cumulative meta-analysis shows that after the addition of the meta-analysis' tenth study in 2013 by Preusche and Wagner-Menghin<sup>[81]</sup>, the outcome overall has trivial variation, indicating stability of the results.

### **Narrative discussion of studies**

Thirteen empathy scales were identified with most of them being used in one study apart from the Jefferson Scale of Physician Empathy, Davis' Interpersonal Reactivity Index and Caring Behaviour Inventory Tool which were used in 34, 3, and 2 studies respectively. Many studies reported that males had lower empathy scores compared to females. This was observed with the Balanced Emotional Empathy Scale<sup>[82]</sup>, the Toronto Composite Empathy Scale<sup>[83]</sup>, the Toronto Empathy Questionnaire<sup>[84]</sup>, the

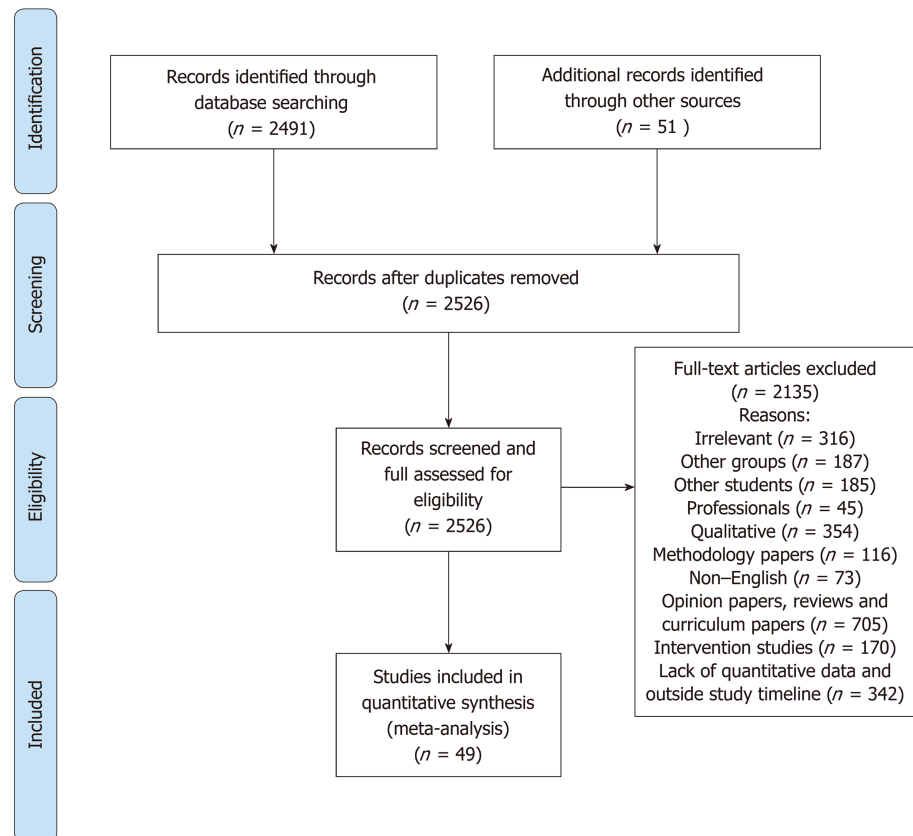


Figure 1 Flow chart of study selection.

Narcissism, Aloofness, Confidence, Empathy (NACE) scale–Empathy<sup>[85]</sup>, and the Jefferson Scale of Physician Empathy<sup>[28,86-90]</sup>. Next, empathy decreased with progression through clinical education and empathy had decreased towards the clinical years with the Caring Ability Inventory<sup>[91]</sup>, the Caring Behaviour Inventory Tool<sup>[92]</sup>, the Toronto Empathy Questionnaire<sup>[84]</sup>, and the Jefferson Scale of Physician Empathy<sup>[86,87,93,94]</sup>.

Regarding correlations between concepts and scales, the Jefferson Scale of Physician Empathy was correlated to Davis' Interpersonal Reactivity Index (0.313)<sup>[95]</sup>, Kiersma-Chen Empathy Scale ( $P < 0.05$ )<sup>[96]</sup> and the Toronto Empathy Questionnaire (0.480)<sup>[84]</sup>. There was a correlation between self-compassion and emotional intelligence (0.400)<sup>[97]</sup>, empathy and interpersonal warmth<sup>[98]</sup>, and empathy and social support<sup>[99]</sup>. Main solutions of the factor analysis of Jefferson scale of Physician Empathy were a two<sup>[100]</sup> or three factor<sup>[101-104]</sup> solution comprising of mainly perspective taking and compassionate care followed standing in the patient's shoes.

## DISCUSSION

In the present study, a systematic review of empathy scales in healthcare students was performed. This is the first systematic review that also performed a meta-analysis of the internal consistency (Cronbach's alpha) of the scales reviewed. Answering the two research questions, 13 scales have been used to assess clinical empathy in healthcare students from 49 studies and total sample size 49384 students. The most frequently used is the Jefferson Scale of Physician Empathy<sup>[25]</sup> followed by Davis' Interpersonal Reactivity Index<sup>[105]</sup>. And secondly, the overall reliability was 0.805 (95%CI 0.786-0.823), which is acceptable, but there was heterogeneity and publication bias. Some heterogeneity was explained by different countries and student types but most heterogeneity remained unexplained. The most reliable scale was the Caring Behaviour Inventory Tool with alpha 0.921; Balanced Emotional Empathy Scale had the lowest reliability of 0.720, while the Jefferson Scale of Physician Empathy and Davis' Interpersonal Reactivity Index were near the average with values of 0.798 and 0.791, respectively.

The present study has certain limitations. First of all, there was heterogeneity in the meta-analysis. This was investigated with subgroup analyses and meta-regression,



**Figure 2 Risk of bias assessment.** A: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies; B: Risk of bias summary: Review authors' judgements about each risk of bias item for each included study.

and a random effects model which sought to reduce the impact of heterogeneity. Heterogeneity could have resulted from population differences and design biases possibly not taken into account. A further limitation was that the meta-analysis was performed at the level of the scale and not the level of subscales for similar scales. The next limitation is the presence of publication bias, as indicated by asymmetry in the

**Table 2** Meta-analytic means of Cronbach's alpha per empathy scale

Scale	N	k	Alpha	95%CI		I <sup>2</sup>	P-value
Balanced Emotional Empathy Scale	237	1	0.72	0.666	0.769	--	--
Toronto Composite Empathy Scale	460	1	0.75	0.716	0.781	--	--
Caring Ability Inventory	598	1	0.77	0.743	0.795	--	--
Pro-Social Personality Battery (Other-Oriented Empathy)	145	1	0.77	0.71	0.821	--	--
Patient-Practitioner Orientation Scale	93	1	0.78	0.707	0.839	--	--
Narcissism, Aloofness, Confidence, Empathy (NACE) scale - Empathy	133	1	0.79	0.734	0.838	--	--
Davis' Interpersonal Reactivity Index	7845	3	0.791	0.742	0.835	96.40%	< 0.001
Jefferson Scale of Physician Empathy	37159	34	0.798	0.774	0.821	98.30%	< 0.001
Professionalism assessment scale - Empathy	122	1	0.84	0.794	0.879	--	--
Toronto Empathy Questionnaire	662	1	0.85	0.833	0.867	--	--
Kiersma-Chen Empathy Scale	216	1	0.86	0.831	0.886	--	--
Emotional Intelligence Assessment Scale - Empathy	471	1	0.87	0.85	0.887	--	--
Caring Behaviour Inventory Tool	1243	2	0.921	0.915	0.927	0%	0.731
Overall	49384	49	0.805	0.786	0.823	98%	< 0.001

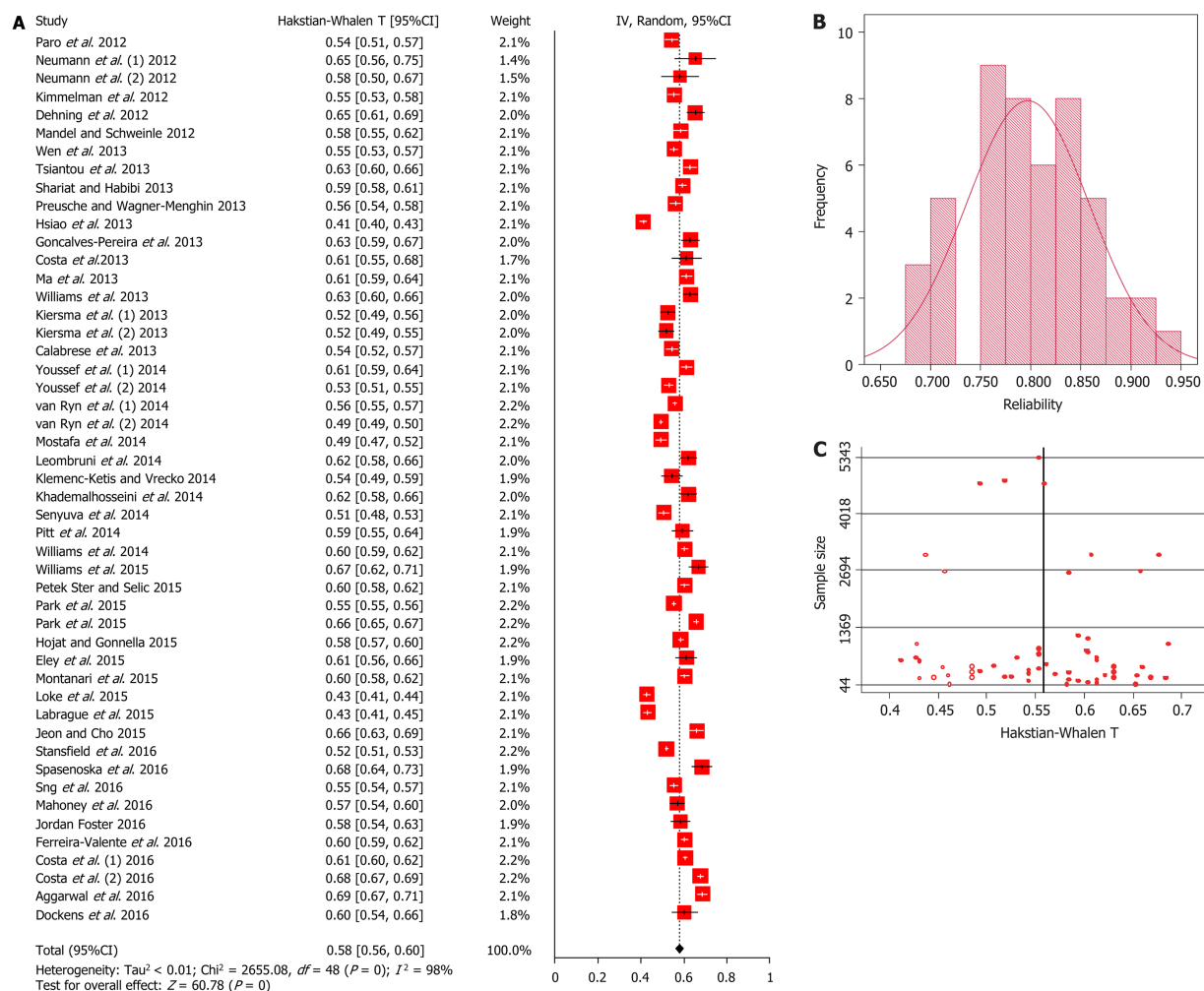
N: Number of participants, k: Number of studies.

funnel plot. This could indicate that there are possibly studies performed that had low internal consistency in their scales and have not been published or accepted for publication. The next important limitation is that the mean values and standard deviations (overall, females, and males) for each scale were not recorded. This is important considering the gender effect in empathy and the variability that this could produce and hence explain heterogeneity<sup>[106]</sup>. Other empathy moderators, not examined in the present study, were other psychological or personality constructs and differences in years. Quality assessment found only minimal impact from insufficient sample sizes. Nevertheless, the sample size in many studies is not sufficiently large which might have led to small study effects<sup>[107]</sup>. This suggests caution when interpreting results because small studies are more likely to report better results<sup>[108]</sup>.

The meta-analytic mean for Cronbach's alpha was within the acceptable range (0.805 which is over 0.700) for scales as described in the literature<sup>[67,68]</sup>. However, it would be preferable if it were closer to 0.900. The most frequent scale used was the Jefferson Scale for Physician Empathy which had an alpha of 0.798. The highest alpha was noted with the Caring Behaviour Inventory Tool which had 0.921. These findings corroborate the concerns mentioned in the Introduction, which suggested that empathy is assessed in many ways with no clear consensus at present with regards to a preferred optimum.

Based on the findings of this meta-analysis, it seems that Caring Behaviour Inventory Tool ought to be utilized more. However, it has been developed mainly for nurses and it hasn't been adapted for other healthcare specialties. This needs to be addressed and further studies are required with this scale tested in other groups. Nonetheless, the Jefferson Scale of Physician Empathy is currently being used most frequently and is currently dominating the clinical empathy literature. Hence, it seems that this scale could be improved with addition of further items that will increase internal consistency, thus increasing the Cronbach's alpha. The third scale currently being used most frequently is Davis' Interpersonal Reactivity Index. Its meta-analytic Cronbach's alpha has the same issues mentioned for the Jefferson Scale of Physician Empathy.

Another practical implication is the need for a consensus to adopt a certain group of scales with the best internal consistency and widespread use. The present Babel of scales is confusing and disruptive for sound research production. The Jefferson Scale of Physician Empathy seems to be close to an integrated definition of empathy incorporating both cognitive and affective traits. It might benefit from the addition of more items similar to Davis' Interpersonal Reactivity Index, thus also increasing its alpha. Moreover, empathy is a massive field and its research extends to social neuroscience and neuropsychology. Hence, we believe that clinical empathy as a field might benefit from borrowing concepts from research studies in the field of social neuroscience and neurobiology<sup>[109]</sup>. This will allow comparative analyses and possible improvements on definition, scales and development of theory. Finally, we believe that more meta-analyses and umbrella reviews are needed with various approaches and research questions in the field of clinical empathy<sup>[110]</sup>. The data produced by the



**Figure 3 Meta-analysis of Cronbach's alpha.** A: Forest plot for all studies; B: Distribution of reliabilities (Cronbach's alpha) in the present study; C: Funnel plot of the effect size against sample size. The empty dots represent the studies from the trim-and-fill analysis. IV: Inverse variance.

literature is large and potentially unmanageable for individual researchers. At the same time, narrative reviews are bound by subjectivity, no matter how in-depth they are. The evidence-based approach will allow the most widely accepted definition and scales of clinical empathy to surface. Also, problematic areas will emerge and will stimulate further research.

In conclusion, this study presented the first meta-analysis of reliability for empathy scales in healthcare education. The results indicate that scales have satisfactory internal consistency but there is a confusion of scales, definitions and empathy components. There is evidently a need to standardize research in clinical empathy with meta-reviews<sup>[110]</sup>. Future research should focus on standardizing scales that are used throughout healthcare education and production of consensus statements on definition of empathy and use of appropriate empathy scales.



Table 3 Meta-regression results

Variable	Coefficient (95%CI)	Standard error	t	P
mean age	-0.00528 (-0.01813, 0.007571)	0.006301	-0.84	0.409
Male %	0.000527 (-0.00077, 0.00182)	0.000634	0.83	0.413
Constant	0.677161 (0.394914, 0.959408)	0.138389	4.89	< 0.001

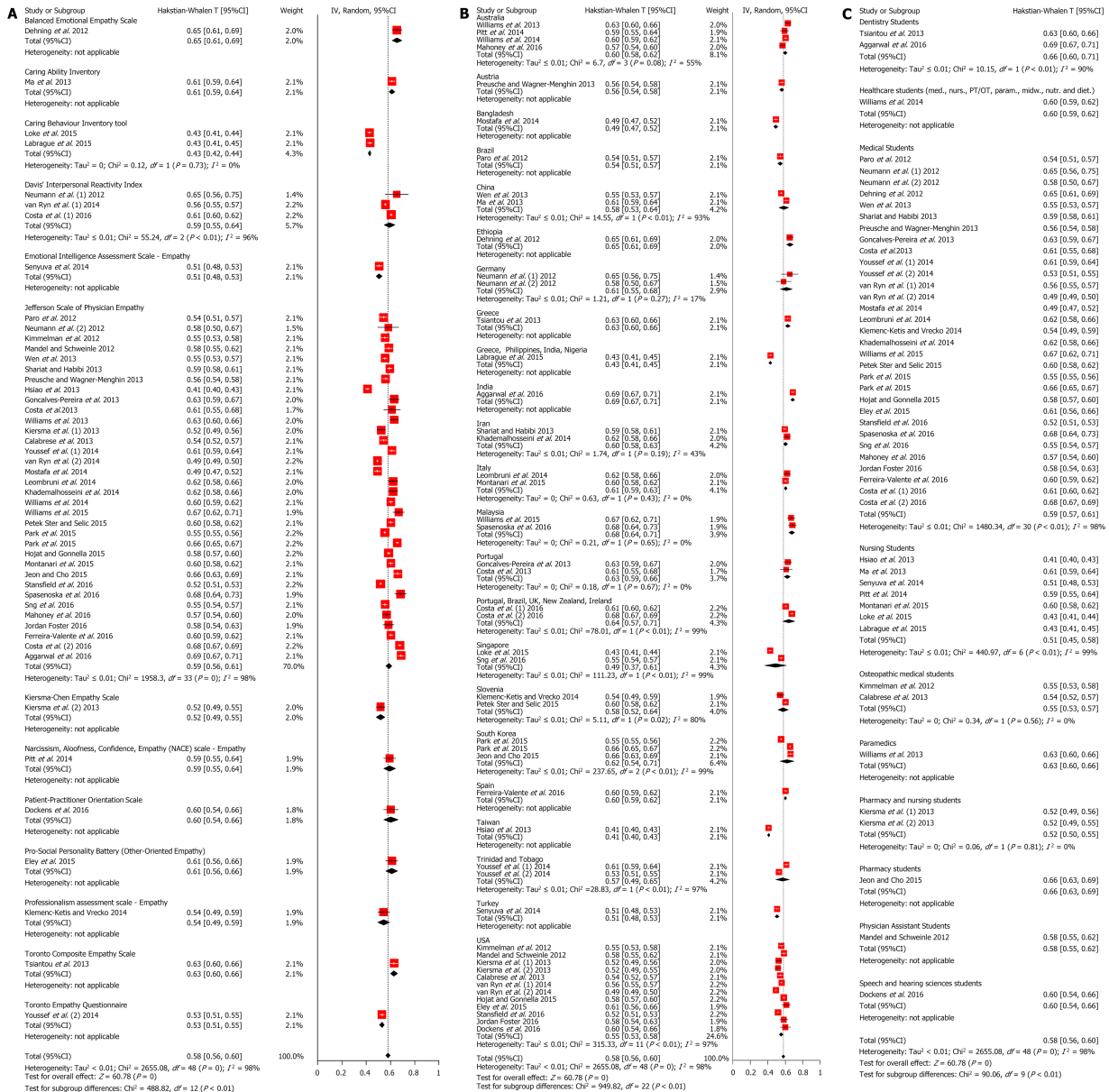
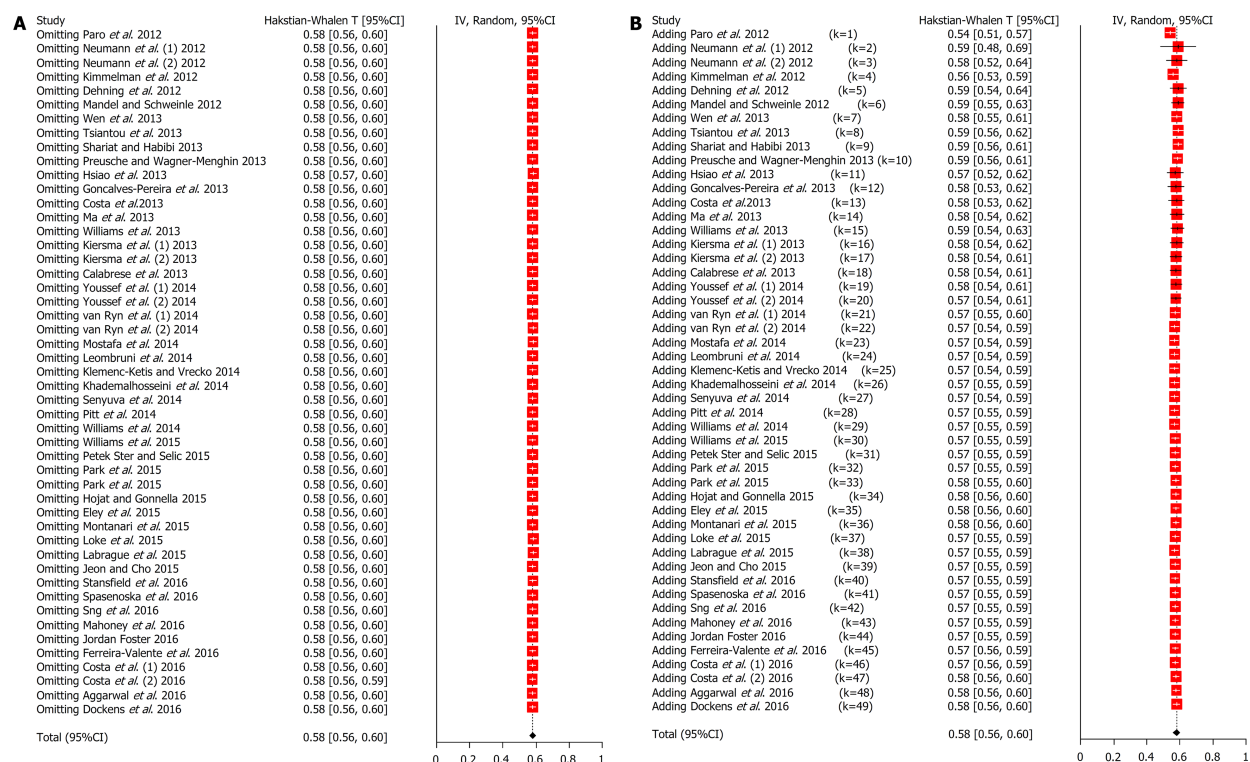


Figure 4 Subgroup analyses. A: Forest plot according to scale; B: Forest plot according to country; C: Forest plot according to field of study. IV: Inverse variance.





**Figure 5 Sensitivity analysis and cumulative meta-analysis.** A: Forest plot for sensitivity meta-analysis; B: Forest plot for cumulative meta-analysis. IV: Inverse variance.

## ARTICLE HIGHLIGHTS

### Research background

Empathy is a particular form of interpersonal understanding and stands for a basic sensitivity to the mindedness of other persons. In general, it is considered that clinical empathy leads to improved patient satisfaction, greater adherence to therapy, better clinical outcomes, greater quality of service delivery perception and lower malpractice liability, while for clinicians and society it promotes the values of humanism, reduces professional burnout and increases diagnostic accuracy, and increases public trust to the healthcare system. Assessing empathy is one of the most important aspects of investigating empathy with over 10 scales currently in use.

### Research motivation

There is a multitude of empathy scales with minimal or no efforts to produce an integrated definition of clinical empathy which can be assessed sufficiently by only a few scales. Next, there are 38 systematic reviews in the topic of empathy but only 6 of them are meta-analyses. The lack of quantitative synthesis does not allow for aggregation of research into meta-studies and umbrella reviews that are better in summarizing evidence and guiding policy and practice.

### Research objectives

Our hypotheses are that multiple empathy scales are currently in use and their results are heterogeneous. The present study's objectives are to answer which empathy scales have been used in the years 2012 to 2016 to assess and measure empathy in healthcare students, what their reliability is and which factors contribute to their heterogeneity.

### Research methods

A systematic review was performed with inclusion criteria any empirical study with quantitative data examining empathy of healthcare students toward patients between 2012 and 2016. A random effects model was used to produce a pooled estimate of the Cronbach's alphas. The Hakstian-Whalen transformation was used for analyses based on the Rodriguez-Maeda method. Heterogeneity was quantified using the  $I^2$  statistic and further investigated with subgroup analysis and meta-regression. Publication bias was assessed using funnel plots, Egger's test, Begg's test, and the trim and fill analysis.

### Research results

Thirteen scales have been used to assess clinical empathy in healthcare students from forty nine studies with total sample size 49384 students. The most frequently used scale is the Jefferson Scale of Physician Empathy followed by Davis' Interpersonal Reactivity Index. The overall reliability was 0.805 (95%CI 0.786-0.823), which is acceptable, but there was heterogeneity and

publication bias. Some heterogeneity was explained by the different countries of the studies under investigation and student types but most heterogeneity remained unexplained.

### Research conclusions

This study is the first meta-analysis of reliability for empathy scales in healthcare education. The results indicate that scales have satisfactory internal consistency but there is a confusion of scales, definitions and empathy components. The meta-analytic mean for Cronbach's alpha was within the acceptable range for scales. The Jefferson Scale of Physician Empathy is currently being used most frequently and is currently dominating the clinical empathy literature. In practice, there is need for consensus to adopt a certain group of scales with best internal consistency and widespread use. The Jefferson Scale of Physician Empathy seems to be close to an integrated definition of empathy incorporating both cognitive and affective traits. It might benefit from the addition of more items similar to Davis' Interpersonal Reactivity Index, thus also increasing its alpha. Finally, more meta-analyses and umbrella reviews are needed with various approaches and research questions in the field of clinical empathy.

### Research perspectives

Future research should focus on standardizing scales that are used throughout healthcare education and production of consensus statements on definition of empathy and use of appropriate empathy scales.

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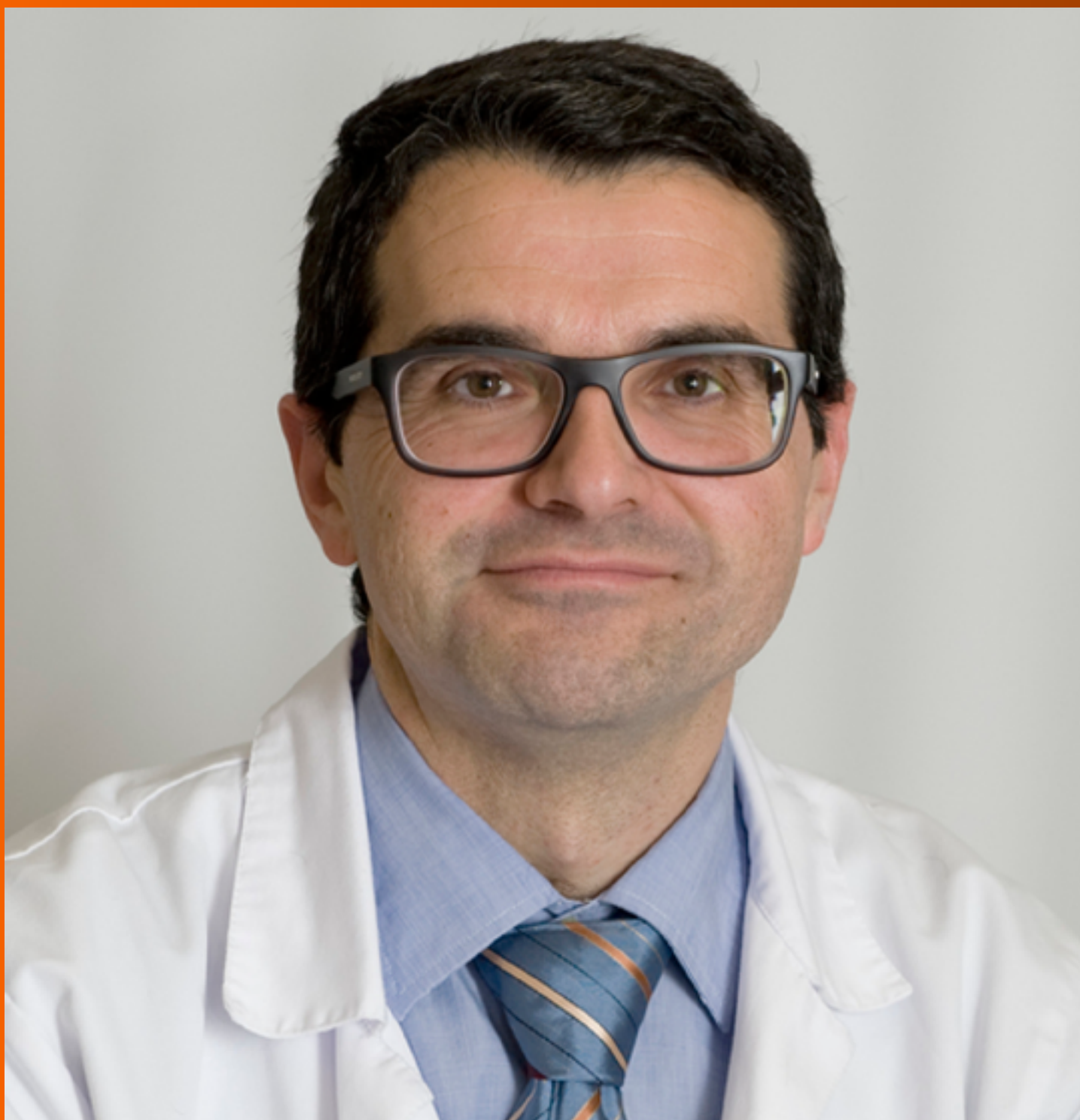


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## Misclassification of smoking habits: An updated review of the literature

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### Abstract

#### BACKGROUND

Misclassification of smoking habits leads to underestimation of true relationships between diseases and active smoking, and overestimation of true relationships with passive smoking. Information on misclassification rates can be obtained from studies using cotinine as a marker.

#### AIM

To estimate overall misclassification rates based on a review and meta-analysis of the available evidence, and to investigate how misclassification rates depend on other factors.

#### METHODS

We searched for studies using cotinine as a marker which involved at least 200 participants and which provided information on high cotinine levels in self-reported non-, never, or ex-smokers or on low levels in self-reported smokers. We estimated overall misclassification rates weighted on sample size and investigated heterogeneity by various study characteristics. Misclassification rates were calculated for two cotinine cut points to distinguish smokers and non-smokers, the higher cut point intended to distinguish regular smoking.

#### RESULTS

After avoiding double counting, 226 reports provided 294 results from 205 studies. A total of 115 results were from North America, 128 from Europe, 25 from Asia and 26 from other countries. A study on 6.2 million life insurance applicants was considered separately. Based on the lower cut point, true current smokers represented 4.96% (95% CI 4.32-5.60%) of reported non-smokers, 3.00% (2.45-3.54%) of reported never smokers, and 10.92% (9.23-12.61%) of reported ex-smokers. As percentages of true current smokers, non-, never and ex-smokers formed, respectively, 14.50% (12.36-16.65%), 5.70% (3.20-8.20%), and 8.93% (6.57-11.29%). Reported current smokers represented 3.65% (2.84-4.45%) of true non-



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smokers. There was considerable heterogeneity between misclassification rates. Rates of claiming never smoking were very high in Asian women smokers, the individual studies reporting rates of 12.5%, 22.4%, 33.3%, 54.2% and 66.3%. False claims of quitting were relatively high in pregnant women, in diseased individuals who may recently have been advised to quit, and in studies considering cigarette smoking rather than any smoking. False claims of smoking were higher in younger populations. Misclassification rates were higher in more recently published studies. There was no clear evidence that rates varied by the body fluid used for the cotinine analysis, the assay method used, or whether the respondent was aware their statements would be validated by cotinine - though here many studies did not provide relevant information. There was only limited evidence that rates were lower in studies classified as being of good quality, based on the extent to which other sources of nicotine were accounted for.

## CONCLUSION

It is important for epidemiologists to consider the possibility of bias due to misclassification of smoking habits, especially in circumstances where rates are likely to be high. The evidence of higher rates in more recent studies suggests that the extent of misclassification bias in studies relating passive smoking to smoking-related disease may have been underestimated.

**Key words:** Misclassification; Smoking; Cotinine; Cigarettes; Tobacco use; E-cigarettes; Passive smoking; Bias; Systematic review; Meta-analysis

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**Core tip:** We update a meta-analysis of evidence on accuracy of reported smoking, using cotinine as a marker. From 200+ studies, we estimated various misclassification rates. True smokers represented 3.00% (2.45%-3.54%) of reported never smokers and 10.92% (9.23%-12.61%) of reported ex-smokers. Reported never and ex-smokers formed 5.70% (3.20%-8.20%) and 8.93% (6.57%-11.29%) of true smokers. Falsely claiming never smoking was extremely common in Asian women. Rates of falsely claiming quitting were high in pregnant women and diseased individuals advised to quit. Smoking misclassification causes overestimation of true passive smoking relationships, a problem exacerbated by increasing misclassification rates in recently published studies.

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## INTRODUCTION

When interviewed, someone may deny current or past smoking habits, or even falsely claim to be a smoker or to have smoked. While random misclassification of smoking habits tends to understate true relationships of disease with smoking, it may overstate relationships with spousal smoking. This overstatement arises because studies of the effects of spousal smoking are typically conducted in self-reported never smokers and because smokers tend to marry smokers. Thus, random misclassification of smoking results in a higher proportion of misclassified true smokers in the group whose spouse smokes<sup>[1]</sup>. In studying the relationship with disease of a variable correlated with smoking, smoking misclassification also affects the extent to which the statistician can adjust for confounding by smoking. Tzonou *et al*<sup>[2]</sup> notes that even a 10% error in a confounding variable leaves about half the confounding effect remaining after adjustment.

To determine the likely extent of bias, it is clearly advantageous to obtain information on the extent of inaccuracy in reported statements on smoking. One approach (not considered here), which gives information on the extent to which smokers may deny past smoking, is to compare statements made at separate time points. A second approach (the subject of this review) is based on studies using

cotinine (a metabolite of nicotine), typically measured in blood, saliva or urine, as an objective indicator of recent smoking. Levels higher than an appropriate cut-off cannot arise from passive smoking or dietary sources of nicotine, and must in practice have arisen from smoking, smokeless tobacco, nicotine replacement therapy or, in recent years, electronic cigarettes<sup>[3-6]</sup>.

Over 20 years ago, Lee and Forey<sup>[7]</sup> reviewed evidence from 35 studies where smoking habits were validated by cotinine, and since then other reviews have considered some of the evidence<sup>[8-12]</sup>. However, these reviews are mostly quite old and, as will become apparent, none consider more than a fraction of the relevant evidence. Here we present a detailed review of the evidence, although, as there are numerous studies using cotinine to validate smoking status, we restrict attention to those measuring cotinine in urine, saliva or blood (serum or plasma) in 200+ participants. Also, as interest is mainly in high cotinine levels in self-reported non-smokers, we exclude studies restricted to self-reported smokers. However, providing a study presents the required data for self-reported non-smokers, we do summarize data on low cotinine levels in self-reported smokers. We also exclude studies of young children, who would have a very low likelihood of smoking.

## MATERIALS AND METHODS

### Study inclusion criteria

These include: At least 200 participants with cotinine levels determined in saliva, urine, blood (serum or plasma); data available on misclassification rates in self-reported non-smokers, never smokers or ex-smokers (or self-reported non-, never- or ex-tobacco users); data in populations reasonably likely to smoke (*i.e.*, not infants or young children); and published in English.

The study also had to provide data for cotinine cut points distinguishing smokers from non-smokers. For plasma, serum and saliva, the lower cut point (Cut 1) had to be in the range 8-35 ng/mL, while for urine it had to be within 50-150 ng/mL, these covering the range of cut points commonly considered appropriate: Reports showing the bimodal distribution of non-smoker and smoker cotinine in saliva<sup>[13,14]</sup>, serum<sup>[15,16]</sup> and urine<sup>[17,18]</sup> support these ranges as do the ranges for non-smokers and smokers found in individual studies<sup>[9]</sup> and the ranges used in other analyses<sup>[13,19]</sup>. For some analyses, we used a higher cut point (Cut 2), as used by some researchers<sup>[20-26]</sup>. To ensure distinct ranges for Cut 1 and Cut 2 we required that, for plasma, serum and saliva Cut 2 had to be at least 50 ng/mL, while for urine it had to be 250-750 ng/mL. Studies using 10%, or 30%, of the mean smoker value for distinguishing smokers were also accepted as providing equivalent data to, respectively, Cut 1 and Cut 2.

### Misclassification rates

Suppose one has data from a study as follows: Self-reported smoking habits [Non-smoker (Participants studied: A, Number misclassified: E); Never-smoker (Participants studied: B, Number misclassified: F); Ex-smoker (Participants studied: C, Number misclassified: G); Current smoker (Participants studied: D, Number misclassified: H)].

Misclassified participants are those with cotinine levels above the defined cut point for non-smokers and those below the cut point for current smokers. Noting that  $A = B + C$  and  $E = F + G$  we sought to derive the following "misclassification rates", with "true" status based on cotinine levels: Rates M1-M3: Percentage of self-reported non-smokers ( $E/A$ ), never smokers ( $F/B$ ) or ex-smokers ( $G/C$ ) whose cotinine implies current smoking ("true current smokers"); Rate M4: Percentage of self-reported current smokers ( $H/D$ ) whose cotinine implies non-smoking ("true non-smokers"). This may include occasional smokers who did not smoke in the days leading up to the sample being taken for cotinine analysis; Rates M5-M7: Percentage of true current smokers who report being non-smokers [ $E/(D - H + E)$ ], never smokers [ $F/(D - H + E)$ ] or ex-smokers [ $G/(D - H + E)$ ]; Rates M8-M10: Percentage of self-reported current smokers plus misclassified non-smokers who report being non-smokers [ $E/(D + E)$ ], never smokers [ $F/(D + E)$ ] or ex-smokers [ $G/(D + E)$ ]; and Rate M11: Percentage of true non-smokers who report being current smokers [ $H/(A - E + H)$ ]. As for rate M4, this may include some occasional smokers.

Not all these rates can be calculated, often because data are unavailable for self-reported current smokers or non-smokers are not separated into never- and ex-smokers. While, assuming that cotinine is the gold standard, rates M5-M7 are theoretically superior to rates M8-M10 for estimating the extent current smokers deny smoking, studies where cotinine is only measured on reported non-smokers provide no estimate of H, so preclude estimation of rates M5-M7.

Where possible, rates were calculated for both Cut 1 and Cut 2. Where a study provided a choice of cut-offs for plasma, serum or saliva, we used that closest to 20 ng/mL for Cut 1 and that closest to 100 ng/mL for Cut 2. For urine, we used cut points closest to 100 ng/mL and 500 ng/mL respectively. These represent the mid-point of the ranges used.

### Literature sources

We considered, in turn, four information sources: A previous attempt to summarize relevant data<sup>[7]</sup>; papers filed under “COT” in the P.N. Lee Statistics and Computing Ltd. database, accumulated over many years; a search on PubMed using the term “cotinine”; and studies referenced in misclassification review papers discovered in our searches. Initially, papers were accepted based on the study inclusion criteria described above, with doubtful cases resolved following intra-author discussions.

### Data recorded

For each study report, data were extracted by one of us and checked by another. Recorded study characteristics included the source reference, location, sexes studied, representativeness of the sample, whether participants were aware their samples would be tested for smoking, body fluid and assay method used for cotinine assay, cut-offs used, whether smoking groups were differentially sampled, whether results were separately available for never and former smokers, and whether the sample was of the general population, pregnant women, from both arms of a case-control study, or of diseased individuals. Data from each study included the numbers of participants in the relevant smoking/non-smoking groups, and the numbers in these groups with cotinine values indicating misclassification. Where necessary, numbers were estimated from data provided in figures.

We also recorded information on the smoking (or tobacco use) index studied, and a study quality measure based on the extent of account taken of other nicotine sources that could produce cotinine levels above the cut point, such as other smoking products (pipes, cigars), smokeless tobacco (snuff, chewing tobacco), nicotine replacement therapy (gums, patches) and e-cigarettes.

The smoking indices considered were cigarette smoking, smoking (of any product) and any tobacco use (smoking or smokeless tobacco use). For all three indices, we recorded whether individuals using nicotine replacement therapy or e-cigarettes had been excluded from the estimation of misclassification rates and, if not, whether the author had referred in the source paper to nicotine replacement therapy or to e-cigarette use as possible confounders. For smokers (of any product) we similarly recorded data on consideration of smokeless tobacco, while for cigarette smokers specifically, we also recorded data on smoking of other products. A study was considered of good quality if users of all non-index tobacco products had been excluded from analysis.

### Adjusting rates for differential sampling

In a few studies, populations were differentially sampled by reported smoking habits. This has no effect on rates M1-M4, as the calculation is within smoking group. However, for rates M5-M11, failure to consider differential sampling would bias rate calculations. We avoided this by calculating adjusted numbers of participants and misclassifieds. Two relevant situations occurred. In the first, results were only available for non-smokers and current smokers, sampled in the ratio 1:S. With asterisks indicating adjusted numbers, we used the formulae  $D^* = D/S$ ,  $H^* = H/S$ ,  $A^* = A$  and  $E^* = E$ .

In the second situation, results were separately available for never-, ex- and current smokers, the groups being sampled in the ratio 1:U:V. Here, the adjusted numbers were  $B^* = ZB$ ,  $C^* = ZC/U$  and  $D^* = ZD/V$ , where  $Z = (B + C)/(B + C/U)$  is a scaling factor set so the adjusted and observed numbers of non-smokers are equal. The adjusted numbers of misclassified individuals were then obtained by multiplying the adjusted numbers of participants by the observed misclassification rate, *i.e.*,  $F^* = B^*F/B$ ,  $G^* = C^*G/C$  and  $H^* = D^*H/D$ .

### Avoiding double-counting

It was necessary to ensure use of the greatest amount of information while avoiding double-counting as far as possible. This was particularly difficult for some large studies where many reports are available. Various rules were defined to avoid double-counting. Thus, results from a single study should not be included in the same analysis for sexes combined and individually, or (except for analysis by body fluid) for more than one body fluid. Also, when study results are reported in multiple publications (or in multiple forms in one publication), we preferred rates based on the most participants, for all four smoking groups than just some, for males and females

separately rather than combined, and results not based on differential sampling.

### **Meta-analyses and meta-regressions**

Mean misclassification rates with 95% CIs were estimated by analysis of variance, weighted by the number of participants the specific rate estimate was based on. Analyses were carried out based on all available results (avoiding double-counting) and, for Cut 1 only, by levels of various factors. These were body fluid, assay method, study type (separating studies of the general population, of pregnant women, and other studies - of diseased individuals and case-control studies), age, participants' awareness that cotinine samples were used to validate their reported smoking habits, period of publication of the source paper, study quality (as described above), for studies of women whether they were pregnant or not, the index of smoking (or tobacco use), sex, location, and the interaction of sex and location. Where rates were estimated by factor level, the significance of differences between factor levels was estimated by a heterogeneity test.

A publication by Palmier *et al.*<sup>[26]</sup> reported results from a study of urine samples of about 6.2 million life insurance applicants, providing data only for Cut 2. This study involved more participants than all the other studies combined so including its results would have meant the overall weighted estimates were dominated by its contribution. We therefore excluded it from the meta-analyses and present its results separately. For each rate we also carried out a multivariate analysis. This involved a stepwise procedure successively including the most significant factor, stopping when no further factor was significant at  $P < 0.01$ .

## **RESULTS**

### **Literature searches**

Figure 1 summarizes the literature searches carried out. Our earlier review<sup>[7]</sup> presented results from 36 studies provided in 30 publications and two personal communications. Four publications<sup>[27-30]</sup> were rejected as reporting studies based on fewer than 200 cotinine measurements, as were two of three studies reported in another publication<sup>[31]</sup>. Two reports<sup>[32,33]</sup> have been replaced by a later fuller report<sup>[11]</sup>. One report<sup>[34]</sup> was superseded by a later report filed under "COT" in our in-house database<sup>[35]</sup>. This left 26 sources reporting 29 studies.

Of 767 publications filed under "COT", 32 were already considered in our earlier review<sup>[7]</sup> and four were reviews of misclassification studies<sup>[9-12]</sup>. This left 731 for further consideration. Of these, 591 failed our inclusion criteria and 33 provided inadequate data (*e.g.*, having a cut point too low, testing using a substance with no accepted cut point, such as hair or umbilical cord serum, or providing too little information). Checking the bibliographies of the four reviews yielded nine additional data sources. This resulted in 116 publications providing useful data for 119 studies.

A PubMed search on "Cotinine" on 5th January 2017 produced 4353 hits. Of these 3577 were rejected from inspecting abstracts and 226 had already been considered. The remaining 550 publications were obtained and examined in more detail. Four hundred and twenty-three failed the inclusion criteria or provided inadequate information, leaving 127 for further consideration, these providing 130 study reports. Examining the reference lists in five further reviews produced no additional relevant references. Overall, therefore, there were 278 study reports from 269 sources.

### **Avoidance of double-counting**

Supplementary File 1 describes our attempts to limit double-counting. It gives details of each study reported by more than one publication and each publication reporting more than one type of misclassification data, such as results at several stages (*e.g.*, early pregnancy, late pregnancy) or for more than one body fluid or cotinine assessment method. For each such study Supplementary File 1 identifies the data available, which are to be excluded from analysis and, for the data to be included, whether it should always be included or only included in some analyses. For rejected reports, the reason for rejection is given, often because it reports a smaller sample size than given elsewhere. Where there is no difference in sample size, other reasons for rejection are given, such as a non-conventional cotinine test method or data for sexes combined when alternative sources give data by sex.

The decisions on inclusion or rejection took account of the smoking categories reported. For example, where one report gave results for non-smokers and current smokers but another for the same study gave only never smoker results, both sources could be included because the calculation of misclassification rates considers either non-smokers or never smokers. By this process, 52 study reports were excluded,

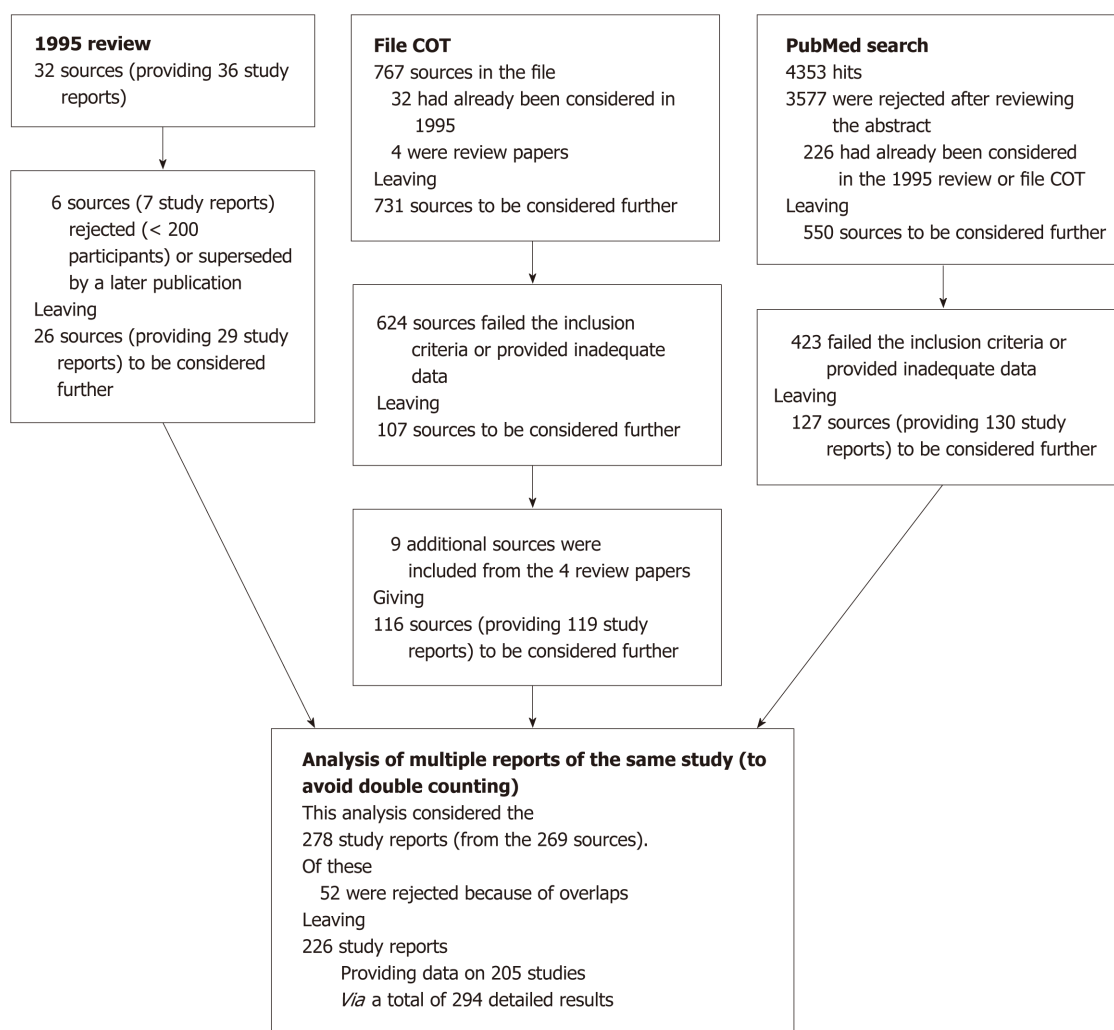


Figure 1 Data sources and processing.

leaving 226 study reports on 205 separate studies.

Often a study report provides multiple results. Many studies report males and females separately. Some reports split their analyses of misclassification by other factors, including race, age, study years (for studies conducted annually) and study arm (pregnant/non-pregnant, cases/controls). Consequently, our dataset of detailed results contains more entries, 294, than there are study reports, 226.

### Study characteristics

Details of the main characteristics of each study and of the study reports used in analysis are given in Supplementary File 2. Table 1 gives the number of results analysed for each characteristic that was used as a factor in analysis. Of the 294 results, 11% were from studies considered in the 1995 review, a further 37% being from studies reported before 2003, the remaining 52% being reported later. Most results (83%) related to studies in Europe and North America, with the rest about equally split between Asia and other locations. Fifty-six percent of results were sex-specific, with more for females (40%) than males (16%), due to the large number of results for pregnant women, 19% of the total. The studies in pregnant women also formed a substantial proportion of results classified as “young”. Most results (64%) related to the general population.

The majority of results (63%) were from studies not specifying whether participants were aware their self-report would be validated, with only 7% (including the very large Palmier *et al*<sup>[26]</sup> study) from studies where participants were aware. Of all the results, 43% were based on blood samples, 31% on saliva, and 27% on urine. Self-report related to cigarette smoking specifically for 37% of results, to any smoking for 54% and to any use of tobacco for the remaining 9%. Only 12% of results were classified as “good” quality.

### Misclassification rates



**Table 1** Distribution of study characteristics among the 294 detailed results

Factor	Level	No. of results analysed <sup>a</sup>
Body fluid	Urine	78 <sup>a</sup>
	Saliva	90
	Blood	126
Assay method	Chromatography	93
	Spectrometry	72
	Immunoassay	108
	Other	21 <sup>a</sup>
Age group <sup>b</sup>	Young	103
	Not young	35
	All ages	108 <sup>a</sup>
	Not stated	48
Study type	General population	189 <sup>a</sup>
	Pregnancy	57
	Diseased or case-control	48
Awareness of validation by cotinine	Yes	22 <sup>a</sup>
	No	47
	Not specified	225
Time of publication	Studies considered in the 1995 review	31
	Studies reported before 2003	109
	Studies reported later	154 <sup>a</sup>
Study quality	Good	36
	Not good	258 <sup>a</sup>
Pregnancy (women only)	Not pregnant	61
	Pregnant	57
Tobacco products considered	Cigarettes	108
	Any smoking	160
	Any tobacco	26 <sup>a</sup>
Sex	Females	118
	Males	48
	Combined	128 <sup>a</sup>
Location	Canada/United States	115 <sup>a</sup>
	Europe	128
	Asia	25
	Other	26

<sup>a</sup>Factor levels with this superscript are the levels applicable to the very large study by Palmier J, Lanzrath B, Dixon A and Idowu O<sup>[26]</sup> considered separately in our analyses.

<sup>b</sup>Studies varied in how they reported the age range studied, sometimes giving a specific range of ages, sometimes a mean age and sometimes no information.

The categories were based on the available age information as follows: Young: Upper age limit < 50 or mean age < 30 or a pregnancy study; Not young: Lower age limit 30+ or mean age 60+, thus excluding young people; All ages: Lower age limit < 30 and upper age limit 50+ or lower age limit < 30 and mean age 30+; Not stated: All other combinations.

Full details of all meta-analyses and meta-regressions are given in Supplementary File 3, while Supplementary File 4 gives a series of tables presenting results for Cut 1 by the levels of each factor, referred to below as Supplementary Tables 1 and 2, *etc.*

### Overall rates

**Table 2** presents overall meta-analysis estimates of each misclassification rate, based on both cut points, as well as estimates from the very large study<sup>[26]</sup>. Using Cut 1 the percentage of reported non-smokers who are true smokers according to cotinine, M1, is 4.96%. The percentage of true smokers is lower for reported never smokers, M2 = 3.00%, and higher for reported ex-smokers, M3 = 10.92%. As expected, these three rates are lower using Cut 2; and M4, the percentage of self-reported current smokers with cotinine level below the cut point, is higher for Cut 2 than Cut 1. Rate M4 is particularly high in the Palmier study<sup>[26]</sup>, where the urine-based cut point was 500

ng/mL.

Using Cut 1, rates M5 to M7, which have cotinine-defined current smokers as the base, are again higher for reporting of ex-smoking than of never smoking and, as for rates M1 to M3, are lower using Cut 2. As for M4, the percentage of true current smokers who report being non-smokers (M5) is high in the Palmier<sup>[26]</sup> study. The pattern of rates for M8 to M10 is similar to that for M5 to M7, though the misclassification rates are somewhat lower. Rate M11, which has cotinine-defined non-smokers as the base, is higher for Cut 2 than for Cut 1, as was noted above for M4.

Table 3 shows, for each rate definition, the distribution of available rate values using Cut 1. This illustrates the variability of the data. Table 3 also indicates where the median value lies. For all eleven rate definitions, some misclassification rate values were lower than 2%, while for all except M2 and M11, some exceeded 50%.

### **Variation in rates by other factors**

Table 4 summarizes, for each factor considered, the significance of the differences in rates between the levels of the factor (using Cut 1, univariate analysis). Supplementary File 4 gives full details of these analyses. These findings are discussed in the following sub-sections.

### **Body fluid**

There is little evidence that misclassification rates vary by whether cotinine was measured in urine, saliva or blood. Only one rate, the percentage of true non-smokers claiming to be current smokers (M11), showed variation significant at  $P < 0.05$ , and then only marginally ( $P = 0.044$ ), rates being somewhat higher for blood (4.6%) than for urine (2.2%) or saliva (2.9%) (Supplementary Table 1).

### **Assay method**

There is little evidence that misclassification rates varied by assay method. Only rates M3 and M4 showed evidence of variation significant at  $P < 0.05$ . Rate M3 was high (19.0%) for the category “other”, representing studies that did not specify their method or used several methods in a single study, compared with 8.8%, 10.1% and 11.5% in the other categories. For M4 the rate was lower using chromatography or immunoassay (6.5% and 9.9% respectively, versus 13.1% and 13.0% for spectrometry and “other” respectively) (Supplementary Table 2).

### **Study type**

Studies were classified as being of the general population, of pregnant women, or “other” (consisting of diseased groups and participants in case-control studies). For some misclassification rates (M2, M6, M7, M9 and M10), there were data from only two studies in pregnancy, most such studies recording cotinine levels in self-reported non-smokers or ex-smokers, not in self-reported never smokers. There were some major sources of variation ( $P < 0.001$ ) by study type. First, reporting of quitting by true current smokers (M3) was higher in pregnant women (22.7%) than in general population (8.7%) or “other” studies (12.0%). Second, the percentage of self-reported current smokers who were true non-smokers according to cotinine (M4), was over twice as high in the “other” group (21.9%) as in the general population or pregnant women (8.0% and 8.5% respectively). The same is true for the percentage of true non-smokers who report being current smokers (M11; 10.5% *vs* 2.9% and 3.5%). Third, the percentage of current smokers who report being ex-smokers is about twice as high in the “other” group as in the general population or pregnant women, whether they be true current smokers (M7; 21.3% versus 6.6% and 9.4%) or self-reported current smokers plus misclassified non-smokers (M10; 10.5% versus 2.9% and 3.5%) (Supplementary Table 3).

### **Age group**

Studies were classified according to whether participants were young, not young, all ages or age not specified. Defining these groups was complicated by there being various ways to present age information in study reports. Participants were classified as young if the upper age limit was at most 50 years or the mean age was at most 30 years or the study was of pregnant women. Studies of not young participants had a lower age limit of at least 30 years or a mean age of at least 60 years. Studies classified as of all ages had an age range that included ages 30 to 50 years, with this inferred for studies with a lower age limit of at most 30 years and a mean age over 30. All other studies were classified as age not specified.

The major sources of variation by age were similar to those for study type. Thus, self-reported quitting among true current smokers (M3) was highest (18.8%) in the young group, which included pregnant women, while the other rates showing clearly

**Table 2** Misclassification rates from Palmier *et al*<sup>[26]</sup> and from the other studies combined by meta-analysis (based on weighted analysis, avoiding double-counting)

Rate		Other studies combined				Palmier
		Cut 1 <sup>a</sup>		Cut 2 <sup>b</sup>		Cut 2 <sup>b</sup>
		<i>n</i>	Rate (95%CI)	<i>n</i>	Rate (95%CI)	Rate
M1	% of self-reported non-smokers whose cotinine implies current smoking	209	4.96 (4.32 to 5.60)	65	3.66 (2.68 to 4.65)	2.01
M2	% of self-reported never smokers whose cotinine implies current smoking	86	3.00 (2.45 to 3.54)	22	2.34 (1.28 to 3.41)	-
M3	% of self-reported ex-smokers whose cotinine implies current smoking	88	10.92 (9.23 to 12.61)	24	6.79 (4.60 to 8.98)	-
M4	% of self-reported current smokers whose cotinine implies non-smoking	142	9.67 (7.73 to 11.61)	44	18.48 (14.46 to 22.50)	53.08
M5	% of true current smokers who report being non-smokers	136	14.50 (12.36 to 16.65)	43	10.42 (5.91 to 14.92)	19.31
M6	% of true current smokers who report being never smokers	52	5.70 (3.20 to 8.20)	13	4.34 (0.19 to 8.49)	-
M7	% of true current smokers who report being ex-smokers	52	8.93 (6.57 to 11.29)	13	7.89 (4.07 to 11.71)	-
M8	% of self-reported current smokers (plus misclassified non-smokers) who report being non-smokers	185	11.59 (10.00 to 13.17)	60	7.92 (5.19 to 10.65)	10.10
M9	% of self-reported current smokers (plus misclassified non-smokers) who report being never smokers	66	4.64 (2.73 to 6.54)	21	4.02 (1.68 to 6.35)	-
M10	% of self-reported current smokers (plus misclassified non-smokers) who report being ex-smokers	66	7.72 (5.95 to 9.50)	21	5.69 (3.54 to 7.84)	-
M11	% of true non-smokers who report being current smokers	137	3.65 (2.84 to 4.45)	43	7.67 (6.14 to 9.20)	8.84

<sup>a</sup>The lower cut point.<sup>b</sup>The higher, more conservative, cut point.

significant variation ( $P < 0.01$ ) - M4, M7, M10 and M11 - were all highest in the not young group, to which the “other” study type group would mainly belong (Supplementary Table 4).

#### **Awareness of validation by cotinine**

In each analysis, the percentage of studies specifying whether or not participants were told that their self-report would be cotinine-validated was quite low, around 25%. The number of studies specifying the participant was told was at most 12 (M1 and M8), and was often only 1 or 2. While one might imagine knowledge of validation would



**Table 3** Distribution of misclassification rate values from the studies included in Table 2 for the lower cut point (Cut 1)

		< 2%	2% to < 5%	5% to < 10%	10% to < 25%	25% to < 50%	> 50%	Total
M1	% of self-reported non-smokers whose cotinine implies current smoking	45	74 <sup>1</sup>	51	31	3	5	209
M2	% of self-reported never smokers whose cotinine implies current smoking	44 <sup>1</sup>	25	13	4	0	0	86
M3	% of self-reported ex-smokers whose cotinine implies current smoking	2	7	31	38 <sup>1</sup>	7	3	88
M4	% of self-reported current smokers whose cotinine implies non-smoking	16	29	34 <sup>1</sup>	42	18	3	142
M5	% of true current smokers who report being non-smokers	5	18	28	52 <sup>1</sup>	22	11	136
M6	% of true current smokers who report being never smokers	18	13 <sup>1</sup>	10	7	2	2	52
M7	% of true current smokers who report being ex-smokers	4	16	17 <sup>1</sup>	7	7	1	52
M8	% of self-reported current smokers (plus misclassified non-smokers) who report being non-smokers	11	30	42	65 <sup>1</sup>	28	9	185
M9	% of self-reported current smokers (plus misclassified non-smokers) who report being never smokers	25	19 <sup>1</sup>	11	7	2	2	66
M10	% of self-reported current smokers (plus misclassified non-smokers) who report being ex-smokers	7	21	22 <sup>1</sup>	10	5	1	66

M11	% of true non-smokers who report being current smokers	45	51 <sup>1</sup>	27	12	2	0	137
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<sup>1</sup>Includes the median.

encourage better self-report, the reverse seemed to be true. For the only analyses where significant variation was seen (M3,  $P = 0.009$  and M8,  $P = 0.022$ ) the misclassification rate was 20.2% and 16.4% respectively among those told, 13.5% and 7.3% among those not told and 10.0% and 12.4% for studies not specifying this. For those other rates where eight or more participants were told (M1, M4, M5, M8 and M11), the percentage misclassified was generally highest in the group that was told, though never significantly (Supplementary Table 5).

### Time of publication

Time of publication, as an approximate indicator of time of study conduct, was divided into three groups: Studies considered in the 1995 review, other studies published before 2003, and studies reported later. For five rates (M4, M5, M7, M8 and M10) there was significant ( $P < 0.05$ ) variation by time of publication, always due to a higher rate in the most recently reported studies, typically by about twofold. These relate to erroneous claims, as judged by cotinine, relevant to self-reported current smoking (M4, 14.5% versus 6.8% and 6.0%), non-smoking (M5, 19.8% versus 10.1% and 10.4%; and M8, 14.9% versus 6.6% and 9.3%) and ex-smoking (M7, 12.8% versus 4.4% and 7.0%, and M10, 10.7% versus 4.4% and 6.9%) (Supplementary Table 6).

### Study quality

Studies were classified as good or not good according to whether they had accounted for other nicotine sources. For some rates (M3, M6, M7, M9 and M10), the number of good studies was four or fewer, not allowing useful analysis. There was no significant ( $P < 0.05$ ) variation by study quality for any of the other rates studied, nor any consistent evidence that misclassification rates were lower in the good studies (Supplementary Table 7).

### Pregnancy

For rates M2, M6, M7, M9 and M10 the analyses were unhelpful, being based on only two studies in pregnant women. For five other rates (M1, M4, M5, M8 and M11), there was no significant difference between pregnant and non-pregnant women. However, the percentage of self-reported ex-smokers who were current smokers according to cotinine (M3) was clearly ( $P < 0.001$ ) higher in pregnant women (22.7%) than in non-pregnant women (7.9%) (Supplementary Table 8).

### Tobacco products considered

Studies were divided by whether cotinine levels were used to check statements made about cigarette smoking, any smoking, or any tobacco use. The number of studies classified under any tobacco use was relatively low, at most 15 (for M1) and was three or fewer for six of the rates. For the misclassification rate (M2) which showed the greatest heterogeneity by group ( $P < 0.001$ ), the percentage of true current smokers among self-reported never smokers was 4.3% for cigarette smoking and 2.1% for any smoking. This is consistent with some self-reported never smokers of cigarettes using other nicotine-containing products not considered in the study, resulting in high cotinine levels. Other misclassification rates showing some evidence of heterogeneity between groups ( $P < 0.1$ , M3, M8) were also higher in studies where the statements checked concerned cigarette smoking (Supplementary Table 9).

### Sex

There was no clear heterogeneity by sex, no  $P$  values being  $< 0.001$ . However, there were some indications of variation, with three  $P$  values  $< 0.05$  and some other values close to 0.1. The percentage of true current smokers who reported never having smoked (M6) was higher for studies in females (12.0%) than for studies in males (3.5%), or studies which only reported combined sex results (4.1%), and a similar pattern was seen for rate M9, where the denominator also included misclassified non-smokers. The percentage of self-reported ex-smokers who proved to be true current smokers (M3) was also highest in females, consistent with the earlier results relating to pregnancy. Exceptionally, rate M7, which concerned true current smokers reporting having quit, and the similar rate M10, were highest where results were

**Table 4** For each factor, the significance of the differences in rates between the factor levels: Cut 1, univariate analyses

Rate		Body fluid	Assay method	Study type	Age group	Aware validated	Time published	Study quality	Pregnancy	Tobacco products	Sex	Location	Sex × location
M1	% of self-reported non-smokers whose cotinine implies current smoking	NS	NS	c1	NS	NS	NS	NS	NS	NS	NS	NS	NS
M2	% of self-reported never smokers whose cotinine implies current smoking	NS	NS	NS	b	NS	NS	a	NS	d1	NS	b	d
M3	% of self-reported ex-smokers whose cotinine implies current smoking	a	b	d1	d	c	a	NS	d	a1	a	c	b
M4	% of self-reported current smokers whose cotinine implies non-smoking	NS	b	d1	d1	NS	d1	NS <sup>1</sup>	NS	NS	NS	NS	NS
M5	% of true current smokers who report being non-smokers	NS	NS	b	NS	NS	d1	NS	NS	NS	NS	NS	c1
M6	% of true current smokers who report being never smokers	NS	NS	NS	NS	NS	NS	NS	NS	NS	b	NS	d1
M7	% of true current smokers who report being ex-smokers	NS	NS	d1	c	NS	b	NS	a	NS	NS	NS	NS

M8	% of self-reported current smokers (plus misclassified non-smokers) who report being non-smokers	NS	NS	NS	a	b	d1	a	NS	b	NS	NS	d1
M9	% of self-reported current smokers (plus misclassified non-smokers) who report being never smokers	a	NS	NS	NS	NS	NS <sup>1</sup>	NS	NS	NS	b	NS	d1
M10	% of self-reported current smokers (plus misclassified non-smokers) who report being ex-smokers	NS	NS	d1	c	NS	b	NS	b	NS	b	NS	NS
M11	% of true non-smokers who report being current smokers	b	NS	d1	d1	NS	NS	NS	NS	NS	NS	a	NS

<sup>d</sup> $P < 0.001$ .<sup>c</sup> $P < 0.01$ .<sup>b</sup> $P < 0.05$ .<sup>a</sup> $P < 0.1$ .NS (not significant):  $P \geq 0.1$ .<sup>1</sup>For each rate (M1-M11) identify the variables that, in multivariate analysis, were independently statistically significantly ( $P < 0.01$ ) associated with the misclassification rate. NS: Not significant.

based on sexes combined (Supplementary Table 10).

### Location

The clearest variation by location ( $P = 0.002$ ) was seen for M3, the percentage of self-reported quitters who were current smokers according to the cotinine test. Here, misclassification rates were 15.2% in Canada/United States, 9.5% in Europe, 5.9% in Asia and 17.8% in other locations. There was also some evidence ( $P = 0.026$ ) of higher rates in the “other” locations of true current smoking among self-reported never smokers (M2) (Supplementary Table 11).

### Interaction between sex and location

It is claimed that some misclassification rates may be particularly high in Asian women, so we looked at the significance of the interaction between the four level location variable and the three level sex variable. Highly significant ( $P < 0.001$ ) variations were seen for rates M2, M6, M8 and M9, with a significant ( $P < 0.01$ ) variation also seen for M5.

Looking first at the percentage of true current smokers who reported being never smokers (M6), it was striking that, whereas mean rates varied from 0% to 6.4% in nine

of the 12 subsets, they were much higher in Asian females (44.3%,  $n = 4$ ), in females in “other” countries (40.6%,  $n = 1$ ) and in females in Canada/United States (17.0%,  $n = 3$ ). Looking further, it was clear that all four rates meta-analysed for Asian females were high (12.5%, 22.4%, 33.3% and 54.2%). The three rates from studies in Canada/United States females were variable (2.5%, 7.8%, 66.3%), with the last very high. This was from a study<sup>[36]</sup> conducted in the United States, but concerning Southeast Asian immigrants. The results are consistent with women who smoke in communities where smoking is culturally unacceptable being very likely to deny ever having done so. Notably, the single high rate (40.6%) for “other” countries comes from a study in the Republic of Karelia, Russia<sup>[37]</sup> the authors commenting on the cultural unacceptability of females smoking in Russia. Essentially similar patterns, based on the same studies with high rates, are seen for rate M9, which also concerns false claims of never smoking.

High rates in Asian females (38.4%,  $n = 6$ ) and in females in “other” countries (26.7%,  $n = 5$ ) were also seen for rate M5, which concerns true current smokers reporting that they are non-smokers. While rates were elevated in each Asian study (range 16.1% to 87.5%), rates in the “other” countries were only markedly elevated in the Karelian study (43.6%), in a study of pregnant women in New Zealand<sup>[38]</sup> (28.0%), and in indigenous females in Australia<sup>[39]</sup> (22.2%). Similar results, based on the same studies with high rates, are seen for rate M8, which also concerns false claims of non-smoking.

For rate M2, the percentage of reported never smokers who were true current smokers, was 3.0% overall. However, rates were again high in Asia, in “other” countries (based only on the Karelian study), and in Canada/United States due mainly to the study of South East Asian immigrants. In each case, rates were high in males as well as in females (Supplementary Table 12).

### Multivariate analyses

Details of these additional analyses are also given in Supplementary File 3, at the end of the section for each rate, under the title “Multivariate analysis”. Factors which remained significant in the multivariate analyses are indicated by underlining the relevant variation in Table 4. As can be seen, some factors do not appear in any final multivariate analysis. These factors (body fluid, assay method, awareness of validation, pregnancy, sex and location) can all be regarded as not clearly associated with any of the 11 rates, as judged by a significance level of  $P < 0.01$ . For most of the rates, these factors were not significant (at  $P < 0.01$ ) in the univariate analysis, though exceptionally, for M3, variations by awareness of validation, pregnancy and location which were significant at  $P < 0.01$  in the univariate analysis were no longer significant at that level in the multivariate analysis.

Table 5 summarizes the results for five factors which showed a significant ( $P < 0.01$ ) independent association with at least one misclassification rate. For each factor, the direction of the major differences is generally the same for each of these rates. Thus, rates are generally higher for studies of diseased populations and case-control studies than for general population studies, for the youngest age group, for studies published from 2003 onwards than for earlier studies, where the study quality is not good, and where the tobacco product considered is cigarettes only. Exceptionally, for study type, the difference between studies of the general population and studies of pregnant women is not in the same direction for all rates. Thus, reporting of quitting by current smokers (M3) was higher in pregnant women, but the percentage of self-reported current smokers who were true non-smokers according to cotinine (M4) was lower.

Independent significant ( $P < 0.01$ ) variation was also seen for the sex by location interaction for four rates (M5, M6, M8 and M9), all relating to smokers reporting non-smoking or never smoking. The variation was predominantly due to the results for females. As shown in Table 5, rates were substantially higher in Asian women than in women in Europe or North America. Rates were also somewhat higher in women in “other” locations, but less clearly, those results being based on relatively few estimates. It should be noted that, for rate M2, the percentage of self-reported never smokers whose cotinine implies current smoking, highly significant ( $P < 0.001$ ) variation for the sex by location interaction in univariate analysis was not significant (at  $P < 0.01$ ) in the multivariate analysis after adjustment for the type of tobacco products considered.

## DISCUSSION

We have attempted to obtain estimates of 11 different misclassification rates and relate them to a range of factors. Although the data are complex, a number of clear

**Table 5 Factors included in the final model for a rate, with the significant differences in misclassification rates (from base level) by factor level: Cut 1, multivariate analysis**

Factor (base level), rates that included the factor in multivariate analysis <sup>a</sup>		Other factor levels: Difference in rate from base level, significance <sup>a</sup>		
<b>Study type (base level = general population)</b>		<b>Pregnancy</b>	<b>Diseased/case-control</b>	
M1	% of self-reported non-smokers whose cotinine implies current smoking		3.92 <sup>++</sup>	
M3	% of self-reported ex-smokers whose cotinine implies current smoking	14.63 <sup>+++</sup>	3.89 <sup>+</sup>	
M4	% of self-reported current smokers whose cotinine implies non-smoking	-14.24 <sup>---</sup>	10.71 <sup>++</sup>	
M7	% of true current smokers who report being ex-smokers		14.74 <sup>+++</sup>	
M10	% of self-reported current smokers (plus misclassified non-smokers) who report being ex-smokers		9.11 <sup>+++</sup>	
M11	% of true non-smokers who report being current smokers	-2.48 <sup>-</sup>	6.92 <sup>+++</sup>	
<b>Age group (base level = young)</b>		<b>Not young</b>	<b>All ages</b>	<b>Not stated</b>
M4	% of self-reported current smokers whose cotinine implies non-smoking	-11.03 <sup>-</sup>	-16.86 <sup>---</sup>	-18.28 <sup>---</sup>
M11	% of true non-smokers who report being current smokers		-3.87 <sup>---</sup>	-4.58 <sup>-</sup>
<b>Time of publication (base level = in 1995 review)</b>		<b>Before 2003</b>	<b>2003 onwards</b>	
M4	% of self-reported current smokers whose cotinine implies non-smoking		11.17 <sup>++</sup>	
M5	% of true current smokers who report being non-smokers		9.61 <sup>(+)</sup>	
M8	% of self-reported current smokers (plus misclassified non-smokers) who report being non-smokers		6.26 <sup>+</sup>	
M9	% of self-reported current smokers (plus misclassified non-smokers) who report being never smokers		6.11 <sup>++</sup>	
<b>Study quality (base level = good)</b>		<b>Not good</b>		
M4	% of self-reported current smokers whose cotinine implies non-smoking	6.32 <sup>++</sup>		
<b>Tobacco products considered (baseline level = cigarettes)</b>		<b>Any smoking</b>	<b>Any tobacco</b>	
M2	% of self-reported never smokers whose cotinine implies current smoking	-2.22 <sup>---</sup>		
M3	% of self-reported ex-smokers whose cotinine implies current smoking	-3.61 <sup>-</sup>		
<b>Location in females (base level = Canada/United States)<sup>c</sup></b>		<b>Europe</b>	<b>Asia</b>	<b>Other</b>
M5	% of true current smokers who report being non-smokers	6.73 <sup>(+)</sup>	23.61 <sup>+++</sup>	
M6	% of true current smokers who report being never smokers	-11.06 <sup>(-)</sup>	27.32 <sup>++</sup>	
M8	% of self-reported current smokers (plus misclassified non-smokers) who report being non-smokers		23.19 <sup>+++</sup>	



M9	% of self-reported current smokers (plus misclassified non-smokers) who report being never smokers	-8.62 <sup>c</sup>	30.96 <sup>+++</sup>	24.27 <sup>+b</sup>
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<sup>a</sup>For each factor, the rates shown are those with which the factor showed a significant ( $P < 0.01$ ) independent association. All differences shown are adjusted for the other factors significant for that rate. They represent the difference in misclassification rate from the rate for the base level. Only statistically significant differences are shown. The significance of the difference is coded as: <sup>+++</sup> $P < 0.001$ ; <sup>++</sup> $P < 0.01$ ; <sup>+</sup> $P < 0.05$ ; <sup>c</sup> $P < 0.1$ .

<sup>b</sup>Result based on three estimates or less.

<sup>c</sup>Variations between location were generally not significant for males or for sexes combined - see Supplementary File 3 for full results for sex  $\times$  location.

conclusions can be drawn. First, there is considerable between-study variation in the level of misclassification.

Second, false claims to have quit smoking are more common than false claims of never having smoked, and it is also clear that the proportion of true current smokers (as judged by cotinine) is higher for self-reported ex-smokers than for self-reported never smokers.

Third, many of the rates vary according to different factors. Notably, false claims of being a non-smoker or a never smoker (rates M5, M6, M8 and M9) are particularly high in Asian females, and for females in other populations where smoking by females is not considered acceptable. This is particularly clear for the percentage of true current smokers who report being never smokers (M6) where individual studies provide rates that sometimes exceed 50% as compared to an overall rate of 5.7%. Not included in our analyses, for reasons described in Supplementary File 1, are results reported from the Health Survey for England specifically for Bangladeshi women<sup>[40]</sup>. Of 227 who reported not being tobacco users, 45 (M1 = 19.8%) had saliva cotinine greater than 15 ng/mL.

There is a clear tendency for many of the rates (particularly M4, M5 and M8) to be higher in more recent studies, though the explanation requires further study. There is also evidence that the percentage of self-reported ex-smokers whose cotinine implies current smoking (M3) is particularly high in pregnant women and younger women, associations which are inter-related, as demonstrated in the multivariate analyses. For a number of the factors (M1, M3, M4, M7, M10 and M11), there is clear evidence that rates are higher in studies of diseased groups and case-control studies than in general population studies, suggesting that circumstances of interview or presence of disease may affect the answers given. Some of these rates (M4 and M11) are also higher in younger populations.

Some other variations in rates also require comment. One is the high percentage of self-reported never smokers whose cotinine implies current smoking (M2) in Asian populations of both sexes, and where only cigarette smoking was considered - to be expected as smoking of other tobacco products may also produce high cotinine levels. An interesting association is the high percentage of self-reported ex-smokers whose cotinine implies current smoking (M3) in populations who were aware they would be tested for cotinine. While this may suggest a tendency for the mention of possible cheating to inadvertently encourage cheating, the multivariate analyses did not include awareness of validation as an independent factor significant at  $P < 0.01$ , so more evidence is needed to confirm this. A problem here is that information on awareness was not available for many of the studies.

The conclusions summarized above were drawn from analysis of the rates based on the lower cut point and did not consider results from the study of about 6.2 million life insurance applicants<sup>[26]</sup> which used a cut point of 500 ng/mL in urine to validate statements made about tobacco use in the knowledge that their responses would be confirmed biochemically. Of 545970 who proved to be cotinine positive, 105,452 (M5 = 19.31%) reported being non-tobacco users, a false-negative self-reporting rate which the authors reported was higher in males and younger participants, and "may be the result of complex interactions among financial incentives, geography and presumptive peer groups, and gender". It is interesting that the authors did not comment on the very high proportion of cotinine negatives (M4 = 498426/938944 = 53.08%) who self-reported tobacco use. The reason for this is not obvious.

It is worth considering the effects of misclassification on the association of disease rates with both active and passive smoking. We consider first associations with current active smoking. Suppose that, in a given population, the proportion of true current smokers is  $P_c$ , the risk of a given disease is 1 unit in true non-smokers and  $R$  units in true current smokers, and the rate of misclassification of true current smokers as non-smokers (M5) is  $M_c$ . Let us initially ignore the reverse misclassification rate (M11), and assume misclassified and non-misclassified smokers have the same disease

risk. Instead of observing the true relative risk of  $R$  we will observe a reduced relative risk of  $R^* = R(P_N + M_C P_C) / (P_N + R M_C P_C)$ , where  $P_N = 1 - P_C$ . Thus, if  $P_C$  is 30%, and  $R$  is 10, setting  $M_C = 10\%$  would be expected to produce observed values of  $P_C^* = 27\%$  and  $R^* = 7.3$ . The bias in the risk estimate increases with increases in both  $M_C$  and  $P_C$ .

Misclassified and non-misclassified smokers may not have the same disease risk for various reasons. Misclassified smokers may have smoked less, suggesting a lower risk than smokers who report their smoking. On the other hand, misclassification may be common in participants advised to quit by their doctor as they were considered to be at higher than average risk. However, assuming the risk for misclassified smokers exceeds that for non-smokers, positive misclassification bias will still occur<sup>[1]</sup>.

In the above calculations we assumed the reverse misclassification rate ( $M_{11}$ ) is zero. Where the true proportions of current smokers and non-smokers are similar, a given value of  $M_{11}$  will bias the relative risk less than will the same value of  $M_5$ . Thus, with 50% smokers, a rate of  $M_5$  of 10% decreases a true relative risk of 10 to an observed 5.5, while a rate of  $M_{11}$  of 10% decreases it to 9.2. However, as the true proportion of current smokers decreases, the biasing effects become more similar.

There are problems in using cotinine data to confirm smoking status. First, cotinine levels do not allow precise estimation of amount smoked, though they are clearly correlated with it. Second, cotinine levels may be increased in a never smoker from environmental tobacco smoke exposure, though in practice this will not produce levels consistent with active smoking. Finally, and most seriously, cotinine levels only relate to current (or quite recent) smoking habits, and do not distinguish never smokers from short, medium or long-term quitters. Those who report never smoking may in fact have smoked until quite recently and have higher risks of smoking-related disease because of this. However, their cotinine levels will not be elevated.

We now consider the effect of misclassification on the relative risk associated with passive smoking. Some years ago, Forey and Lee<sup>[1]</sup> noted that the relationship of passive smoking to lung cancer risk is commonly studied in never smokers, using marriage to a smoker as the index of exposure, and that, as smokers tend to marry smokers, relative risk estimates will be biased if some current or former smokers are misclassified as never smokers. They described in detail how the “misclassification bias” (the apparent risk from spousal smoking if no true effect existed) depends on various factors. They showed that the bias increased with the misclassification rate of ever smokers as never smokers, the relative risk of disease associated with ever smoking, the proportion of participants who have ever smoked, and the concordance ratio between spouses’ smoking habits, and decreased with the proportion of never smokers whose spouse has ever smoked.

The mathematics presented<sup>[1]</sup> also apply to smoking-related diseases other than lung cancer, and to other indices of passive smoking where the index of exposure may be associated with an increased likelihood of smoking. Thus, not only is someone married to a smoker more likely than average to be a smoker themselves, but the same is also true for those whose parents smoke, who live with a smoker, and who work with a smoker.

Application of these results to the misclassification data presented here is not straightforward, as they relate to misclassification of ever smokers, whereas the cotinine data relate to misclassification of current smokers. Denial of past smoking can only be checked from statements made on different occasions by the same individual, evidence for this not being considered here. It is also important to realise that misclassified ever smokers are likely to have lower disease risks than typical ever smokers, as they are more likely to smoke less or be ex-smokers. Lee and Forey<sup>[1]</sup> concluded that the effects of misclassification (taking into account both misclassification of current and ex-smokers as never smokers and the tendency for misclassified ever smokers to have lower risks than non-misclassified ever smokers) were equivalent overall to assuming that about 2.5% of average ever smokers are misclassified as never smokers, though noting that “an appropriate figure is probably in the range 2%-3% but could, not implausibly, be anywhere in the range 1%-4%”.

Recent bias estimations (*e.g.*,<sup>[41]</sup>), have used misclassification rates of 2.5% for studies in Western populations and 10% for studies in Asia. The use of higher rates for Asia was supported by evidence, partly referred to earlier<sup>[1]</sup>, suggesting that misclassification rates are very much higher in Asian women. While the evidence presented here confirms the extremely high misclassification rates in Asian women, they do not suggest the same is true for Asian men. However, given the evidence that misclassification rates are higher in more recently published studies than in the studies considered in the 1996 paper, it seems the estimate of 2.5% for studies in Western populations may be too low.

In conclusion, the combined evidence from 205 studies provides extensive information on the extent to which self-reported smoking habits are confirmed by cotinine levels in blood, saliva or urine and the extent to which true smokers deny

current smoking. Misclassification rates are heterogeneous, with false claims of never smoking much higher in Asian women, and false claims of having quit higher in pregnant women. A number of the rates are higher in diseased groups likely to have been advised to quit. Misclassification rates are higher in more recent studies, which exacerbates problems in determining true relationships of passive smoking with disease.

## ARTICLE HIGHLIGHTS

### Research background

Misclassification of smoking habits leads to underestimation of true relationships between diseases and active smoking, but overestimation of true relationships with passive smoking.

### Research motivation

We estimated overall misclassification rates weighted on sample size and investigated heterogeneity by various study characteristics.

### Research methods

We analysed data from studies using cotinine as a marker which involved at least 200 participants and provided information on high cotinine levels in self-reported non-, never-, or ex-smokers. Information on low levels in self-reported smokers was also analysed.

### Research results

There was considerable heterogeneity between misclassification rates. Rates of claiming never smoking were very high in Asian women smokers, the individual studies reporting rates of 12.5%, 22.4%, 33.3%, 54.2% and 66.3%. False claims of quitting were relatively high in pregnant women, in diseased individuals who may recently have been advised to quit, and in studies considering cigarette smoking rather than any smoking. False claims of smoking were higher in younger populations. There was no clear evidence that rates varied by the body fluid used for the cotinine analysis, the assay method used, or whether the respondent was aware their statements would be validated by cotinine - though here many studies did not provide relevant information. Misclassification rates were higher in more recently published studies.

### Research conclusions

Our demonstration that rates of misclassification of smoking habits are particularly high in some situations underlines the difficulty that epidemiologists have in accurately estimating the increases in risk of various diseases associated with active and passive smoking.

### Research perspectives

Misclassification rates are heterogeneous, with false claims of never smoking much higher in Asian women, and false claims of having quit higher in pregnant women. A number of the rates are higher in diseased groups likely to have been advised to quit. Misclassification rates are higher in more recent studies, which exacerbates problems in determining true relationships of passive smoking with disease.

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## Evaluation of tumor response to antiangiogenic therapy in patients with recurrent gliomas using contrast-enhanced perfusion-weighted magnetic resonance imaging techniques: A meta-analysis

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### Abstract

#### BACKGROUND

It is of vital importance to find radiologic biomarkers that can accurately predict treatment response. Usually, the initiation of antiangiogenic therapy causes a rapid decrease in the contrast enhancing tumor. However, the treatment response is observed only in a fraction of patients due to the partial radiological response secondary to stabilization of abnormal vessels which does not essentially indicate a true antitumor effect. Perfusion-weighted magnetic resonance imaging (PW-MRI) techniques have shown implicitness as a strong imaging biomarker for gliomas since they give hemodynamic information of blood vessels. Hence, there is a rapid expansion of PW-MRI related studies and clinical applications.

#### AIM

To determine the diagnostic performance of PW-MRI techniques including: (A) dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI); and (B) dynamic susceptibility contrast magnetic resonance imaging (DSC-MRI) for evaluating response to antiangiogenic therapy in patients with recurrent gliomas.

#### METHODS

Databases such as PubMed (MEDLINE included), EMBASE, and Google Scholar were searched for relevant original articles. The included studies were assessed for methodological quality with the Quality Assessment of Diagnostic Accuracy Studies 2 tool. Medical imaging follow-up or histopathological analysis was used as the reference standard. The data were extracted by two reviewers independently, and then the sensitivity, specificity, summary receiver operating characteristic curve, area under the curve (AUC), and heterogeneity were calculated using Meta-Disc 1.4 software.



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## RESULTS

This study analyzed a total of six articles. The overall sensitivity for DCE-MRI and DSC-MRI was 0.69 [95% confidence interval (CI): 0.53-0.82], and the specificity was 0.99 (95%CI: 0.93-1) by a random effects model (DerSimonian-Laird model). The likelihood ratio (LR) +, LR-, and diagnostic odds ratio (DOR) were 12.84 (4.54-36.28), 0.35 (0.22-0.53), and 24.44 (7.19-83.06), respectively. The AUC ( $\pm$  SE) was 0.9921 ( $\pm$  0.0120), and the Q\* index ( $\pm$  SE) was 0.9640 ( $\pm$  0.0323). For DSC-MRI, the sensitivity was 0.73, the specificity was 0.98, the LR+ was 7.82, the LR- was 0.32, the DOR was 31.65, the AUC ( $\pm$  SE) was 0.9925 ( $\pm$  0.0132), and the Q\* index was 0.9649 ( $\pm$  0.0363). For DCE-MRI, the sensitivity was 0.41, the specificity was 0.97, the LR+ was 5.34, the LR- was 0.71, the DOR was 8.76, the AUC ( $\pm$  SE) was 0.9922 ( $\pm$  0.2218), and the Q\* index was 0.8935 ( $\pm$  0.3037).

## CONCLUSION

This meta-analysis demonstrated a beneficial value of PW-MRI (DSC-MRI and DCE-MRI) in monitoring the response of recurrent gliomas to antiangiogenic therapy, with reasonable sensitivity, specificity, +LR, and -LR.

**Key words:** Glioma; Perfusion-weighted magnetic resonance imaging; Dynamic contrast-enhanced magnetic resonance imaging; Dynamic susceptibility contrast magnetic resonance imaging; Anti-vascular endothelial growth factor; Antiangiogenic; Meta-analysis

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**Core tip:** Perfusion-weighted magnetic resonance imaging is one of the advanced magnetic resonance (MR) techniques which offer non-invasive and effective ways of grading, differentiating, and assessing therapeutic response and prognosis of brain tumors. This meta-analysis evaluates the clinical applicability of this MR technique in the assessment of the response of recurrent gliomas to antiangiogenic therapy.

**Citation:** Kasenene A, Baidya A, Shams S, Xu HB. Evaluation of tumor response to antiangiogenic therapy in patients with recurrent gliomas using contrast-enhanced perfusion-weighted magnetic resonance imaging techniques: A meta-analysis. *World J Meta-Anal* 2019; 7(2): 51-65

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## INTRODUCTION

Gliomas are the most common primary brain neoplasms in adults<sup>[1]</sup>. They overgrow and are very invasive and angiogenic with a high rate of recurrence and dismal median 15-mo survival regardless of multi-modality management including surgical resection, radiotherapy, and chemotherapy<sup>[2]</sup>. Glioma-associated neovascularization peculiar assembly is an essential factor which contributes to several biological patterns like tumor development, invasiveness, and treatment response<sup>[3]</sup>.

Thanks to the recognition of different signaling pathways and growth factors imperative in tumor angiogenesis, a couple of new anti-angiogenic drugs have so far been manufactured<sup>[4]</sup>. Vascular endothelial growth factor (VEGF) is an arch controller of angiogenesis, vessel development, and vessel permeability<sup>[5]</sup>. The angiogenic action of VEGF is promoted by VEGF receptor-2 (VEGFR-2) which is also the central target for anti-angiogenic therapies, even though added studies have highlighted the significance of signaling *via* VEGFR-1<sup>[6]</sup>.

Bevacizumab (BEV), a humanized monoclonal antibody to the VEGF-A, was the first VEGF inhibitor accredited for cancer treatment by the food and drug authority<sup>[7]</sup>. Also, other multiple approaches for inhibiting VEGF were researched over the past decade. These approaches include neutralizing antibodies to VEGF<sup>[8]</sup>, low-molecular-weight VEGFR tyrosine kinase inhibitors (TKIs)<sup>[9,10]</sup>, and soluble VEGFR constructs (VEGF-Trap)<sup>[11]</sup>. Although antiangiogenic drugs result in a rapid decrease in the contrast enhancing tumor, the treatment response is observed in just a fraction of patients, because the radiological response may partially result from stabilization of

abnormal vessels and does not inherently indicate a true antitumor effect<sup>[12,13]</sup>. Therefore, it is of vital importance to find radiologic biomarkers that can accurately predict treatment response or measure response after the initiation of anti-VEGF therapy<sup>[14]</sup>.

The current imaging modality of choice for clinical use in brain tumors is magnetic resonance imaging (MRI)<sup>[15]</sup>. Although the conventional MRI techniques show excellent anatomical information of tumors, they cannot evaluate quantitatively the blood vessel physiology and exhibit biological characteristics within the tumor molecules and cells, which play a part in tumor grading<sup>[16]</sup>, treatment evaluation<sup>[17]</sup>, and prognosis prediction<sup>[18]</sup>. Moreover, non-enhancing regions representative of edema surrounding the tumor cannot be seen on conventional MRI, hampering the total safe tumor excision and treatment response evaluation<sup>[19,20]</sup>. Perfusion-weighted MRI (PW-MRI) techniques, including dynamic contrast-enhanced MRI (DCE-MRI) and dynamic susceptibility contrast MRI (DSC-MRI), have shown implicitness as a reliable imaging biomarker for gliomas since they give hemodynamic information of blood vessels<sup>[21-23]</sup>. Hence, there is a rapid expansion of PW-MRI application range which explores the association of imaging parameters and the distinctive features of gliomas non-invasively<sup>[24]</sup>.

Recently, a meta-analysis containing 35 studies assessed the diagnostic accuracy of MRI techniques in the evaluation of treatment responses in patients with high-grade gliomas<sup>[25]</sup>. In 23 studies, DSC-MRI and DCE-MRI scans were carried out. The sensitivity and specificity of DSC-MRI (18 reviews, 708 patients) were 87% (82-91%) and 86%, respectively. The pooled analysis showed that the sensitivity and specificity of DCE-MRI (five studies, 207 patients) were 92% (73-98%) and 85%, respectively. However, there is no meta-analysis on the use of PW-MRI in the assessment of the response of recurrent gliomas to antiangiogenic therapy. Therefore, we believe that a meta-analysis of the relevant studies is required to better clarify the role of PW-MRI in predicting and assessing the response of recurrent gliomas to antiangiogenic drugs.

## MATERIALS AND METHODS

The present Meta-analysis was done as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement<sup>[26]</sup>.

### Inclusion criteria

Studies included in this analysis fulfilled these requirements: (A) the study researched the performance of DSC-MRI or DCE-MRI for evaluating antiangiogenic therapy response in recurrent glioma patients; (B) the patients underwent tumor resection and chemotherapy treatment before recurrence and were treated with antiangiogenic therapy after recurrence; (C) The reference standard was either pathology reports or clinical observation or by imaging follow-up; and (D) the primary data were enough to compute the true positive (TP), true negative (TN), false positive (FP), and false negative (FN).

### Exclusion criteria

The studies were excluded for the following reasons: (A) reviews, pre-clinical studies, titles, abstracts, and expert opinions; (B) studies assessing PW-MRI in glioma grading; and (C) inadequate data to generate a 2 × 2 table.

### Study selection

We conducted a comprehensive search in EMBASE, Google Scholar, and Medline (PubMed) for studies up to December 31, 2017 that evaluated the use of PW-MRI in the detection of antiangiogenic response for recurrent gliomas. For PubMed which was the primary database we used to search for studies, MeSH headings used were "MRI", "DCE-MRI", "DSC-MRI", "PW-MRI", "Glioma", "Glioblastoma", "GBM", "Anti-VEGF", "Antiangiogenic", and their combinations.

### Data extraction and quality assessment

Data extraction was carried out by two reviewers (Kasenene A and Baidya A) from each of the selected studies using an Excel spreadsheet.

The extracted data included general characteristics (*i.e.*, first author and year of publication for each study, country, number of patients, median age, study design, imaging modality, imaging biomarkers, MR scanner tesla intensity, type of antiangiogenic therapy given, and the treatment response gold standard used), TP, TN, FP, and FN. Disagreements between investigators concerning outcomes of interest were reviewed later, and the consensus was reached by discussion.

The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool<sup>[27]</sup> was

used to assess the quality of the included studies, where we scored all 14 QUDAS items used to determine the risk of bias as “low risk”, “high risk”, or “uncertain risk”.

### Statistical analysis

We constructed  $2 \times 2$  tables for each study independently. Data from the original publications were used to calculate the sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), diagnostic odds ratio (DOR), 95% confidence intervals (CI), summary receiver operating characteristic (SROC) curves, and Q\* index. Wherever any of TP, FP, FN, and TN values was zero, we added 0.5 to every cell of each study.

We adopted a random effects model (DerSimonian-Laird model) in this study because of the difference in sample size, anti-angiogenic therapy, and reference standards among the included studies.

The analysis was done using Meta-Disc (version 1.4) software and Microsoft Excel 2016 (Microsoft, Seattle, WA, USA)<sup>[28]</sup>.

## RESULTS

### Search strategy and study selection

This meta-analysis included studies which met the selection criteria successfully and were published before December 31, 2017. The search strategy generated a total of 133 clinical studies, of which 44 were investigated further. Out of the 44 studies, we excluded 38 studies, and finally, in this meta-analysis, we included only six studies. The flowchart outlining the process of study selection and exclusion reasons is showed in [Figure 1](#).

### Quality assessment

The quality assessment was performed as per the QUADAS-2 tool and the findings are shown in [Table 1](#). Among the six included studies, four were conducted prospectively, and two were conducted retrospectively. None of the included studies checked all 14 QUDAS risk assessment items.

### Characteristics of the studies

The characteristics and the clinical responses of the six studies encompassed in this meta-analysis are delineated in [Tables 2](#) and [3](#). The studies were all in English, containing 120 patients all together with their age ranging from 25-78 years. The sample size of the studies was 10 to 36 patients.

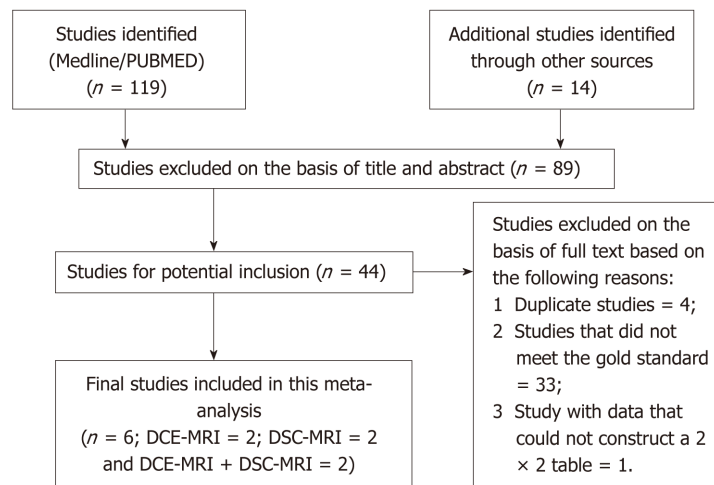
There were two studies on DCE-MRI, two studies on DSC-MRI, and two combined both. All studies performed a sequential DCE-MRI, DSC-MRI, or both before and after the antiangiogenic treatment. Four different types of antiangiogenic therapies were used as the treatment of choice whereby BEV, which is a recombinant humanized monoclonal antibody targeting the VEGF, was used in three studies. VEGF Trap, which acts as a trap by inhibiting the activity of the VEGF subtypes VEGF-A and VEGF-B, was used in one study. Pazopanib, a potent and selective multi-targeted receptor TKI which stops the growth of the tumor and halts angiogenesis, was used in one study. Tivozanib, which is also a TKI, was used in the remaining one study. In four studies, a decrease in volume transfer constant ( $K^{trans}$ ) value was used as the cutoff criterion for response to antiangiogenic therapy. The other studies relied on a reduction of relative cerebral blood volume (rCBV), extracellular volume fraction (EES  $V_e$ ) value, vessel leakage reduction, decrease in tumor volume, tissue oxygenation, or vasogenic edema criteria for therapy response.

### Data analysis

**DSC-MRI and DCE-MRI:** Due to the heterogeneity across all six included studies which evaluated DCE-MRI or DSC-MRI or both, a random effects model (DerSimonian-Laird model) was used to compute the sensitivity, specificity, LR+, LR-, and DOR ([Figures 2-4](#)). The overall sensitivity of DCE-MRI and DSC-MRI was 69%. The specificity was 99%. The detailed sensitivity, specificity, LR+, LR-, and DOR with 95%CI for individual studies are presented in [Table 4](#).

The SROC curves with the Q\* index are shown in [Figure 5](#). Of all six studies, the area under the curve (AUC) ( $\pm$  SE) was 0.9921 ( $\pm$  0.0120), and the Q\* index was 0.9640 ( $\pm$  0.0323).

**DSC-MRI:** Four included studies evaluated DSC-MRI. Also, the random effects model (DerSimonian-Laird model) was used to calculate sensitivity, specificity, LR+, LR-, and DOR ([Figures 6-8](#)). The sensitivity of DSC-MRI to assess antiangiogenic treatment response in gliomas was 73%. The specificity was 98%. The pooled LR+ was



**Figure 1** Flowchart outlining the study selection process according to the Preferred Reporting Items for Systematic Reviews guidelines. DCE-MRI: Dynamic contrast-enhanced magnetic resonance imaging; DSC-MRI: Dynamic susceptibility contrast magnetic resonance imaging.

7.82; LR- was 0.32. The pooled DOR was 31.65.

The SROC curves with the  $Q^*$  index are shown in [Figure 9](#). Of all four studies, the AUC ( $\pm$  SE) was 0.9925 ( $\pm$  0.0132), and the  $Q^*$  index was 0.9649 ( $\pm$  0.0363).

**DCE-MRI:** Four included studies evaluated DCE-MRI, again the random effects model (DerSimonian-Laird model) was used to calculate sensitivity, specificity, LR+, LR-, and DOR (Figures 10-12). The sensitivity of DCE-MRI to assess antiangiogenic treatment response in gliomas ranged from 21% to 64%. The specificity was 97%. The pooled LR+ was 5.34; LR- was 0.71. The pooled DOR was 8.76.

The SROC curves with the  $Q^*$  index are shown in [Figure 13](#). Of all four studies, the AUC ( $\pm$  SE) was 0.9922 ( $\pm$  0.2218), and the  $Q^*$  index was 0.8935 ( $\pm$  0.3037).

## DISCUSSION

PW-MRI is a non-invasive imaging technique which is increasingly used in clinical evaluation and treatment response assessment of brain tumors. The current meta-analysis was conducted to assess the diagnostic performance of PW-MRI techniques (DCE-MRI and DSC-MRI) in the evaluation of glioma response to antiangiogenic therapy.

The overall sensitivity and specificity for DSC-MRI and DCE-MRI were 0.69 and 0.99, respectively. Although the pooled sensitivity for the present study was relatively low, the statistical results still showed that PW-MRI (DSC-MRI and DCE-MRI) techniques are effective ways to assess the response of gliomas to antiangiogenic therapy. On the SROC, the summary points were closer to the left hand, and the AUC was 0.9921, which indicates an excellent diagnostic accuracy. Likelihood ratios are other measures of the diagnostic accuracy, with LR greater than 1 meaning that the test result is associated with the disease presence, and the LR ratio less than 1 meaning that the test result is related to the disease absence. The higher the LR are from 1, the stronger the evidence for the presence or absence of disease; LR > 10 and < 0.1 are thought to provide strong evidence to declare the presence or absence of the disease in most cases<sup>[34]</sup>. The LR+ for the DSC-MRI and DCE-MRI group was 12.84, indicating that there were an approximately 13 times chance of PWI-MRI showing clinically meaningful changes as the result of antiangiogenic therapy. On the other hand, the LR- was 0.35, meaning it was below the cut-off value, indicating a chance of not showing any changes was approximately 0.4 times.

Moreover, for the DSC-MRI group, the sensitivity and specificity were 0.73 and 0.98, respectively, where the sensitivity was slightly higher than in the DSC-MRI and DCE-MRI group. The pooled LR+ was 7.82, which means the chance of DSC-MRI indicating the changes associated with antiangiogenic therapy was approximately 8 times; LR- was 0.32 and below the cut-off point, which suggests that the chance of DSC-MRI not showing treatment-associated changes was 0.3 times, and AUC was 0.9925, indicating an excellent diagnostic accuracy. While for the DCE-MRI group, the sensitivity and specificity were 0.41 and 0.97, respectively; the sensitivity of the DCE-

Table 1 Quality assessment of the included studies

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Iwamoto <i>et al</i> <sup>[29]</sup> , 2010	L	U	H	L	L	L	L
Piludu <i>et al</i> <sup>[30]</sup> , 2015	L	U	U	L	L	L	L
Kalpathy-Cramer <i>et al</i> <sup>[31]</sup> , 2017	L	U	U	L	L	L	L
O'Neill <i>et al</i> <sup>[32]</sup> , 2016	L	U	H	L	L	L	L
Schmainda <i>et al</i> <sup>[4]</sup> , 2014	L	U	U	L	L	L	L
Hilario <i>et al</i> <sup>[33]</sup> , 2016	L	U	U	L	L	L	L

L: Low risk; U: Unclear risk; H: High risk.

group was lower as compared to the previous two groups, and this might be due to the patient sample size variation among the studies in this group. The LR+ was 5.34, indicating a 5 times chance of showing treatment-associated changes; LR- was 0.71 and below the cut-off value, indicating a 0.7 times chance of not showing anything, and the AUC was 0.9922, indicating a higher diagnostic accuracy. All of these values suggest that both DSC-MRI and DCE-MRI have diagnostic value in treatment evaluation of recurrent gliomas.

### Limitations

First, some included studies were too small, which may have led to imprecise and inconclusive results. Second, only articles published in English were included, which may have led to publication bias. Third, there was verification bias or workup bias, because patients were subjected to different reference tests and antiangiogenic medications.

### Conclusion

Regardless of some limitations, this meta-analysis demonstrated a beneficial value of PW-MRI (DSC-MRI and DCE-MRI) in monitoring the response to antiangiogenic therapy in recurrent gliomas, with reasonable sensitivity, specificity, +LR, and -LR.

**Table 2** The basic characteristics of the included studies

Studies	Country	Patient s, n	Median age	Study design	Imaging modality	Imaging biomarkers	MR scanner tesla	Anti- angiogenic treatment	Clinical question	Standard reference	TP	FP	FN	TN
Iwamoto <i>et al</i> <sup>[29]</sup> , 2010	United States	11	53 (29-73)	Pro-spective	DSC, DCE	Ktrans, rCBV	NR	Pazopanib	DSC and DCE vs response	Macdonald criteria RANO criteria	1	0	6	4
Piludu <i>et al</i> <sup>[30]</sup> , 2015	Germany	27	54 (33-77)	Pro-spective	DCE	nIAUGC, Ktrans	3.0 T	Bevacizumab	DCE vs response	RANO criteria	6	0	5	16
Kalpathy-Cramer <i>et al</i> <sup>[31]</sup> , 2017	United States	10	62 (51-74)	Pro-spective	DSC, DCE	Ktrans, rCBV, rCBF	3.0 T	Tivozanib	DSC and DCE vs response	RANO criteria	1	1	4	4
O'Neill <i>et al</i> <sup>[32]</sup> , 2016	United States	12	NR	Pro-spective	DCE	Ktrans, Ve	1.5 T	VEGF Trap	DCE vs response	Macdonald criteria	1	0	0	11
Schmainda <i>et al</i> <sup>[4]</sup> , 2014	United States	36	34 (30-68)	Retrospective	DSC	rCBV, stdrCBV	1.5 T or 3.0 T	Bevacizumab	DSC vs response	Macdonald criteria RANO criteria	6	0	0	30
Hilario <i>et al</i> <sup>[33]</sup> , 2016	Spain	24	52.5 (31-74)	Retrospective	DSC	Leakage volume (CBV-LCCBV)	1.5 T	Bevacizumab	DSC vs response	RANO criteria	14	0	0	10

TP: True positive; FP: False positive; TN: True negative; FN: False negative; DCE: dynamic contrast-enhanced; DSC: Dynamic susceptibility.

**Table 3** Clinical responses of gliomas to antiangiogenic treatments

Study	Patient, n	RANO criteria				Macdonald criteria			
		Responders		Non-responders		Responders		Non-responders	
		CR	PR	SD	PD	CR	PR	SD	PD
O'Neill <i>et al</i> <sup>[32]</sup> , 2016	12	-	-	-	-	0	1	0	11
Piludu <i>et al</i> <sup>[30]</sup> , 2015	27	0	6	5	16	-	-	-	-
Schmainda <i>et al</i> <sup>[4]</sup> , 2014	36	0	6	0	30	-	-	-	-
Iwamoto <i>et al</i> <sup>[29]</sup> , 2010	11	-	-	-	-	0	1	6	4
Hilario <i>et al</i> <sup>[33]</sup> , 2016	24	0	14	0	10	-	-	-	-
Kalpathy-Cramer <i>et al</i> <sup>[31]</sup> , 2017	10	1	1	4	4	-	-	-	-

CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease. \*Response as defined using the Macdonald criteria and Response Assessment in Neuro-Oncology (RANO) working group guidelines.

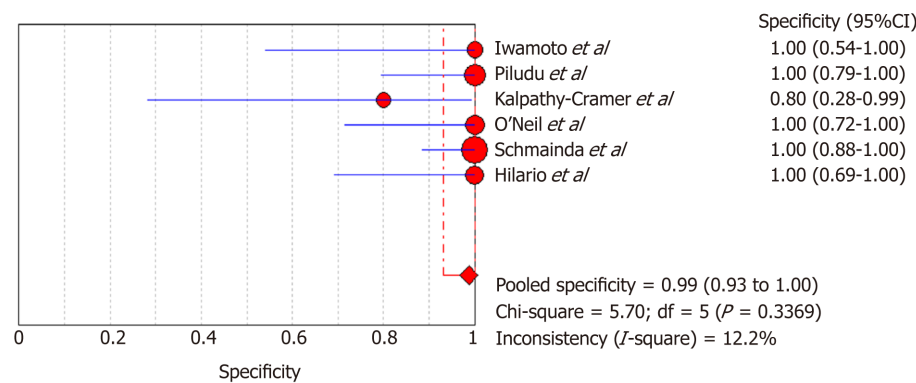
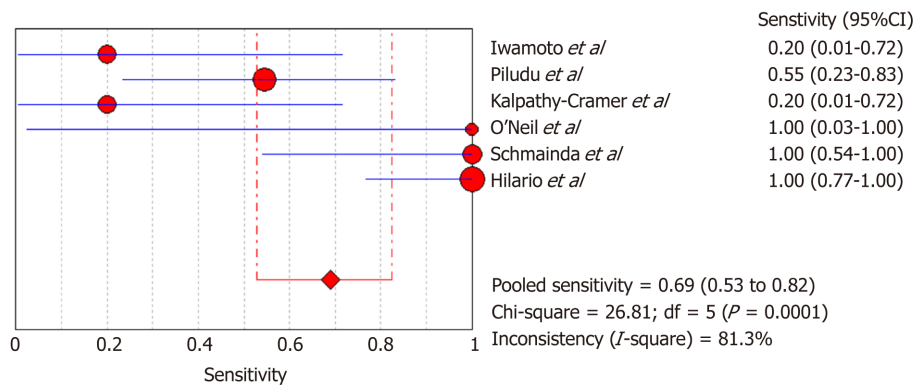
**Table 4** True positive, false positive, true negative, and false negative as well as the sensitivity, specificity, likelihood ratio, and diagnostic odds ratio for the meta-analysis of antiangiogenic treatment evaluation

Study	TP	FP	FN	TN	Sensitivity (95%CI)	Specificity (95%CI)	LR+ (95%CI)	LR- (95%CI)	DOR (95%CI)
Iwamoto <i>et al</i> <sup>[29]</sup> , 2010	1	0	4	6	0.2 (0.01-0.72)	1 (0.54-1)	3.5 (0.17-70.94)	0.81 (0.49-1.340)	4.33 (0.14-132.32)
Piludu <i>et al</i> <sup>[30]</sup> , 2015	6	0	5	16	0.55 (0.23-0.83)	1 (0.79-1)	18.42 (1.14-296.83)	0.47 (0.25-0.880)	39 (1.88-810.44)
Kalpathy-Cramer <i>et al</i> <sup>[31]</sup> , 2017	1	1	4	4	0.2 (0.01-0.72)	0.8 (0.28-0.99)	1 (0.08-11.93)	1 (0.54-1.86)	1 (0.05-22.18)

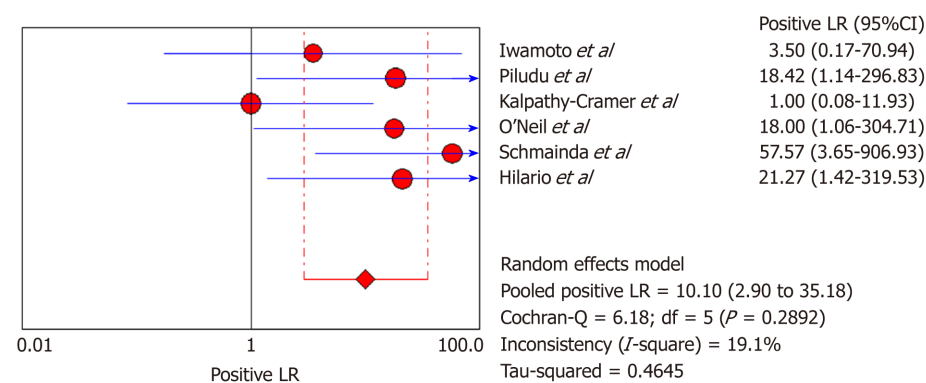


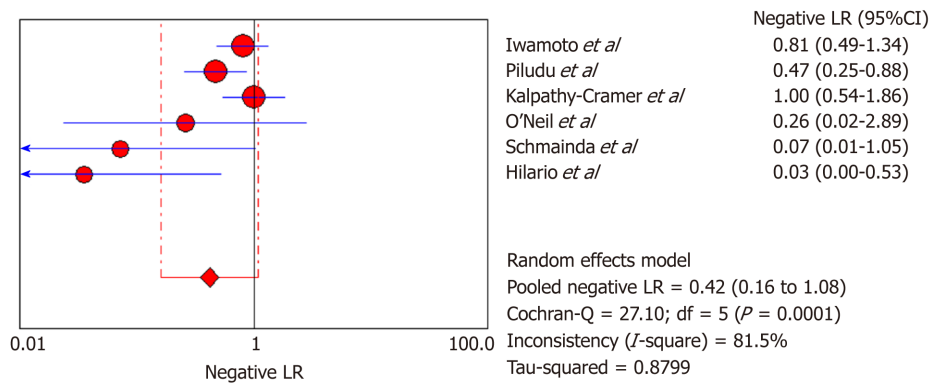
O'Neill <i>et al</i> <sup>[32]</sup> , 2016	1	0	0	11	1 (0.03-1)	1 (0.72-1)	18 (1.06-304.71)	0.26 (0.02-2.89)	69 (0.96-4951.23)
Schmainda <i>et al</i> <sup>[4]</sup> , 2014	6	0	0	30	1 (0.54-1)	1 (0.88-1)	57.57 (3.65-906.93)	0.07 (0.01-1.05)	793 (14.37-43,746.61)
Hilario <i>et al</i> <sup>[33]</sup> , 2016	14	0	0	10	1 (0.77-1)	1 (0.69-1)	21.27 (1.42-319.53)	0.03 (0-0.53)	609 (911.16-33,236.62)
Pooled results	29	1	13	77	0.69 (0.53-0.82)	0.99 (0.93-1)	12.84 (4.54-36.28)	0.35 (0.22-0.53)	24.44 (7.19-83.06)
Heterogeneity test					$I^2 = 81.30\%$ , $P = 0.0001$	$I^2 = 12.2\%$ , $P = 0.3369$	$I^2 = 19.1\%$ , $P = 0.2892$	$I^2 = 81.5\%$ , $P = 0.0001$	$I^2 = 53.5\%$ , $P = 0.0563$

TP: True positive; FP: False positive; TN: True negative; FN: False negative; LR+: Positive likelihood ratio; LR-: Negative likelihood ratio; DOR: Diagnostic odds ratio; CI: Confidence interval.

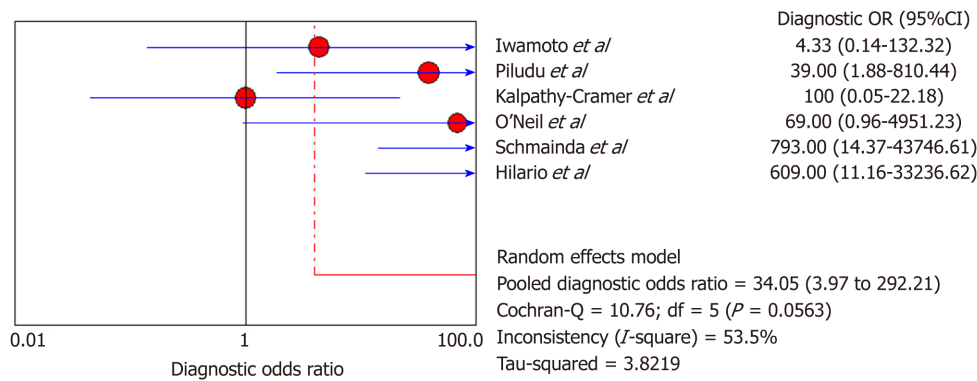


**Figure 2** Sensitivity and specificity of dynamic contrast-enhanced magnetic resonance imaging and dynamic susceptibility contrast magnetic resonance imaging in the evaluation of response to antiangiogenic therapy in recurrent gliomas.

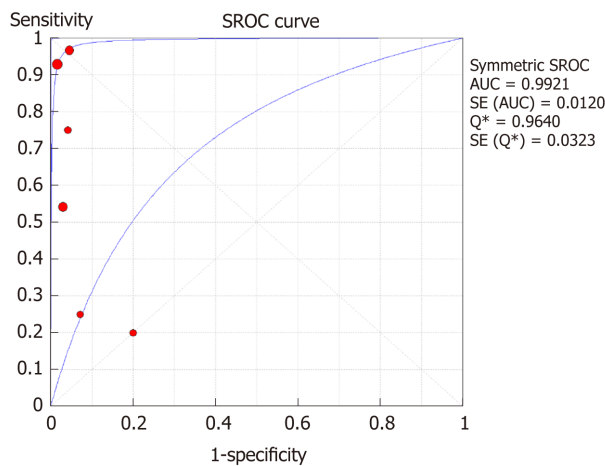




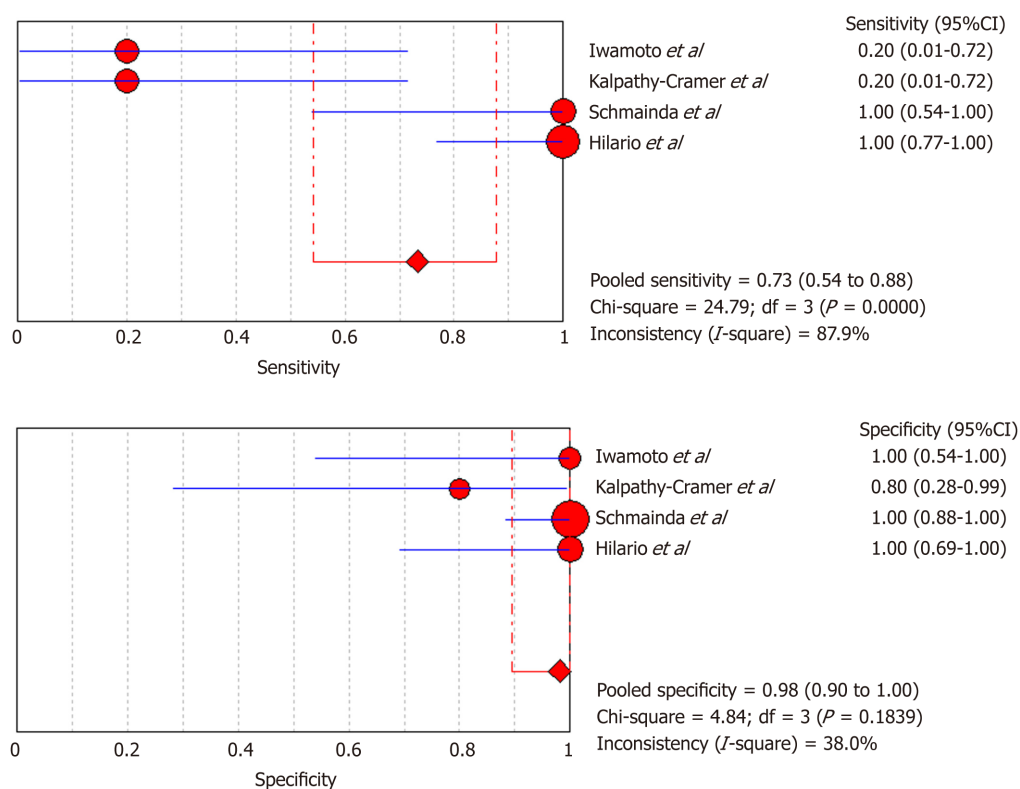
**Figure 3** Likelihood ratio + and likelihood ratio - of dynamic contrast-enhanced magnetic resonance imaging and dynamic susceptibility contrast magnetic resonance imaging in the evaluation of response to antiangiogenic therapy in recurrent gliomas.



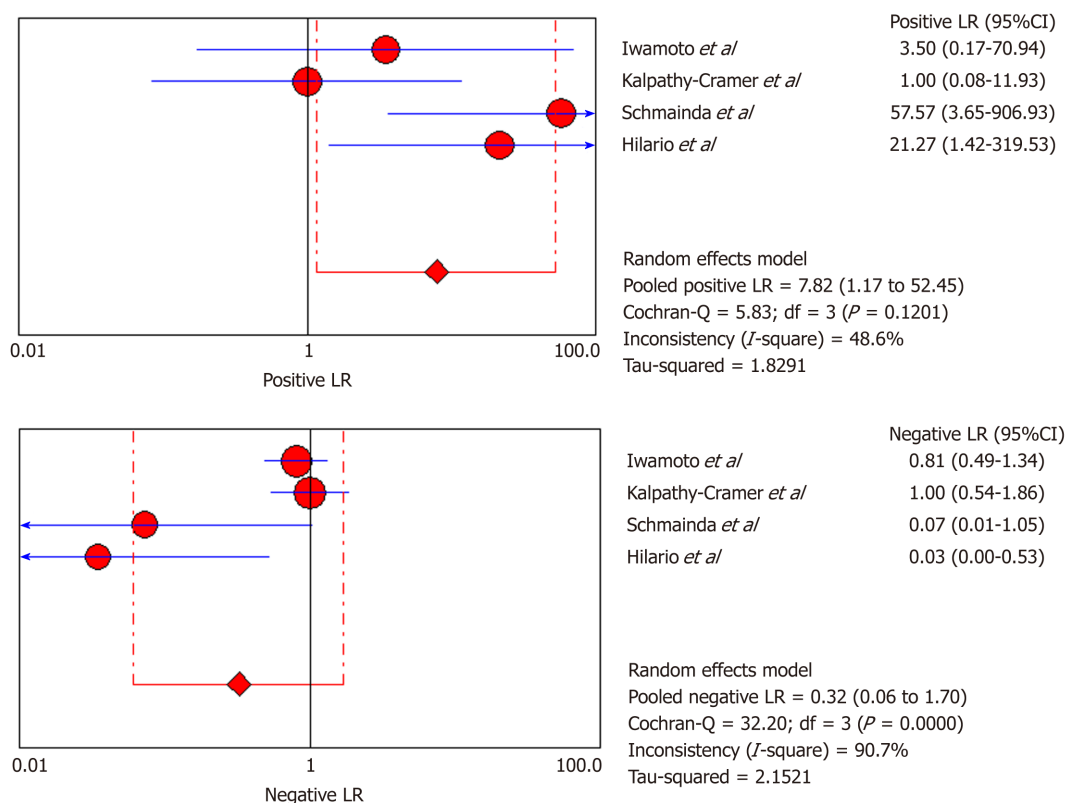
**Figure 4** Diagnostic odds ratio of dynamic contrast-enhanced magnetic resonance imaging and dynamic susceptibility contrast magnetic resonance imaging in the evaluation of response to antiangiogenic therapy in recurrent gliomas.



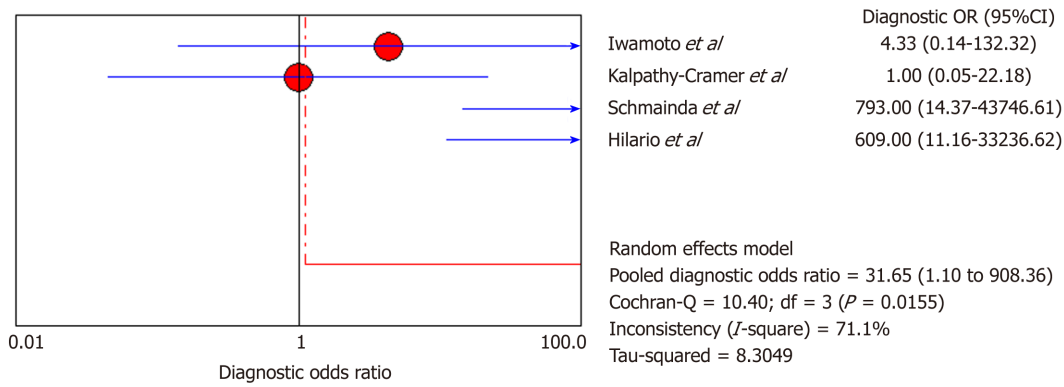
**Figure 5** Summary receiver operating characteristic curve for dynamic contrast-enhanced magnetic resonance imaging and dynamic susceptibility contrast magnetic resonance imaging. Solid circle represents each study in the meta-analysis, and the circle size indicates the study size. SROC: Summary receiver operating characteristic.



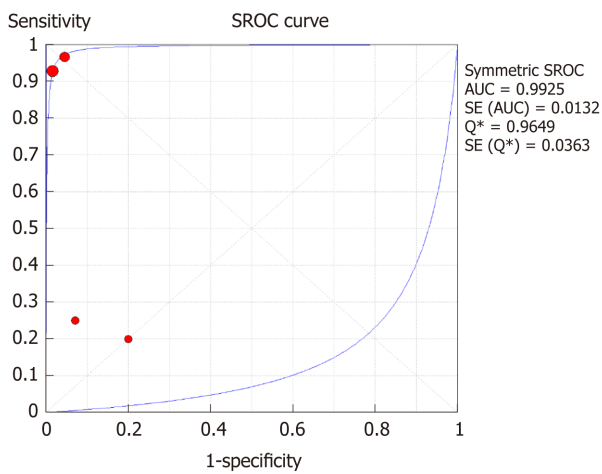
**Figure 6** Sensitivity and specificity of dynamic susceptibility contrast magnetic resonance imaging in the evaluation of response to antiangiogenic therapy in recurrent gliomas.



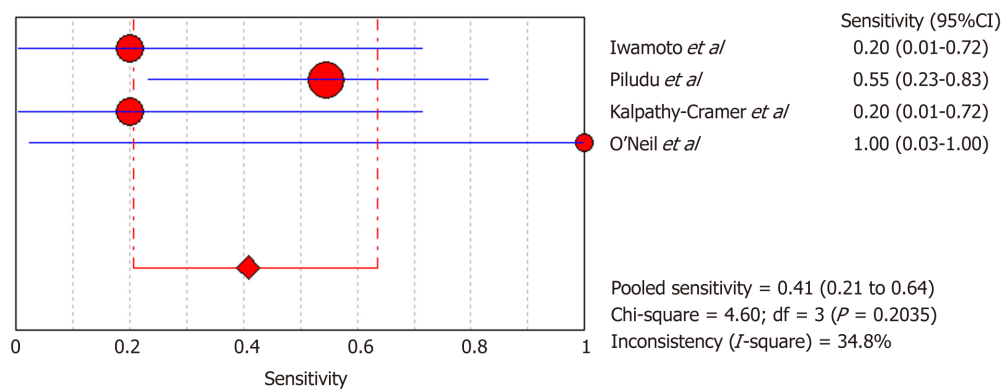
**Figure 7** Likelihood ratio + and likelihood ratio - of dynamic susceptibility contrast magnetic resonance imaging in the evaluation of response to antiangiogenic therapy in recurrent gliomas.

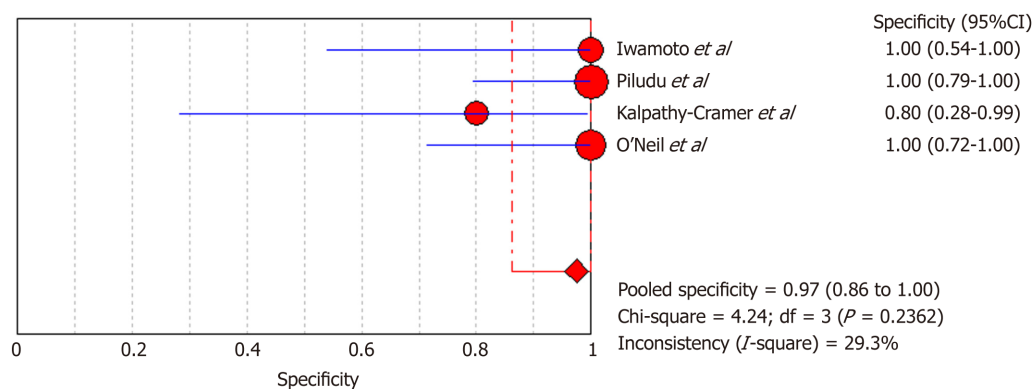


**Figure 8** Diagnostic odds ratio of dynamic susceptibility contrast magnetic resonance imaging in the evaluation of response to antiangiogenic therapy in recurrent gliomas.

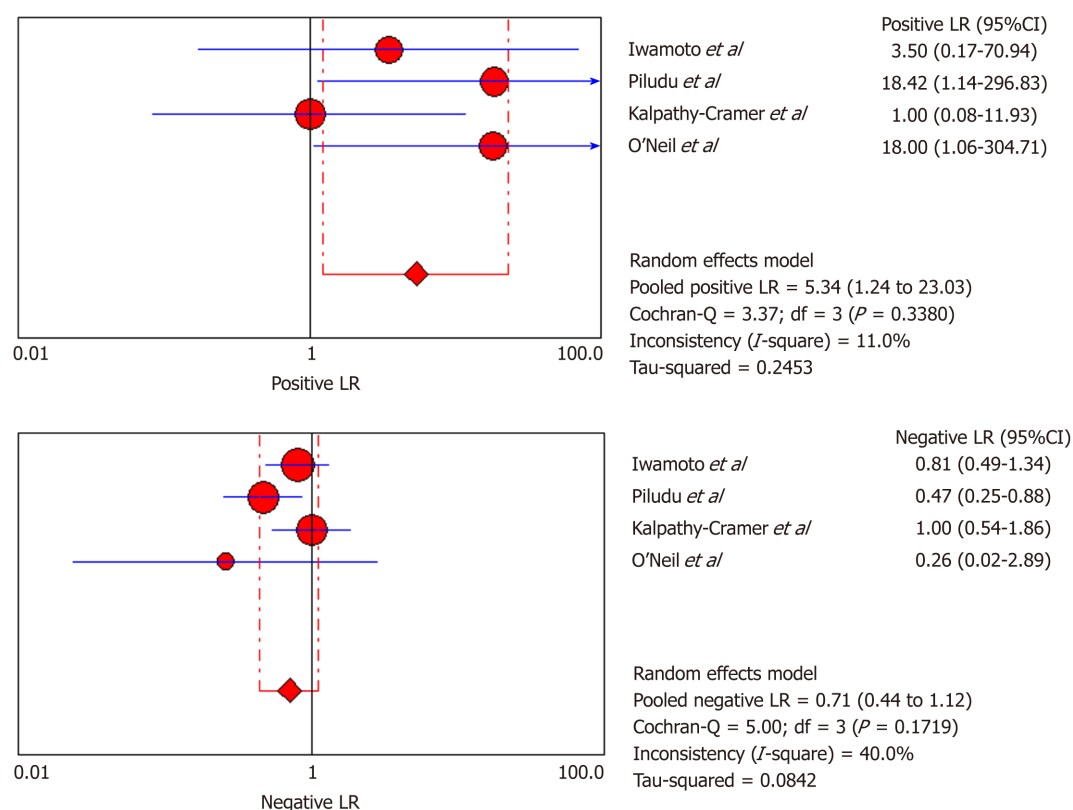


**Figure 9** Summary receiver operating characteristic curve for dynamic susceptibility contrast magnetic resonance imaging. SROC: Summary receiver operating characteristic.





**Figure 10** Sensitivity and specificity of dynamic contrast-enhanced magnetic resonance imaging in the evaluation of response to antiangiogenic therapy in gliomas.



**Figure 11** Likelihood ratio + and likelihood ratio - of dynamic contrast-enhanced magnetic resonance imaging in the evaluation of response to antiangiogenic therapy in recurrent gliomas.

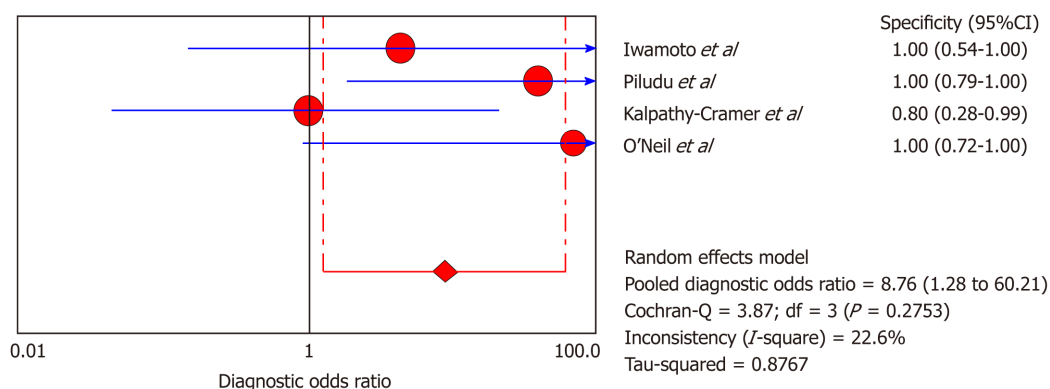


Figure 12 Diagnostic odds ratio of dynamic contrast-enhanced magnetic resonance imaging in the evaluation of response to antiangiogenic therapy in recurrent gliomas.

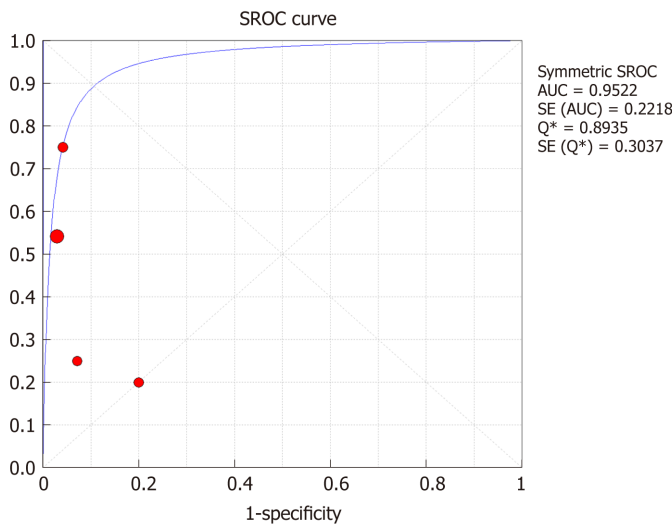


Figure 13 Summary receiver operating characteristic curve for dynamic contrast-enhanced magnetic resonance imaging. SROC: Summary receiver operating characteristic.

## ARTICLE HIGHLIGHTS

### Research background

The perfusion-weighted imaging is an advanced magnetic resonance (MR) technique capable of giving a detailed hemodynamic status of the tumors; this has prompted a surge of studies both *in vitro* and *in vivo* with some of these studies done on humans. The current meta-analysis is exploring the clinical application of this method in recurrent glioma patients after antiangiogenic therapy.

### Research motivation

So far, biopsy, which is too invasive, is the gold standard for diagnosing brain tumors; perfusion-weighted magnetic resonance imaging (PW-MRI) offers a non-invasive way for diagnosing, grading, and assessing progression of brain tumors. Therefore, scientifically proving that this method works is of paramount importance in the spirit of encouraging its incorporation to the daily clinical practices.

### Research objectives

The primary objective of this study was to assess the use of PW-MRI in evaluating the response of recurrent gliomas to antiangiogenic treatment based on the already available literature, and the finding of this study showed that both dynamic contrast-enhanced MRI and dynamic susceptibility contrast MRI could be used for this purpose. These findings would warrant further human-based studies on this MR technique to achieve its universal acceptability for clinical use.

### Research methods

Studies related to this topic were searched in PubMed, Google Scholar, and other scientific search engines. The selected studies were sorted according to the Preferred Reporting Items for Systematic Reviews guidelines, and only six studies meeting the inclusion criteria were included in the current meta-analysis. Meta-Disc 1.4 software and Microsoft Excel were used to analyze the data.

### Research results

The sensitivity and specificity of this study proved that PW-MR techniques are capable of evaluating treatment response in patients with brain tumors, *i.e.*, recurrent gliomas, and these findings, together with other related studies, mean that we are getting a step closer to its complete adaption for clinical use. The only concern is what should be done to improve and perfect this technique.

### Research conclusions

This meta-analysis showed that PW-MRI could be used to assess brain tumor therapeutic response. However, more studies must be conducted because that is the only way we will be sure of its clinical efficacy in diagnosing, follow-up, and treatment of brain tumors.



## Research perspectives

While searching for studies, we realized that very few studies had been carried out on human subjects; this tells us that there is more to be done until it is adopted universally for clinical use. There are many studies carried on animals, which mean the technique is being perfected and improved with each passing day. We need more studies assessing its application on human subjects.

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## Reproducibility and replicability of systematic reviews

Farhad Shokraneh

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**Author contributions:** Shokraneh F is the single author of this manuscript. He started the idea and wrote first draft of the manuscript. He also revised and prepared the paper for the journal.

**Conflict-of-interest statement:** Farhad Shokraneh is campaigning for sharing open data and open methods from systematic reviews. He is also involved in development of reporting guidelines and automation software programs, such as Screen-IT, 2dSearch and Study-Based Registers, to enhance the reproducibility.

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### Abstract

Irreproducibility of research causes a major concern in academia. This concern affects all study designs regardless of scientific fields. Without testing the reproducibility and replicability it is almost impossible to repeat the research and to gain the same or similar results. In addition, irreproducibility limits the translation of research findings into practice where the same results are expected. To find the solutions, the Interacademy Partnership for Health gathered academics from established networks of science, medicine and engineering around a table to introduce seven strategies that can enhance the reproducibility: pre-registration, open methods, open data, collaboration, automation, reporting guidelines, and post-publication reviews. The current editorial discusses the generalisability and practicality of these strategies to systematic reviews and claims that systematic reviews have even a greater potential than other research designs to lead the movement toward the reproducibility of research. Moreover, I discuss the potential of reproducibility, on the other hand, to upgrade the systematic review from review to research. Furthermore, there are references to the successful and ongoing practices from collaborative efforts around the world to encourage the systematic reviewers, the journal editors and publishers, the organizations linked to evidence synthesis, and the funders and policy makers to facilitate this movement and to gain the public trust in research.

**Key words:** Systematic review; Meta-analysis; Reproducibility of results; Automation; Data science; Data anonymization; Datasets; Guideline adherence; Guideline; Peer-review

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**Core tip:** Reproducibility increases the practicality of the research findings and gains the public trust in research. The ongoing developments in automation of systematic reviews, availability of pre-registration platform, dealing more with secondary data or anonymized primary data, the collaboration culture among the organizations who produce systematic reviews, and finally having an update step that mandates replicability

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are all reasons that systematic reviews have the potential to lead the movement toward the reproducibility among the other research designs. Meanwhile, reproducibility can help the systematic reviews to be considered as research design rather than literature review.

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## INTRODUCTION

Systematic reviews are at high levels of evidence hierarchy in clinical practice<sup>[1]</sup>. People who are involved in healthcare systems usually use systematic reviews in research, policy, and practice<sup>[3]</sup> trusting the reproducibility of the results when implemented<sup>[2]</sup>. At the same time, some criticize that the systematic reviews are literature reviews not research<sup>[4,5]</sup>. To utilize the systematic reviews in practice and to call them research studies, we need reproducibility testing; and to ensure that a systematic review is reproducible it is important to design, to record and to report systematic reviews in a transparent and reproducible way and to prioritize and fund reproducible reviews<sup>[6]</sup>. Some suggest that a team independent from the original team can repeat the systematic reviews to ensure the reproducibility<sup>[7]</sup>. Since conducting systematic reviews is already time-consuming<sup>[8]</sup> and resource-rating<sup>[9]</sup>, it is arguable how adding more steps such as reproducibility test that requires more time and resources could reduce waste and increase value.

In context of this paper, reproducibility is re-conducting the same study, using the same methods and data by a different researcher or team and the replicability is re-doing the same study to gather new data or recollect the data<sup>[10]</sup>.

To provide solutions for irreproducibility, the Interacademy Partnership for Health introduced seven strategy to enhance the reproducibility practice in science<sup>[11]</sup>. This editorial discusses the progress with using these strategies in systematic reviewing process and calls for collaboration in all levels of system to enhance the reproducibility of systematic reviews.

## STRATEGY 1: PRE-REGISTRATION

Currently, prospective registration of systematic review protocols in PROSPERO, a register of systematic review protocols, is recommended<sup>[12]</sup>. Compared to clinical trials with at least 17 registries<sup>[13]</sup> there is only one register for systematic reviews; however, unlike clinical trials, it is not yet mandatory to register systematic reviews prospectively<sup>[14]</sup>. Today, PROSPERO covers only 30000 records of conducted, ongoing, awaiting, and abandoned review family (less than a third of 100000 systematic reviews in MEDLINE)<sup>[15]</sup>, it does not support the quality control mechanism<sup>[16]</sup>, and it lacks a rigor follow-up procedure for abandoned systematic reviews<sup>[17]</sup>. To look at the bright side, there is an association between registration of the published reviews and the quality of these reviews<sup>[18]</sup>. Allocating more resources to this register, training and encouraging the systematic reviewers to register their reviews, and making the pre-registration a standard for bias control will push the reproducibility theory toward practice.

## STRATEGY 2: OPEN METHODS

Researchers should share search strategies for all databases<sup>[19]</sup> and analytical codes for meta-analysis<sup>[20]</sup> as part of the methods of systematic reviews. Following to the prospective registration and publication of the protocol, the researchers and the research audiences could assess the reproducibility and detect if any variation from the protocol could have important implementation messages for research, policy and practice<sup>[12]</sup>. This practice is not just to test the reproducibility but also to replicate another analysis or a new update for the systematic review. None of these are possible

without access to all search strategies and statistical codes for meta-analysis.

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### STRATEGY 3: OPEN DATA

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Search results (excluding copyrighted abstract and database-specific meta-data) in Research Information Systems (RIS) format<sup>[21]</sup> and extracted data and meta-data from the studies are the main resulting dataset during the systematic reviewing<sup>[22-24]</sup>. Access to open data from systematic reviews makes it possible to re-screen the search results, to de-duplicate the update searches, to re-run the meta-analyses, and to test the reproducibility of searching, screening, and data analysis steps. Besides, these data will have more value if they have been shared beside their associated meta-data following FAIR guidelines (findable, accessible, inter-operable, and reusable)<sup>[25]</sup>. There have already been calls for sharing the data from systematic reviews but there is no policy or action in place<sup>[22-24]</sup>. Sharing the data from all systematic reviews can lead into data-driven innovations with potential for knowledge discovery and saving the waste of resources and lives.

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### STRATEGY 4: COLLABORATION

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Collaboration among research teams in small or large scale increases the chance for more expertise input and enhances the error detection and fixation practice<sup>[26,27]</sup>. Sharing the data among collaborators or interested research groups could bring together the data and resources for re-analyzing the same data<sup>[20]</sup> or innovations<sup>[23]</sup> that are impossible without such collaboration. It is not good practice to hold the data for years hoping to receive funding or innovating while sharing could result in faster innovation, receiving credits or collaboration in grant applications<sup>[26,27]</sup>. It also raises the morality and mortality question that is it ethical to hold the data when sharing it could lead to decisions that can save public resources and lives, and reduce the waste. The data extracted from other primary research for systematic reviews cannot be owned by the systematic reviewers or organizations that produce the systematic reviews.

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### STRATEGY 5: AUTOMATION

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International Collaboration for the Automation of Systematic Reviews produces annual report of progress for automation of systematic reviews<sup>[28-30]</sup>. This collaboration seems to understand well that the automation is a key for reproducibility and follows Vienna Principles that also emphasize on the replicability of automation activities and sharing the program codes for wider use by the community<sup>[28]</sup>. The value of the automation becomes more obvious looking at reports of human errors in systematic reviews in searching<sup>[31]</sup> and data extraction steps<sup>[32]</sup>. The service provided by machine can speed the process and reduce the waste caused by human errors through standardization of practices such as statistical analysis or systematic review write-up steps<sup>[30,33]</sup>. Despite all technological development, systematic reviewers have underused the automation tools<sup>[34]</sup>. Currently, Systematic Review Data Repository<sup>[35]</sup>, EPPI-Reviewer<sup>[36]</sup>, Study-Based Registers<sup>[37]</sup>, and Evidence Pipeline as semi-automated systems have the potential to evolve into automated systems for systematic reviews.

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### STRATEGY 6: REPORTING GUIDELINES

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Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)<sup>[38]</sup> now celebrates a decade of being used in reporting step of systematic reviews and major journals enforce the systematic reviewers to follow the PRISMA family guidelines in reporting. Such reporting guidelines are helping researchers to report certain items for publications and it is not their primary purpose to advocate the reproducibility<sup>[6]</sup>. There is an update of PRISMA 2019 in progress that will include more items and some these items can maximize the reproducibility practice<sup>[6]</sup>.

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### STRATEGY 7: POST-PUBLICATION REVIEW

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Pre-publication peer-reviews are limited to a few people while post-publication

reviews give chance for wider audience to appraise and comment on some aspect of the research. Post-publication activities take many forms including letter to editor, commentary, blogs, and other social media posts<sup>[26]</sup>. These reviews are separate and independent from the original research and the only connection is through a link or citation. As a result, it is hardly possible to find all these reviews integrated in one place. This problem expands when there are retractions to the original systematic reviews or the findings are published in salami of papers. Such post-publication reviews, however, are encouraged in particular for systematic reviews because they can be taken into account in the next updates of the current systematic review. Having an update step in development of systematic reviews, unlike other published literature, is a unique advantage of systematic reviews allowing the reviewers to correct their mistakes and errors or to consider addition of new data or aspect to the review.

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## OPEN PROCESS: EMBEDDED REPRODUCIBILITY IN AGREEMENT CHECKS

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As an addition to these strategies, it is also important not to overlook the process of the systematic reviewing and its connection to reproducibility. The routine practice in systematic reviews is to involve at least two researchers in screening and data extraction steps to reduce human errors<sup>[32,39]</sup> through double-checking of the decision and to reach an agreement. Such agreement sometimes requires a discussion between two reviewers or inviting the comments from another usually senior researcher. It means the decision on eligibility of studies or accuracy of data extraction is being replicated twice or three times. Since this process itself is replicating part of the review and has value for improving the reproducibility, some of the automation and semi-automation systems allow the researchers to document the process of double- and triple-checking within the system but for transparency purposes, this needs to be shared as well. In other words, the process should be documented and shared publicly.

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## SYSTEMATIC REVIEWS AS ROLE MODEL FOR OTHER RESEARCH DESIGNS

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Systematic reviews have the great potential to lead the reproducibility practice among the rest of study designs in scientific fields because: A. Having an update step allows the systematic reviews to be corrected and helps in advancing 'living systematic reviews'; B. Making a unique progress in automation of systematic reviews helps researchers to save time and resources in every step of systematic reviewing; C. Provision of protocol and methods facilitates the replication of systematic review in update step. To make such role model, the organizations whose main activity includes producing systematic reviews should come together and collaborate on developing policies on reproducibility and sharing the data and methods from within the systematic reviews. On the other hand, these organizations have their own journal platforms and the journal publishers themselves need to engage in this policy development as well. To avoid a meta-waste, *Cochrane Database of Systematic Reviews*, *Systematic Reviews* journal, *World Journal of Meta-Analysis*, *JBIC Database of Systematic Reviews and Implementation Reports*, and *Environmental Evidence* now have a great opportunity to come together and set the bars on reproducibility of systematic reviews.

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## Gastrointestinal stress ulcer prophylaxis in the intensive care unit, where is the data?

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### Abstract

Stress-induced gastrointestinal ulcers are common among patients admitted to the intensive care unit (ICU). These ulcers impose significant morbidity and mortality, therefore, stress ulcer prophylaxis (SUP) is a common clinical practice among healthcare providers dealing with these critically-ill patients. Several strategies for SUP have been suggested over the past four decades, with acid suppressive therapies being the most commonly used in the ICU. Whether SUP is effective and safe, or not, remains a topic of controversy. The data is still conflicting, and provision of a simple answer is not feasible at the present time. Recently, a large phase IV, multicenter, randomized clinical trial (SUP-ICU), negated the benefits (and harms) of proton pump inhibitors as SUP. This article

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reviews some of these controversies.

**Key words:** Gastrointestinal stress ulcers; Proton pump inhibitors; H2-antagonists; Prophylaxis; Complications

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**Core tips:** Stress ulcer prophylaxis (SUP) is a prevalent clinical practice in patients admitted to intensive care unit (ICU). However, there is no high-quality evidence to support its use. Indeed, current data on its efficacy and complications remains conflictive at best, and until an explicit evidence becomes available, health care providers working in the ICU must carefully analyze the advantages and disadvantages of SUP based on each patient's presentation and comorbidities.

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## GASTROINTESTINAL STRESS ULCER PROPHYLAXIS IN THE INTENSIVE CARE UNIT, WHERE IS THE DATA?

Patients with major illnesses (*i.e.*, trauma, shock, sepsis, head injury) are at risk of developing a variety of erosions or erosive lesions in the mucosa of the gastroduodenal tract (known as stress ulcers)<sup>[1]</sup>. These erosions are usually acute, multiple, superficial, and occur mainly in the fundus and body of the stomach, which makes them present as a distinct clinicopathological entity different from other types of ulcers (*i.e.*, reactivation of chronic peptic ulcers, Cushing's ulcers due to head trauma, or drug-induced gastritis)<sup>[1]</sup>. Endoscopic evaluation has revealed that more than 75% of critically ill patients develop gross gastric lesions within 72 h of admission to the intensive care unit (ICU), and almost 100% of them among extremely critical patients<sup>[2]</sup>. However, only a minority of these ulcerations bleed. Indeed, overt bleeding (manifested as bloody nasogastric aspirate, hematemesis, or melena), is reported in 20% of patients without stress ulcer prophylaxis (SUP), while < 5% develop clinically significant bleeding (CSB), defined as overt bleeding with transfusion, hemodynamic instability, and/or the need for intervention<sup>[2]</sup>. These lesions usually remain superficial, and cause sub-epithelial hemorrhages<sup>[3]</sup>. The occurrence of significant bleeding usually indicates a breach of the submucosa and the development of a true ulcer, and it is associated with increased mortality (RR = 2.9, 95% CI = 1.6-5.5), and significant morbidity (increased ICU stay of 6.2 d, 95% CI = 1.0-11.4 d)<sup>[4]</sup>.

It is well known that the most important factor in any gastric ulcer formation is the disruption of balance between gastric acid and gastric wall protective mechanisms, and stress ulcers are no exception. In critically ill patients, activation of sympathetic nervous system, increased catecholamine release and vasoconstriction, hypovolemia, decreased cardiac output, and release of proinflammatory cytokines result in splanchnic hypoperfusion<sup>[5]</sup>. Subsequently, this hypoperfusion leads to a number of deleterious effects including, ischemic damage to the gastric wall integrity, bicarbonate secretion, gastric hypomotility resulting in delayed emptying of acid, delayed mucosal healing, and reperfusion injury after restoration of splanchnic circulation<sup>[5]</sup>. In addition, these effects make the gastric wall vulnerable to damage and ulceration by acid, even if it is within a "normal" pH range.

The compelling morbidity and mortality of gastric ulcers have entailed a prompt action to prevent them among critically ill patients. Acid suppressive therapies seemed to be reasonable options after showing efficacy in decreasing the rate of overt and clinically important bleeding<sup>[6,7]</sup>. They rapidly became a standard critical care practice. A survey of 58 ICUs in North America, mainly in university teaching hospitals, revealed that 84% of patients admitted to the ICUs received SUP, with proton pump inhibitors being the most commonly used agents<sup>[8]</sup>. It also seems that different proton pump inhibitors (PPIs) have similar efficacies in this clinical setting. Messori *et al*<sup>[9]</sup> conducted a meta-analysis and equivalence testing to assess the

difference between intravenous omeprazole and pantoprazole, and they found that these two agents are equivalent according to reasonable equivalence margins. Moreover, several studies have shown that oral and intravenous routes of PPIs administration showed comparable efficacies in suppressing gastric acid secretion<sup>[10-12]</sup>.

However, later clinical evidence had shown that this therapeutic and prophylactic intervention failed to improve the overall clinical outcomes, and a wide debate has started. Ben-Menachem *et al*<sup>[13]</sup> found that SUP with histamine receptor 2 (H2) antagonists neither improved all-cause mortality nor reduced length of stay in ICU patients, when compared to placebo or no intervention. Likewise, Kantorova *et al*<sup>[14]</sup> studied the effect of proton pump inhibitors and H2 antagonists as SUP therapies and found no statistically significant difference in length of ICU stay or mortality. Moreover, the routine use of acid suppressive therapies not only might not improve clinical outcomes, but rather has been reported to cause a variety of potential effects. Gastric acid, in addition to its role in digestion, serves as an important sterilizer of the stomach, and its suppression leads to bacterial and fungal overgrowth and predisposes to nosocomial infections<sup>[15]</sup>. A large prospective study enrolling 63878 in-hospital non-ventilated patients found that acid suppressive therapies were associated with a 30% increase in hospital acquired pneumonia<sup>[16]</sup>. Similarly, several clinical studies have found that acid suppressive therapies are associated with increased risk of *Clostridioides difficile* (*C. difficile*) diarrhea Leonard *et al*<sup>[17]</sup> in a meta-analysis evaluating 2948 patients, revealed a statistically significant association between both PPI and H2 antagonists use and hospital-acquired *C. difficile* infection (OR 1.95, 95%CI: 1.48–2.58). However, this association with *C. difficile* was not established in a larger meta-analysis that enrolled 70862 patients with sepsis, and found no statistically significant correlation between *C. difficile* infection and SUP<sup>[18]</sup>. This conclusion can be reasonable, especially as *C. difficile* spores, are acid resistant and remain viable at gastric pH<sup>[19]</sup>. In addition, the cost of treatment should be considered as well. In one study, the use of acid suppressive therapy in ICU patients showed a cost of 8026 US Dollars per patient<sup>[20]</sup>. Given the number of ICU admissions in the United States alone (5.6 million ICU admissions in 2011), this constitutes an unnecessary heavy economic burden, had this preventive measure been ineffective<sup>[21]</sup>.

A recent multi-center phase IV randomized clinical trial (SUP-ICU) that involved 3298 patients in multiple European countries revealed that intravenous pantoprazole-receiving patients in the ICU were similar to the placebo group in 90-d mortality, rate of *C. Difficile* infection, rate of pneumonia, and surprisingly even in rate of CSB<sup>[22]</sup>. However, the study had several limitations, such as clinical (rather than endoscopic) diagnosis of stress ulcer, limited power to detect differences in the subgroup analyses, and more importantly, the study was powered to detect an absolute mortality reduction of 5%, which is quite high and generally implausible in critical patients<sup>[23]</sup>. Moreover, the incidence of clinically important bleeding in critically ill patients has been reported to be as low as 2.6% in a large multicenter prospective study, further confirming that absolute reduction of mortality of 5% is unreasonable<sup>[24]</sup>.

The large number of conflicting studies make it difficult to draw a simple conclusion regarding the effectiveness of this preventive therapy; therefore, systematic reviews would be suitable means to provide the answer. Several systematic reviews have been conducted. A recent Cochrane systematic review and meta-analysis that included 129 clinical studies, found that SUP interventions, compared to placebo or no intervention, in general decreased the rate of clinically important bleeding, but did not have any effect in the risk of nosocomial pneumonia, all-cause mortality, length of ICU stay, or length of intubation<sup>[25]</sup>. It also concluded that PPIs were superior to H2 antagonists by decreasing CSB.

Another systematic review and network meta-analysis by Alhazzani *et al*<sup>[26]</sup> showed that PPIs are the most effective agents to decrease CSB, followed by H2 antagonists. However, they found that neither PPIs nor H2 antagonists did improve the all-cause mortality or the risk of pneumonia compared to placebo or no prophylaxis<sup>[26]</sup>. Furthermore, a very recent systematic review with meta-analysis and trial sequential analysis (TSA) included 41 trials (included the previously described SUP-ICU) further confirmed previous results, that SUP decreases GI bleeding (overt and clinically important) but not all-cause mortality. However, TSA showed that the evidence of reduction of overt GI bleeding (but not clinically important) was firm. In addition, TSA showed that the required information size to assess the risks of pneumonia and *C. difficile* enteritis had not been reached, therefore, establishing relations is not plausible yet.

Other options have also been sought to prevent stress ulcers. To avoid disrupting the protective mechanism of the acid, ulcer-protective agents without altering gastric pH have been suggested as SUP. Sucralfate, for example, binds to gastric ulcers and erosions and protects them from damaging effects of gastric acid. López-Herce *et al*<sup>[27]</sup>

found that sucralfate had similar effect to H2 antagonists in decreasing the rate of clinically important bleeding in pediatric patients admitted to ICU ( $P < 0.01$ ), when compared to placebo. However, this option didn't hold up enough. Another clinical study, comparing sucralfate to no intervention in patients with head trauma, did not show any statistical difference in risk of gastrointestinal bleeding<sup>[28]</sup>. Moreover, Alhazzani *et al*<sup>[26]</sup> in their meta-analysis, among other studies, found that sucralfate did not improve the rate of clinically important bleeding when compared to placebo.

Although current level of evidence strongly suggests no improvement in all-cause mortality from SUP, some authors argued that cause-specific mortality reduction, rather than all-cause mortality reduction, should be considered when investigating preventive therapies<sup>[29]</sup>. Indeed, had all-cause (rather than cause-specific) mortality used as a measure to determine the efficacy of radiotherapy in the management of breast cancer (a valuable intervention with substantial improvement in overall survival), this intervention might never have been introduced, because initial all-cause mortality reduction was insignificant due equivalent deaths from cardiovascular complications of therapy, that were later abolished with subsequent modifications to radio beam which ensured less exposure of the heart and the major vessels to radiation<sup>[29]</sup>. Meanwhile, recent evidence-based improvements in critical care practice, such as optimal fluid resuscitation (improving splanchnic hypoperfusion) and early provision of enteral feeding, have led to a substantial decrease in rate of CSB, and questions the efficacy of SUP to further decrease the risk of GI bleeding. In a systematic review, Huang and associates analyzed if SUP provided any protections to patients receiving enteral feeding and found no statistically significant difference in rate of gastrointestinal bleeding, mortality, *C. difficile* infection, length of ICU stay, and duration of mechanical ventilation<sup>[30]</sup>. Therefore, we believe that a large scale clinical trial comparing cause-specific mortality and morbidity between a group with early enteral feeding plus SUP to controls with early enteral feeding alone will adequately address this clinical issue. In a nutshell, the current data is neither satisfactory to prove the efficacy of this preventive measure nor to deny it, and until further evidence becomes available, it is at the discretion of the healthcare provider whether to administer SUP to critical patients.

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## Hepatic regeneration in Greek mythology

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### Abstract

The accurate knowledge of surgical anatomy, the amelioration of post-operative processes and the continuously increasing experience of surgeons nowadays allow the performance of severe hepatic operations (e.g., wide liver resections, liver transplantations, etc.). The success of these operations is even more assisted by the great regenerative ability of the liver. Greek mythology, being an important source of information on the beliefs, habits, and phenomena observed during antiquity, reveals that hepatic regeneration was well known to ancient Greeks and this natural ability was established in two tales: the tale of Prometheus and the tale of the Giant Tityus. The main concept of both tales, being the destruction and reconstitution of the liver, is almost the same. Both of the condemned were immortal and their liver regenerated in a night, providing thus, food for the eagles and eternal pain for the sufferers. In conclusion, the tales show that the regenerative ability of the liver was well known from early years and that the trust shown by the Gods in this ability, to cause eternal suffering is on a par with the trust shown by the modern surgeons in it, to assure a successful hepatic operation.

**Key words:** Liver regeneration, Prometheus, Tityus; Greek; Mythology

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**Core tip:** Greek mythology is an important source of information on the beliefs, habits, and phenomena observed during antiquity revealing that hepatic regeneration was well known to ancient Greeks. This natural ability was established in two tales whose main concept was the destruction and reconstitution of the liver: the tale of Prometheus and the tale of the Giant Tityus.

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## INTRODUCTION

The capacity of the liver to fully restore itself after significant hepatic tissue loss either from partial hepatectomy or acute liver injury has been long recognized<sup>[1]</sup>.

The existence of hepatic stem or progenitor cells has been controversial for decades, though it has been presumed that if such cells existed, they would lie within the liver. There is now consensus, however, that not only do facultative hepatic stem cells exist within the liver, but also that cells from extra-hepatic sites, in particular the bone marrow, can contribute to hepatocyte and cholangiocyte regeneration<sup>[2]</sup>. Despite confidence that engraftment of marrow cells in the liver occurs, the mechanical details of this process remain poorly understood<sup>[3]</sup>.

Greek mythology mentions two cases of hepatic regeneration: the myth of Prometheus and the myth of the Giant Tityus. In both myths, the liver was totally destroyed during the day and fully restored during the night.

## MYTH OF PROMETHEUS

According to Hesiod's *Theogony*<sup>[4]</sup>, Prometheus was a Titan, an order of divinities existing before Zeus and thus called the "old Gods." Zeus himself had gained his power with the help and advice of Prometheus. Prometheus, whose name means "forethought," had qualities that made him a master craftsman and creator of mankind. Proud of his creation, he decided to make man perfect, a task that required mankind's possession of fire.

According to legend, after the creation of mankind, the mortals and immortals were gathered for a feast. Prometheus tricked Zeus into eating the less desirable part of the meal, giving the best part to man. In anger, Zeus revoked man's privilege of using fire. Prometheus, ignoring Zeus, stole the fire and gave it back to man. When Zeus discovered the theft, he ordered Hephaestus to seize Prometheus and chain him on a rock on Mount Caucasus. Zeus then sent an eagle to eat his liver. The bird would eat the liver every day and the organ would grow back every night. This punishment lasted for thirteen generations, ending only when Hercules killed the eagle with his arrows.

## MYTH OF THE GIANT TITYUS

The Giant Tityus was the son of Zeus and Elara. It was said that while he was a fetus, his extreme growth caused the death of his mother and instead, Earth gave birth to him with the aid of Zeus. When Tityus grew up, he attacked Leto, mother of Apollo and Artemis, while she headed to Delphi, and violently abducted her. According to Pausanias, Leto's daughter Artemis or Leto's son Apollo sent the Giant Tityus to Hades with their arrows<sup>[5]</sup>.

Homer in the *Odyssey* describes the Giant's bearing in Hades: he lay wounded on the ground, his body being 900 feet long. Two vultures feasted every day on his liver which then grew back during the night<sup>[6]</sup>.

## DISCUSSION

In *Theogony*, Prometheus' liver is immortal not only because Prometheus himself is immortal, but also because the liver is considered the seat of the soul and intelligence. This connection enhances the suffering of both Prometheus and Tityus which is primarily psychic. The pain is aggravated by the temporal dimension and daily repetition of the punishment.

The fact that the liver is the organ attacked in both cases is in all cases bizarre, though the practice of inspection of the viscera could have been the source of information about the abilities of the organ. Nevertheless, even this superficial knowledge in such an early period is admirable. If in ancient mythology the regenerative power of the liver appears as a means of additional punishment, in modern medicine this power is a gift promoting the patient's chances of survival.

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## Prospects for immunotherapy as a novel therapeutic strategy against hepatocellular carcinoma

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### Abstract

Hepatocellular carcinoma (HCC) is a highly aggressive malignant disease, with a poor clinical prognosis. Many standard therapies are often considered for HCC treatment today; however, these conventional therapies often fail to achieve sufficiently effective clinical results. Today, HCC therapy is set to undergo a major revolution, owing to rapid developments in cancer immunotherapy, particularly immune checkpoint inhibitor therapy. Cancer immunotherapy is a novel and promising treatment strategy that differs significantly from conventional therapies in its approach to achieve antitumor effects. In fact, many cancer immunotherapies have been tested worldwide and shown to be effective against various types of cancer; HCC is no exception to this trend. For example, we identified a specific cancer antigen called glypican-3 (GPC3) and performed clinical trials of GPC3-targeted peptide vaccine immunotherapy in patients with HCC. Here, we present an overview of the immune mechanisms for development and progression of HCC, our GPC3-based immunotherapy, and immune checkpoint inhibitor therapy against HCC. Finally, we discuss the future prospects of cancer immunotherapy against HCC. We believe that this review and discussion of cancer immunotherapy against HCC could stimulate more interest in this promising strategy for cancer therapy and help in its further development.

**Key words:** Hepatocellular carcinoma; Cancer immunotherapy; Immune checkpoint inhibitor; Glypican-3; Cancer vaccine; Clinical trials; Cytotoxic T-lymphocytes

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**Core tip:** Hepatocellular carcinoma (HCC) is a highly aggressive malignant disease, with a poor prognosis. Recent developments and advances in cancer immunotherapy, particularly immune checkpoint inhibitor therapy, could lead to a major paradigm shift in standard HCC therapy. This review aims to provide an overview of novel immunotherapies, including antigen-based immunotherapies such as glypican-3-targeted immunotherapy, and immune checkpoint inhibitor therapy against HCC. It also discusses the future prospects of cancer immunotherapy against HCC.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related death<sup>[1]</sup>. Approximately 750000 people are affected and about 700000 result in death in worldwide every year; the incidence is particularly high in Asia and Africa<sup>[2,3]</sup>. Infections with hepatitis B virus (HBV) and hepatitis C virus (HCV), which induce the formation of chronic inflammatory microenvironments in the liver, are considered major risk factors for HCC<sup>[4]</sup>. Other factors, including alcohol intake, obesity, diabetes, and exposure to aflatoxin, have also been implicated in the cause and progression of HCC<sup>[5]</sup>. Early detection can ensure better clinical results for patients with HCC, as evidenced by the high 5-year survival rate (more than 70%) for early-stage HCC patients<sup>[6]</sup>. In contrast, most late-stage HCC patients show poor prognosis, with a 5-year survival rate of less than 16%<sup>[6,7]</sup>. One of the causes of making early diagnosis of HCC difficult is the existence of background liver of patients including chronic liver inflammation and cirrhosis, which makes it difficult to obtain clear images from ultrasonography, computerized tomography (CT), and magnetic resonance imaging (MRI). Furthermore, highly accurate biomarkers for early-stage HCC detection have not yet been established<sup>[8]</sup>. Also, patients with HCC have higher recurrence rates than those with other solid cancers; as HCC is initiated from injured hepatocytes, even after tumor removal, the patient has a high risk of recurrence as long as the background liver disease persists. Therefore, these above factors make HCC one of the most aggressive diseases, with poor survival prognosis.

Currently, there are various options for HCC therapy, depending on the clinical stage. Surgical hepatic resection and radiofrequency ablation therapy (RFA) is considered as ideal for early-stage HCC patients, who have adequate liver function and no evidence of portal hypertension or vascular invasion<sup>[9-13]</sup>. For early-stage HCC patients with relatively poor liver function, liver transplantation is an effective therapeutic procedure<sup>[14]</sup>. Also, many reports have demonstrated the effectiveness of RFA and transarterial embolization (TAE) for HCC patients. However, these conventional strategies are limited by considerations of tumor size, number of intrahepatic metastases, and adequate hepatic reserve capacity; they are therefore unsuitable for many patients<sup>[15,16]</sup>. Meanwhile, for patients with advanced HCC, transcatheter arterial chemoembolization and molecular targeted drugs have been conducted. Systemic chemotherapy, which is other treatment method, and has been reported to show a high frequency of adverse events and strong tolerance, with poor clinical effectivity<sup>[17]</sup>. Molecular targeted therapy, one of the more modern strategies, is based on specific molecular attributes of cancer types. Sorafenib, an inhibitor of tyrosine kinase, is the first molecular targeted drug against HCC that is approved by the Food and Drug Administration (FDA). Indeed, in a phase-III clinical trial for advanced HCC, patients who received sorafenib had better overall survival (OS) than placebo-treated patients (10.7 mo *vs* 7.9 mo)<sup>[18-21]</sup>. However, because of its low response rate for HCC patients and the relatively high incidence of adverse events, sorafenib may not be the most suitable strategy for HCC therapy<sup>[18-20,22,23]</sup>. Today, the development of other molecular targeted drugs is under way<sup>[24,25]</sup>. Indeed, as novel molecular targeted drug against HCC, Regorafenib and Lenvatinib were approved by the FDA in April 2017 and August 2018, respectively. In any case, the development of a new therapeutic strategy of HCC with adequate antitumor effect and few adverse events would be urgent<sup>[26,27]</sup>.

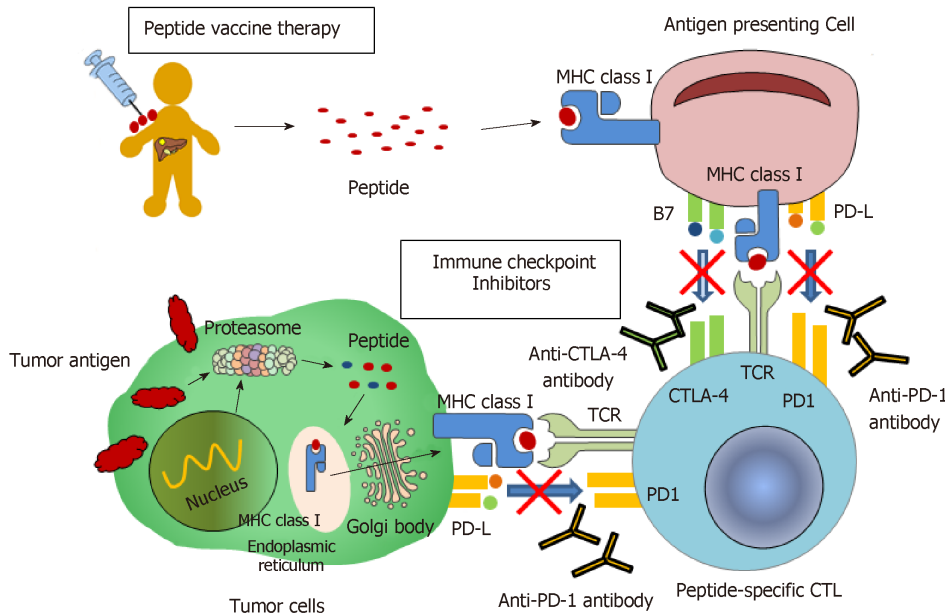
In recent years, immunotherapy has attracted a lot of attention from both basic scientists and clinicians as a promising new method of cancer therapy. It has been demonstrated that several immunotherapies have high antitumor effects against various cancer types, including malignant melanomas and hematological malignancies<sup>[28,29]</sup>. Also, immunotherapy could theoretically be ideal for HCC therapy, as: (1) it exerts antitumor effects through mechanisms different from those of existing therapies; and (2) it produces recurrence prevention effects along with curative effects<sup>[30-32]</sup>. Indeed, immunotherapy against HCC has been studied for decades<sup>[33,34]</sup>, and many clinical trials have been performed on HCC patients<sup>[35,36]</sup>. Several randomized controlled trials have established the use of immunotherapy as an adjuvant therapy to reduce the risk of cancer recurrence<sup>[37-39]</sup>. Therefore, it is almost certain that immunotherapy will be one of the major options for HCC therapy in the near future. Here, we introduce novel immunotherapeutic strategies for HCC therapy, including immune checkpoint inhibitor therapy; we also elaborate on immunotherapy using GPC3, which is a cancer-specific antigen we identified (Figure 1). We believe this review could awaken more interest in immunotherapy for HCC, which would help improve the survival prognosis of HCC patients.

## IMMUNE RESPONSE AND IMMUNE ESCAPE IN HCC

The liver has a role as a lymphatic system of the whole body, and HCC occurring in these tissues often has properties different from other cancers. Commonly, most HCC patients have a background of chronic hepatitis or liver cirrhosis. Liver cirrhosis is often a highly genotoxic environment with persistent inflammation and fibrosis, which could promote the onset of HCC. Generally, HCC is a malignant disease induced by inflammation; the carcinogenesis of HCC usually involves DNA oxidative injury accumulated under a sustained inflammatory environment caused by HBV, HCV, or other factors.

During the development and progression of HCC, patients show a unique anti- or pro-tumor response<sup>[29]</sup>. Previously, it had been reported that patients with HCC showed spontaneous T-cell response to many tumor antigens, including alpha fetoprotein (AFP), glypican-3 (GPC3), NY-ESO-1, SSX-2, MAGE-A-10, and p53<sup>[40-46]</sup>. However, the anti-tumor effects of these immune responses are not sufficient to cause tumor regression or inhibit disease progression. Several mechanisms have been proposed to explain this phenomenon. First, there would be a change in the expression levels of major histocompatibility complex (MHC) class I, which plays a role in antigen presentation to cytotoxic T-lymphocytes (CTLs). Indeed, the downregulation of MHC class I has been reported in many advanced cancers, including HCC<sup>[47-49]</sup>. However, changes in the expression levels of HLA class I have not been consistent in previous studies, and remain unclear. In addition, a decrease in the expression of co-stimulatory molecules B7-1 and B7-2 has been reported in HCC<sup>[50]</sup>. Second, there would be excessive activation of immunosuppressive cells, including regulatory T-cells (Tregs) and bone marrow-derived suppressor cells (MDSCs)<sup>[51]</sup>. Tregs are the most well-known players in cancer evasion from immunosurveillance. In fact, Tregs have been shown to suppress anti-tumor immunity<sup>[52,53]</sup>. Patients with HCC have increased number of Treg in peripheral blood mononuclear cells (PBMCs) and tumor-infiltrating lymphocytes (TILs), which is correlated with disease progression and poor prognosis<sup>[54-56]</sup>. MDSCs are the heterogeneous cell population of early bone marrow progenitor cells; they suppress the cytotoxic activity of NK cells and adaptive immune responses mediated by CD4<sup>+</sup> and CD8<sup>+</sup> T-cells. Specifically, MDSCs have been reported to induce the production of Foxp3 and interleukin-10 in CD4<sup>+</sup> T-cells through arginase activity; they also suppress T-cell function through the induction of Tregs. In addition, the frequency of MDSCs in the peripheral blood is positively correlated with the risk of recurrence of HCC in patients who received RFA therapy<sup>[57]</sup>. Third, there is the presence of immune checkpoint factors. It has been shown that, due to escape from immune monitoring, especially from specific T-cell responses for tumor antigens, several immune checkpoint pathways could be actively exploited by tumors. Indeed, some immune checkpoint molecules such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death-1 (PD-1) have been detected in the tumor microenvironment and are often overexpressed<sup>[58-61]</sup>. Recent years have seen a rapid advance in research on these immune checkpoint molecules. In some cancers, particularly malignant melanomas, inhibitors of immune checkpoint molecules have been shown to cause a major antitumor effect. Today, inhibitors targeting immune checkpoint molecules are being extensively studied worldwide. Finally, persistent infection of the liver could lead to immune escape mechanisms. It is well known that





**Figure 1 A scheme of mechanisms of peptide vaccine therapy and immune checkpoint inhibition therapy.** In peptide vaccine therapy, a injected tumor-associated peptides induce peptide-specific cytotoxic T-lymphocyte via antigen presenting cells, with resulting in an antitumor effect on cancer cells. Immune checkpoint inhibition therapy could result in anti-tumor effects by inhibiting the mechanism that negatively suppresses the immune response to tumor cells; anti-CTLA-4 antibody and anti-PD-1 antibody play a role of blocking the PD-1/PDL1 and CTLA4/B7 pathways, respectively. MHC: Major histocompatibility complex; CTLA-4: Cytotoxic T-lymphocyte-associated antigen 4; PD-1: Programmed cell death-1; CTL: Cytotoxic T-lymphocyte.

NK cells, which kill cancer cells by recognizing them as “non-self,” can inhibit the progression of cancer. A recent report showed increased blood levels of MHC class I-related chain A, which is involved in the recognition of cancer cells by NK cells, during the progression of chronic liver disease; this attenuates the NKG2D-mediated cytotoxic activity of NK cells, which could contribute to the development of liver cancer<sup>[62,63]</sup>. In addition, the presence of the unique liver cells such as sinusoidal endothelial cells and Kupffer cells (macrophages that act as antigen-presenting cells) is known to induce immune tolerance. Thus, hindering these immune escape mechanisms and promoting immune response with stronger antitumor effects could improve the clinical efficiency of cancer therapies.

Recent studies have focused on immune responses in patients who received existing conventional therapies for HCC. Briefly, anti-cancer immune response induced by tumor-specific T-cells have been confirmed in HCC patients treated with RFA and TAE<sup>[64-67]</sup>. In addition, the frequency of natural killer T-cells (NKT cells), which have anti-tumor effect, has been reported to increase in the peripheral blood after RFA treatment<sup>[68]</sup>. These results suggest that traditional therapies for HCC are, at least in some cases, involved in immune responses; this opens up the possibility of combining with novel immunotherapy.

## CANCER IMMUNOTHERAPY TARGETING GPC3

### GPC3

In 2001, we identified GPC3, a cancer-specific antigen expressed in some cancer cells, from tens of thousands of genes collected using cDNA microarray against several cancer tissues, several surrounding non-cancer tissues, and various normal tissues<sup>[69]</sup>. GPC3, a 65-kDa protein comprising 580 amino acids, is a heparan sulfate proteoglycan bound to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. It is hardly expressed in any normal tissue, except in the embryonic liver, embryonic kidney, placenta, and parts of the kidney tubules. However, it is expressed in tissues of HCC, ovarian clear cell carcinoma, malignant melanoma, lung squamous cell carcinoma, hepatoblastoma, Wilms tumor, and AFP-producing gastric cancer, but not in other cancer tissues. There are two types of GPC3, a membrane-bound type and a secreted type, but it is not clear how these types influence the development and progression of cancer. In HCC, they are known to be involved in neoplastic transformation<sup>[70]</sup>. The expression of GPC3 in HCC has also been reported to be associated with clinical prognosis<sup>[71]</sup>. We had evaluated the relationship between



GPC3 expression in HCC and long-term prognosis in 33 HCC patients who underwent radical surgery (in preparation). The 5-year survival rates were 42.9% and 83.3% in GPC3-positive (21 cases) and -negative (12 cases) patients, respectively. Thus, GPC3-negative patients had significantly better OS than GPC3-positive patients (log-rank test,  $P = 0.02$ ). This result was also consistent with that of a previous report<sup>[71]</sup>. With such high cancer-specificity, GPC3 could be an ideal target for immunotherapy against cancer. Nakano *et al.*<sup>[72]</sup> also independently identified GPC3 as a cancer-specific antigen almost at the same time as our group. They also developed an antibody therapy targeting GPC3, and are being progressed research using its antibody<sup>[72]</sup>. In addition, phase I/II clinical trials of GC33, a novel GPC-3 antibody, are ongoing under world scale in patients with advanced HCC (Table 1)<sup>[73,74]</sup>. We also showed that GPC3-derived peptides could restrict HLA-A24 and HLA-A2, thus inducing peptide-specific CTLs<sup>[43,75]</sup>. HLA-A24 is present in approximately 60% of the Japanese population, while HLA-A2 is present in 40% of the Japanese population and is also a major haplotype in the Caucasian population<sup>[43,75]</sup>. Further, we performed clinical trials for a peptide vaccine therapy using HLA-A24- and HLA-A2-restricted GPC3-derived peptides (Table 1)<sup>[76]</sup>. Here, we present the results of these trials and our attempts to develop novel immunotherapies using GPC-3.

### **Clinical trials using GPC3 peptide vaccine against HCC**

**Phase I clinical trial of GPC3 peptide vaccine for advanced HCC:** A Phase I clinical trial for a GPC3 peptide vaccine was performed between February 2007 and November 2009 in 33 patients with advanced HCC at the National Cancer Research Center East Hospital (Kashiwa, Japan) (UMIN Clinical Trials Registry: 000001395)<sup>[29,77]</sup>. The primary endpoint was the safety of the GPC3 vaccine and the immune response against it. No dose-limiting toxicity was observed in any of the enrolled patients, and the GPC3 vaccine showed high tolerability. In addition, IFN- $\gamma$  ELISPOT assay revealed that the GPC3 vaccine induced adequate number of GPC3 peptide-specific CTLs in 30 (90.1%) out of 33 patients. Also, the disease control rate (DCR) was 60.6% after 2 months after administration of vaccine, and the median time to progression (TTP) and OS was 3.4 and 9.0 mo, respectively. We also demonstrated that GPC3 vaccination could induce immunological responses, including a decrease in tumor markers and an increase in GPC3 peptide-specific CTLs in peripheral blood. In addition, we evaluated the immunological changes before and after vaccination using biopsy specimens of the tumor. We observed that more CTLs had infiltrated into tumor in the post-vaccination than the pre-vaccination, which proved that the vaccination caused immunological effects<sup>[29,77]</sup>.

In a subsequent phase I trial, we investigated the extent of CTL infiltration into the PBMCs and tumors in 11 patients with advanced HCC who had undergone GPC3 vaccination and were resistant to sorafenib treatment (UMIN Clinical Trials Registry: 000005093)<sup>[78]</sup>. We found that the number of GPC3-peptide specific CTLs in the PBMCs increased after GPC3 vaccination in 9 of 11 cases. In addition, tumor biopsy specimens after vaccination were obtained from 3 patients, and we observed infiltration of CTLs into tumors in all of them. These results confirmed that GPC3 vaccination could induce the infiltration of GPC3 peptide-specific CTLs into tumor. Remarkably, we observed a valuable case in which multiple HCC tissues became inflamed and then necrotic, after 2 times injections of the vaccine. This was a promising result implying that the peptide vaccine therapy could potentially obtain not only immunological response, but also sufficient clinical antitumor effect<sup>[79]</sup>. We also established several GPC3 peptide-specific CTL clones from tumor biopsy specimens collected after the vaccination<sup>[78]</sup>.

**Phase II clinical trial of GPC3 peptide vaccine as a recurrence preventive effect in HCC patients after radical treatment:** To evaluate the efficiency of our peptide vaccine in preventing recurrence, we performed a single-arm phase II clinical trial for the GPC3 peptide vaccine as an adjuvant therapy; the trial was performed in 35 patients with HCC after radical treatment (UMIN Clinical Trials Registry: 000002614)<sup>[80]</sup>. We found that the 1 - and 2-year recurrence rates (the primary endpoints in the trial) in the enrolled patients were 24.4% and 53.7%, respectively. This result showed that the GPC3 peptide vaccine could be useful as adjunctive therapy for HCC after radical therapy. Also, we evaluated the long-term survival prognosis in the enrolled patients. The median PFS and OS were 20.4 and 72.8 mo, respectively, while the 5-year survival rate was 70.6% (in preparation). In addition, the two cases showed recurrence, despite increased peptide-specific CTLs in the peripheral blood after the vaccination. In these HCCs, GPC3 was mostly expressed in the primary tumor before the vaccination; however, its expression was almost absent in the recurrent tumor after vaccination<sup>[80]</sup>. These results indicate that peptide vaccine therapy targeting tumor-associated antigens could eradicate cancer cells expressing the antigen, but might be ineffective

**Table 1 Review of clinical trials of immune checkpoint inhibitor therapy and glypican-3-based immunotherapy in patients with hepatocellular carcinoma**

Immunotherapy	Title	Trial no.	Phase	n	Primary endpoint	Result	Status	Ref.
<b>GPC3-based immunotherapy</b>								
<b>GPC3 peptide vaccination</b>								
HLA-A 24:02-restricted GPC3 <sub>298-306</sub> peptide vaccine, and HLA-A 02:01-restricted GPC3 <sub>144-152</sub> peptide vaccine	Phase I trial of a glypican-3-derived peptide vaccine for advanced HCC	UMIN000001395	I	33	The safety and immune response to GPC3 vaccination	Well-tolerated. The GPC3 vaccine induced a GPC3-specific CTL response in 90.1% patients (30/33)	Completed	[29]
HLA-A 24:02-restricted GPC3 <sub>298-306</sub> peptide vaccine, and HLA-A 02:01-restricted GPC3 <sub>144-152</sub> peptide vaccine	Immunological efficacy of glypican-3 peptide vaccine in patients with advanced HCC	UMIN000005093	I	11	The frequency of peptide-specific CD8 <sup>+</sup> T-cells in PBMCs and infiltration into the tumor after vaccination	The number of peptide-specific CD8 <sup>+</sup> T-cells in PBMCs increased in 9 out of 11 cases. In 3 cases, they infiltrated into the tumor after the vaccination	Completed	[78]
HLA-A 24:02-restricted GPC3 <sub>298-306</sub> peptide vaccine, and HLA-A 02:01-restricted GPC3 <sub>144-152</sub> peptide vaccine	Phase II study of the GPC3-derived peptide vaccine as an adjuvant therapy for HCC patients	UMIN000002614	II	35	The 1- and 2-y recurrence rate	The 1- and 2-yr recurrence rates were 24.4% and 53.7%, respectively	Completed	[80]
<b>Anti GPC3 antibody</b>								
GC33	First-in-man Phase I study of GC33, a novel recombinant antibody against GPC3, in patients with advanced HCC	NCT00746317	I	20	Tolerability and tumor response	Well-tolerated. The median TTP was 26.0 wk in patients with GPC3-high HCC	Completed	[73]
GC33	Japanese phase I study of GC33, a humanized antibody against GPC3 for advanced HCC	Japic CTI-101255	I	13	Determined maximum tolerated dose of GC13	Well-tolerated for GC33 dose of 20 mg/kg in Japanese patients with HCC	Completed	[74]
GC33	-	NCT01507168	II	185			Ongoing	-

Anti-GPC3 CAR-T based GC33	-	NCT0239525 0	I	13			Ongoing	-
<b>Immune checkpoint inhibitor therapy</b>								
<b>Anti-PD-1 antibody</b>								
Nivolumab	Nivolumab in patients with advanced HCC: an open-label, noncompara tive, phase 1/2 dose escalation and expansion trial	NCT0165887 8	I / II	262	Safety, tolerability, and clinical efficacy, including ORR, DCR, DOR, and PFS	ORR was 20%. DCR was 64% (CR and PR; 3 and 39 cases). The median DOR and PFS was 9.9 and 4.0 mo, respectively	Completed	[94]
Nivolumab	-	NCT0257650 9	III	726			Ongoing	-
Pembrolizu mab	Pembrolizu mab in patients with advanced HCC previously treated with sorafenib: non- randomised, open-label phase 2 trial	NCT0270241 4	II	104	Clinical efficacy, including ORR, DCR, DOR, and PFS	ORR was 16.3%. DCR was 61.5% (CR and PR; 1 and 16 cases). The median DOR and PFS were 2.1 and 4.8 mo, respectively	Completed	[96]
Pembrolizu mab	-	NCT0270240 1	III	408			Ongoing	-
Pembrolizu mab (with Lenvatinib)	-	NCT0300692 6	I	104			Ongoing	-
<b>Anti-CTLA-4 antibody</b>								
Tremelimu mab	A clinical trial of CTLA-4 blockade with tremelimum ab in patients with HCC and chronic hepatitis C	NCT0100835 8	II	21	Clinical efficacy, including ORR, TTP, and OS	PR ( <i>n</i> = 3) and SD ( <i>n</i> = 10) rate were 17.6% and 58.8%, respectively. The median TTP and OS were 6.48 and 8.2 mo, respectively	Completed	[97]
Tremelimu mab (with RFA or TAE)	Tremelimu mab in combination with ablation in patients with advanced HCC	NCT0185361 8	II	32	Clinical efficacy as adjuvant therapy after RFA or TAE	PR rate was 26%. The median TTP and OS were 7.4 and 12.3 mo, respectively	Completed	[101]
Tremelimu mab (with Durvalumab)	-	NCT0251934 8	II	144			Ongoing	-

GPC3: Glypican-3; CAR-T: Chimeric antigen receptor modified T cells; PD-1: Programmed cell death-1; CTLA-4: Cytotoxic T-lymphocyte-associated protein-4; RFA: Radiofrequency ablation; TAE: Transcatheter arterial embolization; HCC: Hepatocellular carcinoma; ORR: Objective response rate; DCR: Disease control rate; DOR: Duration of response; PFS: Progression free survival; TTP: Time to progression; OS: Overall survival; PBMC: Peripheral blood mononuclear cell; CR: Complete response; PR: Partial response; SD: Stable disease; CTL: Cytotoxic T lymphocyte.

against cancer cells that do not express the antigen or those that have lost antigen expression. Therefore, vaccine therapies targeting multiple cancer-related antigens will be key for future research in this field; combination of immunotherapies with other therapies should also be explored.

**Novel approach for enhancing the antitumor effect of peptide vaccine therapy**

As mentioned above, although we could show the safety and immunological response of the peptide vaccine, its anti-tumor effect remains limited. Even if an excellent peptide vaccine could sufficiently induce peptide-specific CTLs, the anti-tumor effect would depend critically on the number of peptides presented to HLA class I molecules on the surface of cancer cells. To improve this defect, we developed an intra-tumor peptide injection therapy, where the peptide vaccine is directly administered into tumor tissues<sup>[81]</sup>. Direct injection into the tumor allows a sufficient number of peptides to be presented to HLA class I molecules on the tumor cells, thus enhancing the killing capacity of peptide-specific CTLs. In addition, the peptide vaccine itself could induce the infiltration of peptide-specific CTLs into the tumor. Indeed, we showed that intra-tumor peptide injection therapy effectively suppressed tumor growth in a tumor-transplanted mouse model. We also showed that the combination of subcutaneous and intra-tumor injection of the peptide vaccine resulted in higher anti-tumor effect than either of the therapy.

We also found that combining the peptide vaccine with anti-PD-1 antibody<sup>[82]</sup> or anti-CD4 antibody<sup>[83]</sup> could enhance the induction of peptide-specific CTLs and result in higher antitumor effects than peptide vaccine therapy alone. The use of anti-CD4 antibodies could remove Tregs and weakly CD4-positive macrophages that suppress the positive immune response against cancers. We expect that these approaches would bring about a breakthrough for peptide vaccine therapy in the future.

**Development of T-cell receptor-engineered T-cell therapy and chimeric antigen receptor-transduced T-cell therapy targeting GPC3**

We had established multiple GPC3 peptide-specific CTL clones from the peripheral blood and cancer tissues of HCC patients vaccinated in the clinical trials<sup>[77,78,84]</sup>. Several of these CTL clones had high capability for killing cancer cells that express GPC3 peptides. In collaboration with Mizukoshi *et al.*<sup>[64]</sup>, we generated induced pluripotent stem cell-derived T-cells transduced with GPC3-specific T-cell receptors (TCRs), which were derived from the most suitable of our CTL clones. TCR-engineered T-cell therapy has been said to be superior to peptide vaccine therapy in terms of safety and anti-tumor effect. Thus, TCR-engineered T-cell therapy based on GPC3 might be a promising novel therapy for patients with advanced HCC in the future.

It was recently shown that chimeric antigen receptor (CAR)-transduced T-cell therapy was remarkably effective against blood malignant disease, with a clinical response rate of more than 80%. However, its efficacy against solid cancers has not been established. Phase I clinical trials of anti-GPC3 CAR-modified T-cells based on GPC3 are currently underway in China for patients with refractory or relapsed GPC3<sup>+</sup> HCC<sup>[85]</sup>. In collaboration with Ishida *et al.*<sup>[86]</sup>, we are also developing a next-generation CAR-transduced T-cell therapy based on a novel GPC3 antibody. To overcome the disadvantages of the conventional methods of this therapy, we combined FITC-conjugated cancer-specific antibodies with CAR-transduced T-cells that react with FITC. This approach allows us to precisely control the cancer-killing ability of CAR-transduced T-cells by adjusting the dose of FITC-conjugated antibodies; this also helps in the survival of CAR-transduced T-cells.

These studies continue to help us strive toward the goal of establishing a practical clinical application for TCR-engineered T-cells and CAR-transduced T-cells for treating solid cancers.

**Development of serum full-length GPC3 as biomarkers for peptide-based immunotherapy**

Previous studies have shown that secreted GPC3 is released into the serum in the patients with HCC. Recently, we developed an assay to quantify serum full-length GPC3 in cooperation with a private company. This assay could measure serum GPC3, which could be useful as a biomarker for early diagnosis, prediction of recurrence, and evaluation of the effect of anti-GPC3 therapy against HCC. In fact, we showed that the vaccinated group ( $n = 9$ ) had better PFS and OS than the non-vaccinated group ( $n = 12$ ) in GPC3-positive HCC patients who had high GPC concentration in the peripheral blood after radical surgery (log-rank test,  $P = 0.075$ ,  $P < 0.01$ , respectively) (in preparation). This result suggested that serum GPC3 concentration after radical surgery could be a useful biomarker for predicting the clinical effect of GPC3 vaccination.

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**IMMUNE CHECKPOINT INHIBITORS**

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The use of immune checkpoint inhibitors such as anti-PD-1 and anti-CTLA-4

antibodies has attracted attention as a novel cancer therapy that can provide dramatic and long-term anti-tumor effect through an approach that is different to that of conventional therapies. In 1992, Ishida *et al*<sup>[86]</sup> found that the expression of PD-1 was elevated when T-cells underwent apoptosis; after then, they clarified that PD-1 is a receptor that negatively regulates the immune response against cancer cells. In addition, blocking the PD-1/PD-L1 pathway has been shown to cause an anti-tumor effect by excluding the tumor-induced immune suppressive system and promoting the immune response against tumors.

In 1995, Krummel *et al*<sup>[87]</sup> found that CTLA-4 was essential for self-tolerance in immunity; its deficiency caused serious autoimmune diseases. In addition, blocking the interaction between CTLA-4 and B7-1/B7-2 induced tumor rejection in mice<sup>[88]</sup>. These immune checkpoint inhibitor therapies could be another promising option for cancer therapy that ensures safe and efficient therapeutic effects against cancers that are resistant to conventional chemotherapy and radiation therapy. In fact, nivolumab, an anti-PD-1 antibody, and ipilimumab, an anti-CTLA-4 antibody, were the first antibodies to be approved for use against advanced malignant melanomas resistant to conventional chemotherapy and radiation therapy. Since then, based on clinical trials on patients with various types of cancer, these therapies have gradually expanded their application for several cancers<sup>[89-91]</sup>. However, the response rate for these therapies are still low; for malignant melanomas, excluding Hodgkin's lymphoma, it was near 30%, while for other cancers, it ranges from 10% to 30%. Thus, despite many favorable signs, these therapies still benefit only a limited number of patients. Therefore, there is an urgent need to establish appropriate biomarkers for predicting the clinical response of patients against immune checkpoint inhibitors. Number of mutations in the patient has been considered as biomarkers to predict the clinical efficacy of these immunotherapies. Also, it has been reported that PD-L1 expression levels could be involved in the clinical effect of immunotherapy in patients with non-small cell lung cancer, but these relevance has not been reported in those with HCC. In May 2017, pembrolizumab was approved by the FDA for patients with malignant diseases who showed high microsatellite instability or incomplete mismatch repair; this was the first approval in which genetic abnormalities, and not the type of cancer, was the adaptation condition<sup>[92,93]</sup>. Future studies should therefore focus on identifying biomarkers with better accuracy for predicting treatment effects, and on establishing treatment-selective algorithms to use them.

In recent years, against HCC patients, clinical trials for immune checkpoint inhibitor therapy alone and in combination with other conventional therapies have been proposed (Table 1). Originally, there are many immune cells in the liver. In addition, chronic inflammation such as liver cirrhosis and viral hepatitis, which could be the host of HCC, could induce immunosuppression against HCC. These two mechanisms would imply that immune checkpoint inhibitors could have a sufficient therapeutic effect in patients with HCC. In the following sections, we introduce clinical trials for immune checkpoint inhibitor therapy in HCC patients. The development of immune checkpoint inhibitor therapies could trigger a major revolution in conventional hepatocarcinotherapy, and future developments in these therapies look promising.

### Anti-PD1 antibody

Nivolumab is the first humanized monoclonal IgG4 antibody against human PD-1. It has been approved for use against many types of cancer, including malignant melanoma, non-small cell lung cancer, renal cell cancer, and gastric cancer. A phase I/II clinical trial for the use of nivolumab against HCC was performed between November 2012 and August 2016 in patients with advanced HCC (Checkmate-040)<sup>[94]</sup>. The results of this trial confirmed the tolerability of nivolumab in HCC patients. In addition, the results showed an objective response rate (ORR) of 20% ( $n = 42/214$ ) and a DCR of 64% ( $n = 138/214$ ). The median duration of response and progression-free survival were 9.9 and 4.0 mo respectively, and OS rate at 9 mo was 74%. A subsequent result published in ASCO 2017 revealed that the median OS was 15.6 mo in 145 cases that were co-treated with sorafenib and 28.6 mo in 80 cases not given sorafenib<sup>[95]</sup>. In addition, nivolumab therapy had a sustained long-term clinical effect in patients who had partial response (PR) and stable disease (SD)<sup>[94]</sup>. Based on this promising result, the FDA approved nivolumab for patients with HCC who had previously been treated with sorafenib in September 2017. Currently, phase III clinical trials for the safety and clinical efficacy of the combination of nivolumab and sorafenib as a first-line standard therapy for patients with advanced HCC are being performed, and the results are awaited (Checkmate-459).

Pembrolizumab is another anti-PD1 antibody. Recently, phase II clinical trials for pembrolizumab were performed on 104 patients with advanced HCC who had received sorafenib treatment (KEYNOTE-224)<sup>[96]</sup>. The results showed 16.3% ORR and



61.5% DCR. The median duration of response and RFS were 2.1 and 4.8 mo, respectively, indicating that pembrolizumab was almost as effective as nivolumab. Currently, phase III clinical trials are underway for evaluating the efficacy of pembrolizumab as a secondary treatment after sorafenib (KEY NOTE-240).

### **Anti-CTLA-4 antibody**

CTLA-4 is a receptor on the cell membrane of T-cells that suppresses antigen presentation by dendritic cells. Treatment with anti-CTLA-4 antibodies could enhance the antitumor effect of cancer-specific T-cells by inhibiting CTLA-4 activity. Currently, ipilimumab, an anti-CTLA-4 antibody, has approval for use in patients with malignant melanoma. Recently, a phase II clinical trial was conducted to evaluate the clinical efficacy of tremelimumab, another anti-CTLA-4 antibody, in patients with HCC<sup>[97]</sup>. After 8 wk of treatment, 3 and 10 patients (among a cohort of 21 HCC patients) showed PR and SD, respectively. Median PFS and OS were 6.5 and 8.2 mo, respectively.

### **Combination therapy using immune checkpoint inhibitors**

To achieve a sufficiently strong antitumor immune response, a combination therapy using anti-PD-1 and anti-CTLA-4 antibodies is being developed for HCC treatment. This combination therapy has already been shown to have strong antitumor effects in patients with malignant melanoma<sup>[98]</sup>. The anti-CTLA-4 antibody increases the number of CTLs that infiltrate into tumor by blocking the CTLA-4/B7 pathway. The anti-PD-1 antibody enhances the anti-tumor immune response of cancer-specific CTLs by blocking the PD-1/PD-L1 pathway. The combination of these two mechanisms of immune responses allows this combination therapy to produce an enhanced antitumor effect.

Also, a phase I/II clinical trial for evaluating the efficacy and safety of a combination therapy using tremelimumab (anti-CTLA-4 antibody) and durvalumab (anti-PD-L1 antibody) is underway in 40 patients with advanced HCC. Interim results of this clinical trial announced at ASCO 2017 revealed that the ORR was 25%<sup>[99]</sup>. In addition, a phase III clinical trial of these combination therapies as first line therapy in the advanced HCC patients is currently ongoing (NCT 03298451).

In recent years, combination therapies of molecular targeted drugs with immune checkpoint inhibitors have attracted attention. This promising approach could achieve additive therapeutic effects of the two drugs and a synergistic effect by improving the tumor-induced immunosuppressive microenvironment<sup>[100]</sup>. In fact, clinical trials investigating the efficacy of such combination therapies against HCC have already started in Japan and the United States of America (NCT 03006926).

Several therapies combining local regional methods such as RFA and TAE with immunity checkpoint inhibition therapy have also been reported. Clinical trials for an anti-CTLA-4 antibody as an adjuvant therapy after RFA and TAE showed promising anti-tumor effects, with a PR rate of 26%, a median PFS of 7.4 mo, and a median OS of 12.3 mo<sup>[101]</sup>. Thus, immune checkpoint inhibitor therapy shows great promise as preventive measure for HCC recurrence. This therapy also has the potential for application to a wide range of treatment strategies, from first-line standard therapy to adjuvant/neoadjuvant therapy.

## **FUTURE PROSPECTS OF IMMUNOTHERAPY IN HCC**

Immunotherapy is now widely considered a landmark therapeutic strategy that could radically alter conventional cancer therapy. Unfortunately, patients who can benefit from it remain limited, even in the highly promising immune checkpoint inhibitor therapy. Therefore, further understanding of therapeutic effect prediction and resistance mechanism for immunotherapy could be necessary. Immunotherapy, including immune checkpoint inhibition, cannot provide any anti-tumor effect unless T-cells interact with the cancer-associated peptide and the MHC molecules, thus recognizing cancer cells. Therefore, there have been many efforts to identify and develop more promising tumor-associated peptides and their epitopes. Peptide vaccine therapy using cancer-specific antigens such as GPC3 could induce peptide-specific CTLs against several cancers. Thus, peptide vaccine therapy could be a revolutionary therapeutic strategy by combining with other therapies.

Today, advances in next-generation sequencing and bioinformatics have made it possible to catalog all genomic mutations in individual patients. It has been reported that patients who accumulate more genetic mutations show stronger immune response and better anti-tumor effects after adoptive immunotherapy using TILs<sup>[102-106]</sup> and immune checkpoint inhibitor therapy<sup>[92,107,108]</sup> than those with fewer mutations. We are currently developing individualized vaccine therapy targeting neo-antigens,



which are gene mutation antigens, in several solid tumors, including HCC. Clinical trials are already underway for individualized vaccine therapy in Europe, North America, and China in several cancers, and the preliminary results are beginning to be reported<sup>[109-113]</sup>.

The liver, which contains many immune cells, is unique in that it has a developed immune escape mechanism. The novel immunotherapies could exploit this unique characteristic of the liver and will be ideal candidates for treating HCC in the future. In fact, the widespread acceptance and application of immune checkpoint inhibitor therapy could result in a major paradigm shift in HCC therapy. Cancer immunotherapy and its combination with conventional methods are being rapidly developed worldwide. Meanwhile, immunotherapy has the possibility of causing immune-related adverse events different from conventional therapy, and more severe management may be required. Therefore, for optimization of immunotherapy, we believe that it is urgent to product more strict novel algorithms for treatment selection and management of HCC. We believe that further development of novel cancer immunotherapy can have innovative benefits against many patients with HCC who have been suffering.

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## Early immune response in post endoscopic retrograde cholangiopancreatography pancreatitis as a model for acute pancreatitis

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### Abstract

This opinion review summarizes comparison of clinical presentation and immunology of post-endoscopic pancreatitis and acute pancreatitis (AP) of other etiology. The rationale for this topic was found in studies that mention differences in clinical presentation between these entities, stating that severe form of AP after endoscopic retrograde cholangiopancreatography was more severe than AP of other etiology. Found difference in clinical presentation may have a background in different immunology that needs to be further investigated.

**Key words:** Innate immunity; Pancreatitis immunology; Post endoscopic retrograde cholangiopancreatography pancreatitis

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**Core tip:** Innate immunity plays an immense role in the development of acute pancreatitis (AP) and may determine the course of the disease. Information about the role of innate immunity in patients with post-endoscopic pancreatitis (PEP) is still deficient. PEP may serve as an ideal model for further research of innate immunity function in AP development.

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## INTRODUCTION

Acute pancreatitis (AP) is the most common gastrointestinal cause of morbidity and mortality, with a reported incidence that varies between 4.9 and 73.4 cases per 100000 worldwide<sup>[1]</sup>. The most common cause of AP are gallstones, followed by alcohol abuse as an independent risk factor.

Endoscopic retrograde cholangiopancreatography (ERCP) is an invasive diagnostic and therapeutic technique, which carries certain complication risks. Acute pancreatitis is the most common one. According to the European Society of Gastrointestinal Endoscopy guidelines, the reported incidence of post-endoscopic pancreatitis (PEP) is 3.5% in unselected patients<sup>[2]</sup>.

The severity of AP can be divided into mild, moderately severe or severe based on the presence or absence of persistent organ failure and local and systemic complications. In 90% of cases, PEP is of a mild or moderate severity<sup>[2]</sup>.

Although, the prevalence of the severe form of PEP is reported to be low, Testoni *et al*<sup>[3]</sup> in their study reported that the overall and severe pancreatitis-related mortality was approximately double after ERCP in comparison with AP of other causes. Also, the length of hospital stay in severe cases was longer for post ERCP pancreatitis.

Treatment of AP regardless of the cause, is primarily supportive and implies a certain economic burden to the healthcare system worldwide. Even more, if it develops into a severe form<sup>[4]</sup>.

More thorough clarification of the disease pathogenesis is needed, in order to find an adequate immune target to predict and consequently prevent the severe form of the disease<sup>[5]</sup>.

## CLINICAL PRESENTATION

Admission in hospital varies between patients who develop AP, therefore the exact time of injury is not known. In post-ERCP AP there is the opposite situation, the exact time of injury can be foreseen. Messmann *et al.* concluded that post-ERCP AP represents an adequate model for the evaluation of early immune response in AP. The researchers linked higher values of IL-6 and CRP with the development of AP after ERCP, and concluded that the severity of the disease is not only reflected by a higher, but also earlier peaking, IL-6 serum concentration<sup>[6]</sup>. The role of IL-6 in predicting disease severity was also recognised in patients with AP<sup>[7,8]</sup>. Time is a limit for IL-6, since most of the studies confirmed its effectiveness as a disease severity marker between 12-24 h after ERCP<sup>[9,10]</sup>. Opposite to IL-6 and CRP values, amylase and lipase values were unselectively elevated in all patients after ERCP with an earlier peak in their higher values<sup>[6,10]</sup>. Amylase and lipase are released into the systemic circulation due to a disturbance in transport and an increase in ductal permeability; however, they are not thought to be responsible for inducing further inflammation. They can't discriminate between patients with the potential to develop the mild or severe form of the disease<sup>[10]</sup>. According to the present guidelines, use of the Cotton criteria is recommended and amylase values are evaluated after 24 h for the diagnosis of PEP<sup>[2]</sup>.

Differences between the mild and severe form of PEP and non-ERCP were found, they are summarised in **Table 1**. (With permission: Plavsic *et al*<sup>[5]</sup>)

Testoni *et al*<sup>[3]</sup> reported that clinicians may overestimate the presence of true

**Table 1 Differences in clinical presentation of post-endoscopic pancreatitis vs acute pancreatitis**

	Post-endoscopic pancreatitis		Acute pancreatitis	Conclusion
Fung <i>et al</i> <sup>[11]</sup> endoscopic retrograde cholangiopancreatography - induced acute necrotising pancreatitis <i>vs</i> acute necrotising pancreatitis induced by other causes.	Higher APACHE II scores on admission		Lower APACHE II scores on admission	acute necrotising pancreatitis is more severe when induced by endoscopic retrograde cholangiopancreatography
	More extensive pancreatic necrosis		Less extensive pancreatic necrosis	
	Higher rate of infected necrosis		Lower rate of infected necrosis	
Testoni <i>et al</i> <sup>[3]</sup> endoscopic retrograde cholangiopancreatography induced acute pancreatitis <i>vs</i> non endoscopic retrograde cholangiopancreatography induced acute pancreatitis	No statistical difference: (1) the severity of the pancreatitis; (2) the mortality rate (double in severe post-endoscopic pancreatitis); (3) hospitalisation			There was a statistical difference ( <i>P</i> < 0.001). Mild form of post-endoscopic pancreatitis, a sort of pancreatic reaction, instead of a true episode of acute pancreatitis
	In the mild form of acute pancreatitis, serum amylase fell by 50% in 38.9 h. Peak serum amylase halved within 48 h in 92% of patients	In the mild form of acute pancreatitis, serum amylase fell by 50% in 46.4 h. Peak serum amylase halved within 48 h in 73.6% of patients		
Abid <i>et al</i> <sup>[12]</sup> mild form: Endoscopic retrograde cholangiopancreatography induced acute pancreatitis <i>vs</i> non endoscopic retrograde cholangiopancreatography induced acute pancreatitis	Shorter duration of pain; Shorter time of intravenous hydration; Shorter time to the resumption of an oral diet; Shorter hospital stay. ( <i>P</i> < 0.001).		Endoscopic retrograde cholangiopancreatography-induced acute pancreatitis mild attacks run a significantly shorter and milder course than non- endoscopic retrograde cholangiopancreatography related mild attacks	

pancreatic acute damage in mild PEP, based on significant differences found in the dynamics of serum amylase values measured in patients with PEP and non- ERCP AP. Severe PEP was associated with a higher mortality rate and a longer hospital stay, although with no significant differences.

## IMMUNOLOGY

Disease immunology was extensively studied in both groups of patients and certain components of the immune system emerged as a potential disease severity marker. (Table 2)

Systemic inflammatory response syndrome causes the activation of the compensatory anti-inflammatory response syndrome (CARS). Too strong a CARS, paradoxically leads to immunosuppression and a higher possibility of infection<sup>[22]</sup>. A fall in the co-expression of HLA-DR on CD14+ monocytes is considered a standard laboratory indicator of a CARS<sup>[23]</sup> Analysis of this immune component is linked to the severe form of AP and immunosuppression.

Dysregulated host inflammatory response was included in the new sepsis definition by the Society for Critical Care Medicine and the European Society of Intensive Care in 2016. A recent review article analysed the role of the major innate lymphocyte population, Natural Killer (NK) cells, in the dysregulated host inflammatory response on infection. They concluded that NK cells appear to be critical for the elimination of pathogens during the early phase of sepsis and lead to the prevention of secondary infection during the immunosuppressive phase. This opinion suggests that they may be suitable as new immunotherapeutic agents<sup>[24]</sup>.

Infection is considered to be the most important prognostic factor for disease severity in AP, regardless of the cause. In non-ERCP AP, infection is considered to be the secondary event, while in PEP it's considered to be the primary event<sup>[3]</sup>.

## CONCLUSION

It has been proven that innate immunity plays an immense role in AP and the imbalance of innate immunity may determine the severity of the disease early in the course of the disease<sup>[16,25,26]</sup>. As Table 2 shows, most of the studies that researched the role of immune cells in innate immunity, used patients with AP as the research subjects. Answers about the role of immune cells in patients with PEP are still insufficient.

On the contrary, the role of different cytokines in both groups of patients was extensively studied.

Table 2 Role of immune components in predicting disease severity

	Acute pancreatitis	Post-endoscopic pancreatitis
Monocytes and macrophages	(1) Expression of HLA-DR on monocytes gives a good insight into monocyte function; (2) Decreased monocyte HLA-DR expression may serve as an indicator of immunosuppression <sup>[13]</sup> ; and (3) Decreased monocyte HLA-DR expression predicts the development of organ dysfunction in severe acute pancreatitis <sup>[13]</sup> .	
T cells	(1) CD4 <sup>+</sup> lymphocytes are reported to have a direct cytotoxic effect on acinar cells <sup>[14]</sup> ; (2) Depletion of CD4 <sup>+</sup> lymphocytes reduces the severity of acute pancreatitis <sup>[15]</sup> ; and (3) Reduction in the number of cytotoxic T lymphocytes (CD3+CD8+) in severe form of acute pancreatitis <sup>[16]</sup> .	
Natural Killer cells	(1) Depletion of the natural killer cell population on the first day of severe acute pancreatitis <sup>[16]</sup> ; and (2) No significant change in natural killer cell number in mild acute pancreatitis <sup>[16]</sup> .	
IL-10	Predictive marker of organ failure in severe acute pancreatitis <sup>[17]</sup> .	Conflicting results about reducing the incidence of post endoscopic retrograde cholangiopancreatography acute pancreatitis after IL-10 usage <sup>[18,19]</sup> .
IL-6	Independent factor for predicting severity in acute non-endoscopic retrograde cholangiopancreatography pancreatitis <sup>[7]</sup> .	(1) Peak value 24-48 h after clinical expression of post endoscopic pancreatitis; and (2) In necrotising post endoscopic pancreatitis, the peak levels of IL-6 occur after 24 h <sup>[6]</sup> .
IL-1 $\beta$	(1) Required for full pancreatic and distal organ injury and inflammation <sup>[20]</sup> ; and (2) Values peak after 24 h and are larger in patients with severe acute pancreatitis compared to mild acute pancreatitis, although a strong correlation with acute pancreatitis severity in humans wasn't found <sup>[21]</sup> .	

Further investigation of innate immunity cells and their function in PEP is important. Especially, as already mentioned, the exact time of injury in PEP is known and therefore it may represent a good model for evaluation of the early immune response in AP<sup>[6,27]</sup>.

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## PD-1/PD-L1 antagonists in gastric cancer: Current studies and perspectives

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### Abstract

Immune checkpoints release suppressive signals for T cells, which enable the tumors to escape from immune destruction and provide a new concept that uses the capabilities of the immune system as a therapeutic target for tumors. At present, programmed death receptor 1 (PD-1)/programmed death ligand-1 (PD-L1) has become the most promising therapeutic target. PD-1/PD-L1 blockades exhibit long-lasting antitumor efficacy and safety in patients with various cancers, such as melanoma and non-small-cell lung cancer. Moreover, PD-L1 is highly expressed in the peripheral blood and tumor specimens of patients with cancer, and the expression of PD-L1 is positively correlated with various pathological features and may serve as a predictor of poor prognosis or a diagnostic tool. Clinical trials have verified that PD-1/PD-L1 blockade therapy benefits patients with advanced gastric cancer or gastroesophageal junction cancer. Furthermore, there are many molecules involved in the regulation of PD-1/PD-L1 expression, and the modification of these molecules *via* drugs and combinations with PD-1/PD-L1 inhibitors may further improve the efficacy of immunotherapy for gastric cancer. In this review, the efficacy, safety, and possible combination treatment options of PD-1/PD-L1 in gastric cancer are reviewed in experimental and clinical settings.

**Key words:** Immunotherapy; PD-1/PD-L1 inhibitors; Programmed death-ligand 1; Gastric cancer

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**Core tip:** Programmed death receptor 1 (PD-1)/programmed death ligand-1 (PD-L1) inhibitors have been defined as a distinct type of immunotherapy for various cancers. A growing number of studies have investigated the role of PD-1/PD-L1 inhibitors in gastric cancer. This manuscript presents a comprehensive overview of the mechanism of PD-1/PD-L1 blockade therapy, summarizes the efficacy and safety of some critical clinical



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trials, and highlights possible combination treatment options in gastric cancer. This manuscript also provides insight into the current research limitations and indicates the development direction for future research of PD-1/PD-L1 checkpoint inhibitors.

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## INTRODUCTION

Cancer immunotherapy is one of the most successful therapies in the field of cancer treatment in recent years<sup>[1]</sup>. Following surgery, radiation, chemotherapy, and targeted therapy, immunotherapy has become a new method for the treatment of cancer. As early as 2013, cancer immunotherapy was rated as one of the top ten scientific breakthroughs<sup>[2]</sup>. Evading immune destruction is one of the hallmarks of cancer, as illustrated by Hanahan and Weinberg<sup>[3]</sup>. The concept that the immune system can recognize and control the growth of tumors can be traced back to 1893 when William Coley used live bacteria as an immunostimulant to treat cancer. However, due to its limited clinical efficacy, cancer immunotherapy has been met with moderate enthusiasm. This limited efficacy results from the ability of the tumor cells to avoid being identified and eliminated by the immune system, which allows the tumor cells to be part of the host<sup>[4]</sup>. Over the past few decades, enormous progress has been made in illuminating how cancer evades the immune system, which in turn provides novel methods to stop cancer immune evasion by eliminating cancer cells. Cancer immunotherapy utilizes the host's natural defense mechanism to enhance antitumor immunity for a stronger antitumor effect. At present, cancer immunotherapy includes adoptive cellular immunotherapy, checkpoint inhibitors, and therapeutic cancer vaccines<sup>[5-7]</sup>. The immunological checkpoint molecules include programmed cell death-1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4). The application of immune checkpoint inhibitors has shown significant antitumor effects on several cancers and has tremendously changed the treatment of melanoma, lung cancer, and kidney cancer. Compared with CTLA-4 inhibitors, checkpoint blockades targeting the PD-1/programmed death ligand-1 (PD-L1) axis have more advantages in efficacy and fewer side effects<sup>[8]</sup>, which allows PD-1/PD-L1 antagonists to be developed into a more promising and efficient approach for anticancer therapy.

Gastric cancer is a malignant cancer of the digestive tract that has serious implications for human health worldwide<sup>[9]</sup>. According to the recent global statistics, gastric cancer remains a significant cancer globally, and there were more than 1000000 newly diagnosed gastric cancer cases in 2018 along with an estimated 783000 deaths (1 in every 12 deaths globally), making it the fifth most frequently diagnosed cancer and the third leading cause of cancer death<sup>[10]</sup>. *Helicobacter pylori* is the main risk factor for gastric cancer, with almost 90% of new cases of stomach cancer being ascribed to an infection with *Helicobacter pylori*<sup>[11,12]</sup>. Due to the lack of specific symptoms and signs in the early stage, most patients are diagnosed with gastric cancer at the advanced stage, which severely affects the prognosis of the patients. Although there are some advances in early gastric cancer screening and treatment, the 5-year survival rate of patients with advanced gastric cancer is still below 10%<sup>[13]</sup>. Endoscopic resection is a critical method in the treatment of early gastric cancer. The treatment of advanced gastric cancer is usually based on surgery and supplemented by chemotherapy<sup>[14]</sup>. Combination chemotherapy is the first-line treatment for patients with advanced gastric cancer. Several cytotoxic drugs, such as fluoropyrimidine, platinum, paclitaxel, and irinotecan, have curative effects on gastric cancer. Although tremendous progress has been made in chemotherapy, the overall prognosis is still poor<sup>[15]</sup>. Furthermore, an increasing number of patients who were initially sensitive to chemotherapy gradually acquired drug resistance during treatment, which limits the further application of chemotherapy drugs. Additionally, targeted therapies against HER2 and VEGFR have also been approved for the treatment of advanced gastric cancer, but the 5-year overall survival (OS) is only 20%-30%<sup>[16]</sup>.

Recently, cancer immunotherapy, especially with checkpoint antagonists, has been transforming the entire field of cancer treatment and has achieved optimistic effects in solid tumors such as melanoma, lung cancer, and kidney cancer. Furthermore,

PubMed and The American Society of Clinical Oncology Annual Meeting data suggest that PD-1/PD-L1 inhibitors may lead us into a new era for the treatment of gastric cancer<sup>[16]</sup>. Herein, we review the mechanisms of PD-1/PD-L1 blockade therapy, summarize the present knowledge of PD-1/PD-L1 inhibitors to reveal their efficacy and safety in stomach cancer or gastroesophageal junctional cancer, and highlight the possible combination of conventional therapy with PD-1/PD-L1 checkpoint inhibitors.

## MECHANISM OF ACTION OF PD-1/PD-L1 BLOCKADE THERAPY

PD-1, a transmembrane receptor on T cells, was initially identified from apoptotic T cell hybridomas through a subtractive method and has become known as a predominant negative regulator of antitumor T cell effector function when engaged by its ligand PD-L1, which is expressed on the surface of cells within tumors<sup>[17]</sup>. PD-1 bears its name from its earliest description that it was expressed as a receptor involved in cell death<sup>[18]</sup>. As a significant member of the B7/CD28 costimulatory molecule superfamily, PD-1 is primarily expressed on the surface of activated T and B cells. Both PD-L1 (also known as B7-H1) and PD-L2 (also known as B7-DC) are ligands of PD-1, and they are essential members of the costimulatory molecules in the B7 family<sup>[19,20]</sup>. PD-L1 is usually expressed in antigen-presenting cells (APCs), such as macrophages and DCs; however, in the presence of inflammatory factors, such as interferon (IFN) or interleukin 4 (IL-4), PD-L1 is also expressed in epithelial and skin cells<sup>[21]</sup>. PD-L2 has more exclusive expression than PD-L1 in APCs<sup>[22,23]</sup>. The binding of PD-1 to its ligands functions as an immune checkpoint and regulates the host's costimulatory or inhibitory signals to exert effects on T lymphocytes, thereby modulating the magnitude and duration of T lymphocyte responses.

Under physiological conditions, the combination of PD-1/PD-L1 produces an inhibitory signal to prevent the host from developing autoimmune disease. However, when an inflammatory response occurs in the host, the binding of PD-1 prevents the spread of inflammation, thus localizing tissue damage and preventing the excessive inflammatory reaction. Furthermore, within the background of the tumor microenvironment, antitumor T cells continuously recognize cognate tumor antigens from when cancer develops in the primary stage to the formation of metastatic lesions. Activation of the TCR gives rise to the production of proinflammatory cytokines, including IFN- $\gamma$ , which is the most potent driver of reactive PD-L1 expression<sup>[22,24]</sup>. Moreover, the chronic expression of IFN- $\gamma$  in the microenvironment induces the elevated expression of PD-1 on the infiltrated T cells. The recognition of PD-1 on antitumor T cells by the highly expressed PD-L1 on tumor cells not only inhibits secretion of T cell immune stimulating cytokines (IL-2, IFN- $\gamma$ , and tumour necrosis factor- $\alpha$ ) but also promotes the secretion of the immunosuppressive cytokines (IL-10), thus inhibiting T cell activation and proliferation<sup>[25]</sup>. Eventually, the tumor cells evade immune destruction. PD-1 is therefore a negative regulator of immune responses and is becoming a promising therapeutic target in cancer immunotherapy.

## PD-1/PD-L1 EXPRESSION AND ITS CORRELATION WITH CLINICOPATHOLOGIC FEATURES OR PROGNOSIS IN PATIENTS WITH GASTRIC CANCER

A growing number of studies have been conducted to illuminate the correlation between PD-1/PD-L1 expression and clinicopathologic features or prognosis in patients with gastric cancer. Wang *et al*<sup>[26]</sup> obtained tissues from 509 patients who underwent gastrectomy, and all tissues were collected and analyzed in the form of a tumor microarray (TMA). In that study, the authors found that a positive PD-L1 status was correlated with high CD3+ and CD8+ T cell invasion. Positive expression of PD-L1 and CD8+ T cells was associated with long OS time in stomach cancer patients, but there were no significant differences noted between the groups with high and low PD-1 and CD3 expression. These results suggest that PD-L1 expression and a high density of CD8+ T cells may serve as prognostic indicators in patients with advanced gastric cancer. Moreover, in a cellular and specimen-based study, Amatatsu *et al*<sup>[27]</sup> investigated PD-L1 mRNA expression in three gastric cancer cell lines and 124 blood specimens from patients with gastric cancer by qRT-PCR assays. It was demonstrated that a high level of PD-L1 expression significantly correlated with deep

tumor invasion, distant metastasis, and advanced stage ( $P = 0.001$ ,  $P < 0.001$ , and  $P < 0.001$ , respectively). In terms of diagnostic performance, surprisingly, the area under the ROC curve for predicting patients with distant metastasis was 0.772. The sensitivity and specificity of PD-L1 mRNA expression for predicting distant metastasis were 0.814 and 0.667, respectively. In addition, compared with patients with low PD-L1 expression, patients with high PD-L1 expression had a significantly lower 5-year survival rate (84.1% *vs* 50.0%,  $P < 0.0001$ ). Univariate and multivariate analyses of survival revealed that PD-L1 expression was significantly associated with postoperative survival ( $P < 0.0001$ ) and could be selected as an independent prognostic factor ( $P = 0.024$ ). Similarly, in a study that involved 465 patients, Böger *et al.*<sup>[28]</sup> reported that the immunohistochemical analysis results of a TMA exhibited a close relationship between the protein expression of PD-L1 and some important prognostic clinicopathological factors, including depth of tumor invasion, distant metastasis, and UICC stage. These findings imply that the assessment of PD-L1 expression has potential clinical application for monitoring tumor properties and progression in patients with stomach cancer. Moreover, the cancer genome atlas (TCGA) classifies gastric cancer into the following four molecular subtypes: (1) Epstein-Barr virus (EBV)-positive; (2) microsatellite instability (MSI); (3) chromosomal instability; and (4) genomically stable<sup>[29]</sup>. Identification of these subtypes offers a roadmap for patient stratification, and trials of targeted therapies also provide the necessary molecular tools to realize individualized treatment in cancer. A total of 15 eligible studies that included 3291 patients were selected for a meta-analysis<sup>[30]</sup>, which showed that PD-L1 expression was associated with OS in gastric cancer (HR = 1.46, 95%CI: 1.08 ± 1.98;  $P = 0.01$ ). The authors also found that EBV infection-positive (EBV+) and MSI tumors are more likely to express PD-L1 than the other types of gastric cancer tumors, which is consistent with the results of previous reports<sup>[31-34]</sup>. This result may provide evidence that gastric cancer patients, especially those with the subtypes of EBV+ and MSI tumors, may be prime candidates for PD-1 blockade therapy. Nevertheless, an original study from Japan showed that EBV-positive gastric cancer cells that express high levels of PD-L1 inhibited T-cell proliferation, and the IFN- $\gamma$  signaling pathway played an important role in the expression of PD-L1<sup>[35]</sup>.

However, the heterogeneous expression of PD-L1 within primary tumor sites is one of the critical obstacles to the clinical treatment of PD-1/PD-L1 checkpoint blockades<sup>[36]</sup>. The KEYNOTE-010 study suggested that the level of PD-L1 expression could act as a useful molecular tool to distinguish responders from nonresponders in PD-1/PD-L1 immunotherapy<sup>[37]</sup>. As mentioned above, the status of PD-L1 expression in blood specimens or tissue specimens is not only associated with clinicopathological features, prognosis, and diagnostic performance but is also associated with the therapeutic effects of PD-1/PD-L1 checkpoint blockades.

## CLINICAL EFFECTS OF PD-1/PD-L1 INHIBITORS IN GASTRIC CANCER

PD-1/PD-L1 checkpoint blockades have dramatically transformed the landscape for conventional treatments in patients with gastric cancer. At present, there are five anti-PD-1 or anti-PD-L1 antibodies approved by the FDA for approximately 11 cancer indications<sup>[17]</sup>; these approved antibodies include two antibodies for PD-1, nivolumab and pembrolizumab, and three antibodies for PD-L1, atezolizumab, avelumab, and durvalumab. These drugs are still in the early stages of clinical research. The current PD-1 inhibitors are mainly used for the treatment of melanoma, non-small-cell lung cancer (NSCLC), and urothelial cancer. Based on the efficacy of PD-1 blockers in NSCLC and melanoma patients, PD-1/PD-L1 inhibitors will hopefully continue to expand their range of applications. Clinical trials with PD-1/PD-L1 antibodies have been initiated in multiple studies. These studies investigated the efficacy and safety of PD-1/PD-L1 antibodies in the treatment of melanoma, urinary tract cancer, digestive tract tumors, and malignant gliomas. Here, we focus on the treatment of gastric or gastroesophageal junctional cancer with PD-1/PD-L1 inhibitors.

The KEYNOTE-012 study<sup>[38]</sup>, a multicenter phase Ib trial using the anti-PD-1 antibody pembrolizumab, included 162 patients with recurrent or metastatic adenocarcinoma of the stomach or gastroesophageal junction cancer. The PD-L1 positive rate in these patients was 40%. All 39 patients were treated with pembrolizumab at 10 mg/kg once every 2 wk for 24 mo, and the endpoints included trial completion (24 mo), complete remission (CR), cancer progression, or the occurrence of unacceptable toxic effects. The results showed that the objective response rate (ORR) was 22.2% (95%CI: 10.1%-39.2%), the rate of grade 3 or 4 treatment-related adverse events (TRAES) was 13%, the median progression-free

survival (PFS) time was 1.9 mo (95%CI: 1.8-3.5), and the median OS time was 11.4 mo. At the six month follow-up, the PFS rate was 24%, the OS rate was 69%, and nearly 53% patients experienced tumor shrinkage to some degree. Moreover, no patients withdrew from the trial due to immune-related adverse reactions. Although this trial did not include a control group, the side effects were still in the acceptable range, and these results warranted further study in phase II and III trials. CheckMate-032<sup>[39]</sup> was a multicenter phase I/II cohort study that treated advanced gastric cancer patients with nivolumab. This trial enrolled 59 patients diagnosed with advanced and metastatic (A/M) gastric or gastroesophageal junction cancer, and the positive rate of PD-L1 expression in all patients was 38%. All patients were prescribed 3 mg/kg of nivolumab every 2 wk until unacceptable toxicity effects occurred. The results showed that the ORR was 14%, the OS was 5 mo (95%CI: 3.4-12.4), and the 12-mo OS rate was 36%. Moreover, no treatment-related deaths occurred in this study, and all adverse reactions were controllable. The analysis of the classified data demonstrated that the ORRs in the PD-L1-positive and PD-L1-negative patients were 27% and 12%, respectively. Nivolumab had improved efficacy in the PD-L1-positive patients than in the PD-L1-negative patients. In a randomized controlled phase III trial published in *Lancet*, 493 participants were randomized into a nivolumab-treated group and a placebo-treated group. The OS in the two groups was 5.26 mo and 4.14 mo, respectively (HR: 0.63; 95%CI: 0.51-0.78;  $P < 0.0001$ ); in comparison, the OS for the PD-L1-positive patients in the treatment and control groups was 5.22 mo and 3.82 mo, respectively (HR: 0.51; 95%CI: 0.21-1.25). The 12-mo OS rate in the treatment group was significantly higher than that in the control group (26.2% *vs* 10.9%). Although most of these clinical trials did not list positive PD-L1 expression as one of the inclusion criteria, the results did provide solid evidence that, compared with conventional therapy, PD-1/PD-L1 inhibitors brought new hope for gastric cancer patients with highly expressed PD-L1.

The tumor microenvironment is complicated and interacts with multiple signaling pathways, both of which jointly regulate the initiation and progression of cancers and even the responses to specific therapies. Studies have demonstrated that there are a variety of signaling pathways involved in cancer immunotherapy and that these pathways may interact with each other<sup>[40]</sup>. Acquired resistance after a period of response is one of major problems with checkpoint blockade therapy as well<sup>[41]</sup>. Therefore, it is often difficult to achieve the desired clinical effects with the long-term application of PD-1/PD-L1 inhibitors or single-agent treatment. To maximize the benefits of cancer therapy, the combination of different immunotherapies or immunotherapy with conventional therapies such as radiotherapy, chemotherapy, and oncogene-targeted therapy, has been shown to alter the immunosuppressive tumor microenvironment and enhance the ability to eliminate cancers, which is the future direction for cancer therapy<sup>[42]</sup>.

A clinical trial was designed based on the foundation of the CTL-4 and PD-1 pathways having coinhibitory roles after preclinical studies showed evidence of synergy in syngeneic mouse models. In this trial, the patients were treated with a combination of ipilimumab and nivolumab to block CTLA-4 and PD-1, respectively<sup>[42]</sup>. The data showed that in the single treatment group, the time to progression (TTP) was 6.9 mo, and the ORR was 57.6%, while in the combination treatment group, the TTP was 11.5 mo, and the ORR was 57.6%<sup>[43]</sup>. Furthermore, KEYNOTE-059<sup>[44]</sup> was a phase II cohort clinical trial that studied pembrolizumab alone or in combination with cisplatin/5-FU among advanced gastric cancer patients. The data showed that the PD-L1 positive rate in the 25 enrolled patients was 64%, the ORR was 60% (95%CI: 38.7~78.9), the ORR of PD-L1-positive patients was 68.8%, the ORR of PD-L1-negative patients was 37.5%, the PFS was 6.6 mo (95%CI: 5.9-10.6), and the OS was 13.8 mo. Despite the fact that there were no adverse events related to death, the rate of grade III-IV TRAEs remained high (76%) and included diarrhea, dysgeusia, thyroid disorders, and nausea. Clearly, the coinhibitory group benefitted more than the single inhibitory or traditional therapy group. On the basis of improving safety and efficacy, minimizing the adverse event rate is a major problem that is needed to address in combination treatments.

Several studies have demonstrated that the PD-L1 positive rate in gastric cancer tissues was over 40%<sup>[45-47]</sup>. We have noted that according to the molecular characteristics of gastric cancer, the TCGA divided stomach cancer into four molecular subtypes in 2014. The EBV-positive type accounts for 9% of all gastric cancers and displays recurrent PIK3CA mutations, extreme DNA hypermethylation, and high expression of PD-L1/2<sup>[29]</sup>. This classification provides a theoretical basis for the simultaneous treatment of PD-L1 inhibitors and anti-EB virus therapy. Furthermore, CD40 is one of the critical costimulatory molecules in the antitumor treatment immune response, but the effects of CD40 monoclonal antibody from clinical trials were unsatisfactory<sup>[48]</sup>. One explanation for this phenomenon is that the



expression of PD-L1 on the surface of tumor cells was also elevated with the use of a CD40 agonist. Therefore, when conducting research to illuminate the mechanism of costimulatory molecules, blocking the PD-L1 pathway is of great importance. In addition, numerous studies have revealed that dysregulation of the Wnt/ $\beta$ -catenin signaling pathway occurred in more than 70% of gastric cancer patients<sup>[49]</sup>. Activation of the Wnt/ $\beta$ -catenin signaling pathway is not only involved in the physiological processes of proliferation, invasion, metastasis, and drug resistance<sup>[50-52]</sup> but is also negatively correlated with T cell invasion within many tumors such as colorectal cancer<sup>[53]</sup>, melanoma<sup>[54]</sup>, ovarian cancer<sup>[55]</sup>, and prostate cancer<sup>[56]</sup>. In contrast, the inhibition of the Wnt/ $\beta$ -catenin signaling pathway significantly suppressed proliferation and metastasis both *in vitro* and *in vivo*<sup>[57]</sup>. These studies suggest that Wnt/ $\beta$ -catenin signaling pathway inhibitors may stimulate immune cells and enhance T cell infiltration in tumors, allowing tumors to respond to immunotherapy. It is apparent that more novel studies are needed to identify potential therapeutic targets to promote the exploration and realization of the potency of combination therapy.

## CONCLUSION

Although considerable progress has been made in cancer therapy and the treatment of cancers has entered the new era of immunotherapy, the efficacy and safety of PD-1/PD-L1 inhibitors in advanced gastric cancer patients still need to be further explored by in-depth research in clinical settings. First, most of the clinical trials were primarily limited to early stage I or II disease, and the number of PD-L1-positive patients in the treatment group remained relatively low, which prevents gathering enough direct and potent evidence to validate the curative effects. Second, given the results of the clinical trials up to now, TRAES may become one of the critical factors that thwarts the future application of PD-1/PD-L1 blockade therapy. Identifying and understanding the mechanism of adverse events are of great importance in preventing the occurrence of side effects. Third, after the safety and efficacy of cancer immunotherapy have been validated, the next question is how to select the best treatment method for specific patients. According to the comprehensive molecular characterization of gastric adenocarcinoma, the single-cell sequencing technique would help researchers to recognize different subtypes, discriminate responders, and design the best treatment strategy for patients, allowing individualized cancer therapy to become a reality. Thus, it is essential to list PD-L1-positive gastric cancer patients in the inclusion criteria, minimize the rate of adverse events, and use molecular tools to identify specific patient subpopulations in the research of cancer immunotherapy.

The efficacy of the combination of PD-1/PD-L1 blockade therapy with other traditional therapies remains to be fully elucidated. Combination therapy provides a new direction for research and is a new aspect of cancer immunotherapy. The combination of radiation and anti-PD-1/PD-L1 therapy is as an example of this new area. Ahmed *et al*<sup>[58]</sup> retrospectively reviewed patients who received stereotactic radiosurgery (SRS) for melanoma brain metastases (BM). The patients were treated with SRS before, during, or after nivolumab therapy. Their results demonstrated that when compared with SRS alone, the combination therapy was better tolerated with no unexpected neurotoxicity. In addition, these patients had superior out-of-field BM control and OS compared with those who received the current standard treatment for melanoma<sup>[58]</sup>. Although no prospective trials have been published, there are currently several cumulative trials evaluating the safety and efficacy of PD-1/PD-L1 inhibitors combined with radiation therapy in various malignancies<sup>[59]</sup>. Preliminary reports from some of these trials have shown promising outcomes<sup>[60,61]</sup>. Consequently, efforts still need to be made in the exploration of combination therapy, new molecular targets, or already identified targets. Based on the recent successes of the field of immunotherapy, continuing to incorporate knowledge from mechanistic basic science research is essential to achieving therapeutic success.

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## Higher dose of simethicone decreases colonic bubbles and increases prep tolerance and quality of bowel prep: Meta-analysis of randomized controlled trials

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### Abstract

#### BACKGROUND

Antifoaming agents, such as simethicone, may facilitate mucosal inspection during colonoscopy. However, conflicting results have been reported with regard to the impact of simethicone on quality of bowel preparation and adenoma detection rate (ADR).

#### AIM

To perform a meta-analysis of trials that have compared simethicone *vs* placebo during colonoscopy.

#### METHODS

A reproducible literature search of multiple medical databases yielded eleven studies ( $n = 2605$ ) for inclusion. Studies were compared for quality of bowel preparation, bubbles quality, ADR, and tolerability. Two reviewers independently scored the identified studies for methodology and abstracted pertinent data. Pooling was conducted by both fixed-effects and random-effects models. Relative risk (RR) estimates with a 95% confidence interval (CI) were calculated. Heterogeneity was assessed by  $I^2$ -squared index ( $I^2$ ) statistics.

#### RESULTS

Patients' demographic characteristics were comparable in all studies. Of the 2605 patients, 1300 were in the simethicone group, whereas 1305 were in the placebo group. Inadequate bowel preparation was much lower in the simethicone group than in the placebo group [13% *vs* 24.6%; RR = 0.51 (0.31-0.82);  $P < 0.0001$ ]. The placebo group was more likely to have significant colonic bubbles than was the

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simethicone group [35% *vs* 8%; RR = 1.49 (1.25-1.76);  $P = 0.0001$ ]. Use of simethicone resulted in a slight, statistically significant increase in ADR compared with the placebo group [26.6% *vs* 21.6%, RR = 1.07 (1.01-1.13);  $P = 0.02$ ]. Higher doses of simethicone (> 478 mg) were more likely to result in significant reduction of inadequate bowel preparation, colonic bubbles, and to improve ADR.

### CONCLUSION

Adding simethicone improved the quality of bowel preparation, visualization, tolerability, and, eventually, ADR.

**Key words:** Simethicone; Colonoscopy; Bubbles; Bowel preparation; Adenoma detection rate

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**Core tip:** Colonoscopy is an essential tool in the screening for and preventing colon cancer, but inadequate colon preparation limits visualization, prolongs procedure time, and, affects exam quality. An antifoaming agent, simethicone, has been a promising addition to bowel preparation. This meta-analysis of randomized controlled trials assessed the effect of its addition to commonly utilized bowel preparations and analyzed the optimum effective dose. We concluded that the addition of simethicone to bowel preparation significantly reduced inadequate bowel preparation, increased adenoma detection rate and trended to improved tolerability of the prep. Higher doses of simethicone were more likely to achieve those outcomes.

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## INTRODUCTION

Colorectal cancer (CRC) is the third leading type of cancer worldwide, with 1-2 million new cases every year and a mortality rate of 600000/year<sup>[1]</sup>. Colonoscopy is an important tool for CRC screening and surveillance<sup>[2]</sup>. The presence of fecal residue and bubbles during the exam may limit visualization, prolong the procedure time, and, hence, affect the quality of the exam<sup>[3]</sup>. An antifoaming agent, simethicone, has been a promising addition to bowel preparation. It reduces the surface tension of air bubbles and has been shown to reduce bloating and abdominal pain, as well as improve mucosal visualization<sup>[4]</sup>. Results regarding improvement in the quality of bowel preparation and adenoma detection rate (ADR), however, have been mixed<sup>[4]</sup>. The aim of this study was to encompass recent randomized controlled trials in a meta-analysis to assess the effect of simethicone on bowel preparation, ADR, and patient compliance and to assess the optimal dose of simethicone to achieve aforementioned effects.

## MATERIALS AND METHODS

The methods of our analysis and inclusion criteria were based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses recommendations.

### Study identification

One investigator (MM) and a research librarian independently designed and conducted a computer-assisted scan of multiple medical literature databases (including PubMed and OVID EMBASE) for relevant papers published from the start of 1947 through October 2018. A reproducible systematic literature search strategy was employed that searched for the term: "colonoscopy" AND "simethicone" OR "anti-foaming agent". Two investigators (MM, HM) examined all published studies that compared simethicone with placebo in the improvement of bowel preparation,

bubbles quality, ADR, and tolerability.

### **Study eligibility**

Investigators were not blinded to journal titles, author names, or institutional affiliations. Both inclusion and exclusion criteria were drafted before the initiation of literature review. Titles and abstracts were screened initially for potentially relevant studies. Once these articles were listed, the studies that clearly did not meet the eligibility criteria were excluded. Later, full manuscripts were studied in detail to ensure that the selected studies were appropriate for our analysis. Any disagreement was resolved by the senior investigator.

For a trial of simethicone compared with placebo to qualify for inclusion in this meta-analysis, it should have met the following criteria: (1) prospective randomized controlled trial; (2) comparison of simethicone with placebo; and (3) either bowel preparation quality, bubbles score, ADR, or a combination of any of these parameters was studied.

### **Data extraction**

One author (MM) extracted data from studies in tabulated data extraction forms and validated by a second author (MH). Extracted data was compared to the original research papers. The following data were collected: first author, publication year, country of origin, multi-center participation, number of subjects in each group, trainees' involvement, sedation, pre-procedure diet, split dosing, blinding, patient demographics, indications of colonoscopy, quality and method used to assess bowel preparation, scoring of bubbles in lumen, ADR, polyp detection rate, and side effects and tolerance of prep material. Discrepancies in data extraction were resolved by consensus.

### **Outcomes for analysis**

Rate of inadequate bowel preparation was the primary outcome. The definition of inadequate bowel preparation was either based on Boston Bowel Preparation score [(BBPS) < 6] or subjective reporting of "fair" or "poor" or "inadequate" in the included studies. Secondary outcomes included significant presence of colonic bubbles (more than minimal bubbles), ADR, and tolerability of bowel preparation, including bloating, nausea, vomiting, and abdominal pain.

### **Assessment of study quality**

Bias was assessed by utilizing the Cochrane Collaboration Risk tool which is available in Review Manager 5. There are six criteria which this tool uses to evaluate four bias sources. To assess selection bias, it evaluates adequate sequence generation and allocation concealment. To assess detection and performance bias, it checks whether blinding is effective with respect to personnel, participant, and outcome assessors. To assess attrition bias, it assesses completeness of outcome data. To assess reporting bias, it assesses if selective reporting is present. It also has a protocol to assess other biases such as early withdrawal or extreme baseline imbalances. If a trial excelled in the aforementioned domains it was categorized to lowest risk of bias. If any disagreements occurred among the extracting authors, they were solved by consensus.

### **Data synthesis and statistical analysis**

The software utilized to conduct this meta-analysis is Review Manager (RevMan) v5.3 (The Nordic Cochrane Centre, Copenhagen, the Cochrane Collaboration). Assessments were made under Mantel-Haenszel fixed-effects method with summary risk ratio and 95% confidence interval. Random-effects model was used to combine estimates. If no significant heterogeneity ( $P > 0.1$ ) was noted, fixed-effects model was rendered. Statistical tests were 2-sided and  $P < 0.05$  was considered significant. Effort was made to report 95% CIs with the pooled data. A funnel plot was utilized to evaluate publication bias (inverse standard error for each study was plotted against natural log of the RR (lnRR)). Heterogeneity was assessed by  $I^2$  statistics, with value of more than 40% reported as substantial heterogeneity.

## **RESULTS**

### **Study identifications and selection**

The literature search yielded eighteen potential studies for inclusion. Full text was only available for fifteen studies. Two studies were immediately excluded after initial review, because they compared different volumes in the experimental and control arms<sup>[5,6]</sup>. Two studies examined simethicone *vs* placebo, but were not included in this



meta-analysis; one study included only mean data<sup>[7]</sup>, and the other was a prospective, but not randomized, clinical trial<sup>[8]</sup>. Eleven studies were included in the final analysis (Figure 1). On manual review of the references of retrieved manuscripts, no other studies meeting inclusion criteria were identified.

### Description of variation in study methods

There were four studies from Asia<sup>[9-12]</sup>, two from Europe<sup>[13,14]</sup>, and five from North America (Table 1)<sup>[15-19]</sup>. All studies provided some sort of patient demographic information. Five studies provided data regarding sex distribution and there was no difference in male/female distribution across arms ( $P = 0.91$ )<sup>[9-12,15]</sup>. Four studies provided data regarding age distribution which was not different either ( $P = 0.85$ )<sup>[9-12]</sup>. The number of participants in these studies ranged from 42 to 294. One study involved only patients with inflammatory bowel disease<sup>[13]</sup>. Symptomatic indication was the highest reason for colonoscopy (67%) among the five studies with detailed information regarding indication<sup>[9-12,15]</sup>. All studies but two commented on the quality of bowel preparation<sup>[17,19]</sup>. Three studies used the Boston Bowel Preparation Score (BBPS)<sup>[9-11]</sup>; adequate preparation was defined as total score of  $\geq 6$ . Three studies used a subjective tool (excellent-good-fair-poor)<sup>[12,13,15]</sup>; adequate preparation was defined as scores of "excellent" or "good". One study used a dichotomous subjective tool (good or poor)<sup>[18]</sup>, with adequate preparation defined as a score of "good". One study used a 0 to 4 scoring system, where 0 represented hard stool and 4 represented no stool; a score of 3 or 4 was considered adequate<sup>[14]</sup>. All studies but two examined the degree of air bubbles<sup>[14,18]</sup>. The degree of air bubbles was summarized as significant (no or minimal bubbles) *vs* not significant (more than minimal bubbles) to accommodate the various definitions used in these trials. Only two studies assessed the relationship of simethicone use to ADR<sup>[9,10]</sup>.

Three studies used a 2-l PEG solution with ascorbic acid, Moviprep (Norgine, Amsterdam, the Netherlands), in a split dose fashion<sup>[11,15,16]</sup>. Two studies used a 2-l PEG solution only on the morning of procedure<sup>[9,10]</sup>. Three studies used sodium phosphate solution (Nap)<sup>[12,14,17]</sup>. Two studies used 4-l PEG solution<sup>[13,18]</sup>. One study used 5-l PEG<sup>[18]</sup>, and another used 6-l PEG<sup>[19]</sup>. Among the various studies, the dose of simethicone ranged from 120 mg to 900 mg daily for five days.

### Assessment of study quality

When assessing domains in risk of bias, we noted that all trials had inadequate bias control (Figure 2). The principle risks of bias noted were allocation concealment and participant blinding.

### Data synthesis

Eleven studies met the inclusion criteria ( $n = 2605$ ). Of the 2605 patients, 1300 were in the simethicone group, whereas 1305 were in the placebo group. Given that the between-study variability was substantially high for the pooled RR of inadequate bowel preparation, significant colonic bubbles, abdominal pain and distension, the random effects model was employed. Since the I<sup>2</sup> of the pooled RR of the ADR, nausea, and vomiting was less than 40%, the fixed effects model was utilized for these analyses.

The rate of inadequate bowel preparation was much lower in the simethicone group than in the placebo group [13% *vs* 24.6%; RR = 0.51 (0.31-0.82);  $P < 0.0001$ ; Figure 3]. The placebo group was more likely to have significant colonic bubbles than was the simethicone group [34.9% *vs* 8%; RR = 1.49 (1.25-1.76);  $P = 0.0001$ ; Figure 4]. Use of simethicone resulted in a slight, statistically significant increase in ADR compared with the placebo group [26.6% *vs* 21.6%, RR = 1.07 (1.01-1.13);  $P = 0.02$ ; Figure 5].

With regards to tolerability, simethicone use resulted in less abdominal distension [16.6% *vs* 30%, RR = 0.58 (0.43-0.78);  $P = 0.0003$ ] and trends towards less abdominal pain [8.1% *vs* 12.3%, RR = 0.66 (0.42-1.03);  $P = 0.07$ ]. There was no difference between the two groups with regard to nausea [23.6% *vs* 22.8%, RR = 1.02 (0.86-1.21);  $P = 0.78$ ] or vomiting [8.4% *vs* 8.4%, RR = 0.99 (0.71-1.38);  $P = 0.97$ ].

With regards to simethicone dose, the mean dose was calculated to be 478mg. Sensitivity analysis was conducted to compare inadequate bowel preparation, significant bubbles and ADR among PEG based, NaP based bowel preparations and simethicone dose above and below the mean dose (478 mg). Although both dose categories resulted in significant reduction in colonic bubbles, higher than mean dose of simethicone were significantly associated with adequate bowel preparation and ADR (Table 2). No significant publication bias was noted when inadequate bowel preparation outcome was analyzed via funnel plot (Figure 6).



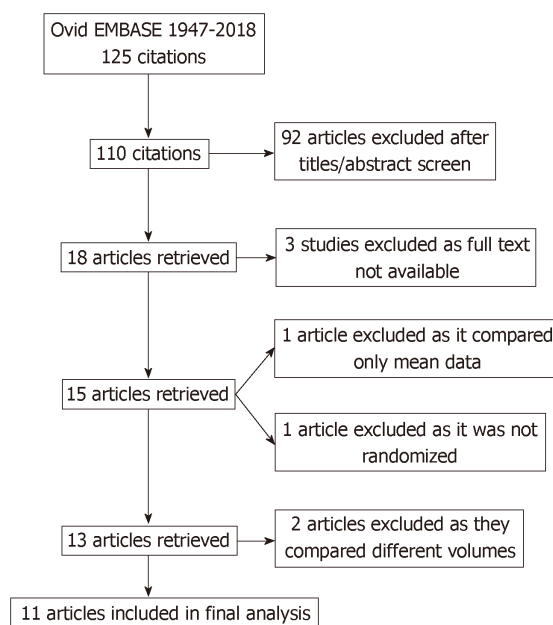


Figure 1 Flowchart of the literature search and study selection.

## DISCUSSION

Simethicone is polydimethylsiloxane mixture that is frequently prescribed to reduce abdominal discomfort from excessive gas in the bowel. This anti-foaming agent reduces the surface tension of air bubbles so they merge and are easily passed via belching or flatulence.

In this meta-analysis, simethicone use resulted in a 50% reduction in inadequate bowel preparation and an almost 50% improvement in colonic bubbles during colonoscopy. Improvement in bowel preparation quality is likely attributed to reduced foam formation and reduced possibility of residual stool adherence to the colon, enhancing its expulsion from the gastrointestinal tract<sup>[10]</sup>. In the trials included, there were significant disparities in simethicone dose (120 to 1200 mg), mode of administration (mostly, mixed with the purgative solution), and timing of administration (depending on the timing of purgative used; mostly, split dosing was utilized).

Two earlier meta-analyses related to simethicone and colonoscopy quality were published<sup>[20,21]</sup>. Wu *et al*<sup>[20]</sup> noted no improvement in the quality of bowel preparation when using simethicone in addition to regular purgatives. However, of the 13 studies included in that meta-analysis, seven evaluated colonoscopies specifically, and all had relatively small sample sizes (18-82 patients in each arm) and significant variability in bowel preparation utilized. Recently, Pan *et al*<sup>[21]</sup> examined the impact of simethicone use on ADRs in 1855 patients undergoing colonoscopies. This meta-analysis was restricted to trials that used PEG as the bowel purgative. Two of the six trials included in the meta-analysis used different PEG volumes in the placebo and control arms.

Our meta-analysis results concur with those of Pan *et al*<sup>[21]</sup> with regard to significant improvement of ADR. However, we included only RCTs that used the same volume and type of purgatives in each arm, regardless of whether the purgative was PEG-based. We included non-PEG-based alternatives as well, which confirms the broad applicability of simethicone. We also examined other outcomes, including quality of bowel preparation, significant bubbles, and tolerability. Additionally, we performed multiple sensitivity analyses to evaluate the impact of simethicone on quality of bowel preparation, colonic bubbles, and ADR relative to the type of purgative or the dose of simethicone. We found that the beneficial effect of simethicone on the improvement of the quality of bowel preparation was more pronounced with PEG-based purgatives than with purgatives based on sodium phosphate. We also found that simethicone doses above the mean (478 mg) were more likely to result in significant reduction of inadequate bowel preparation, colonic bubbles, and ADR (Table 2). We feel this highlights an important area of research, as studies evaluating optimal dose of simethicone are lacking.

Simethicone is a well-tolerated and safe drug. It has very few side effects, given that the drug is not absorbed into the blood stream<sup>[22]</sup>. In our meta-analysis, we found that

Table 1 Characteristics of studies included in the meta-analysis

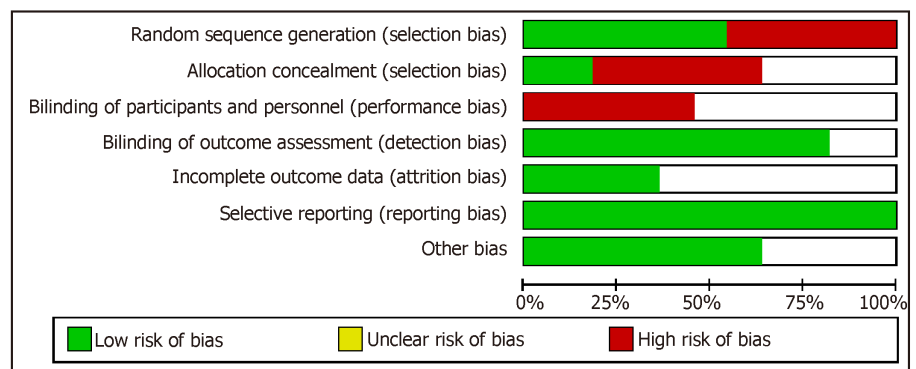
Ref.	Country	No. of patients Simethicone/placebo	Bowel preparation used	Simethicone	Timing of Simethicone	Timing of bowel preparation	Diet prior to colonoscopy
McNally <i>et al</i> <sup>[19]</sup>	United States	49/48	6L PEG	160 mg	Mixed with PEG	Not clear	NA
Shaver <i>et al</i> <sup>[18]</sup>	United States	102/102	5L PEG	75 mL (30% simethicone)	45 mL before and 30 mL after ingestion of PEG	Night before	NA
Lazzaroni <i>et al</i> <sup>[13]</sup>	Italy	57/48	4L PEG	120 mg	Mixed with PEG	Split dose	NA
Sudduth <i>et al</i> <sup>[17]</sup>	United States	42/44	45 mL NaP × 2 doses	320 mg	160 mg after each dose of 45 mL NaP	Split dose	Clear liquid the day before
Altintas <i>et al</i> <sup>[14]</sup>	Turkey	82/83	90 mL NaP/133 mL NaP	300 mg	Capsule containing simethicone given 3 times/d × 5 d	Split dose	Watery or low fiber diet × 5 d
Tongprasert <i>et al</i> <sup>[12]</sup>	Thailand	62/60	45 mL NaP × 2 doses	480 mg	240 mg with each 45 mL NaP	NA	Low residue the day before
Jansen <i>et al</i> <sup>[16]</sup>	United States	177/193	4L PEG/PEG-Asc	200 mg (20 mL 10 mg/mL)	20 mL with the last L of PEG	Split dose	NA
Matro <i>et al</i> <sup>[15]</sup>	United States	62/61	PEG-Asc	400 mg	Added to PEG	Split dose	Low residue for breakfast (the day before), followed by clear liquid
Yoo <i>et al</i> <sup>[11]</sup>	South Korea	130/130	PEG-Asc	400 mg	Added to the last 500 mL of clear fluid	Split dose	Low residue × 3 days/clear liquid the night before
Zhang <i>et al</i> <sup>[9]</sup>	China	289/290	2L PEG	1200 mg	Added to PEG	Day of procedure	Low residue the day before
Bai <i>et al</i> <sup>[10]</sup>	China	294/289	2L PEG	1200 mg (30 mL, 40 mg/mL)	Added to PEG	Day of procedure	Low residue/clear liquid the night before

PEG: Polyethylene glycol; NaP: Sodium phosphate; N/A: Not available.

simethicone improved bowel prep tolerability. Even though no difference was observed between the two groups with regards to nausea or vomiting, simethicone resulted in less abdominal distension ( $P = 0.0003$ ) and showed a trend toward less abdominal pain ( $P = 0.07$ ). This would suggest an increased likelihood of patients completing prep, hence improving the quality of bowel preparation and ultimately reducing the need for repeat colonoscopies.

Our meta-analysis has several strengths. When compared with the two previous meta-analyses conducted on this topic, our sample size was the largest. We analyzed in detail the dose of simethicone, as well as its effect on bowel preparation, colonic bubbles, and ADR. Most of the included studies suggest statistically insignificant trends which, when pooled together, do reach statistical significance. Taking into account the multiple countries included, the patient diversity, and uncomplicated administration of simethicone, we believe these results to be generalizable. The significant heterogeneity between the studies may be a result of multiple factors, including variation in bowel preparation used, dosage and timing of simethicone administration, the different methods used to assess the quality of bowel preparation and the severity of colonic bubbles, and the diet permitted during the few days prior colonoscopy.

There are some limitations. First, as this was a group-level study, it is susceptible to ecological bias. Second, all but one of the studies was only single-blinded. Recent literature has cast doubt on the safety of simethicone use during endoscopy. There are concerns that particles will deposit in the working channel and may be a harbinger of infection despite reprocessing. A recent study by Barakat *et al*<sup>[23]</sup> noted increased ATP bioluminescence after using medium and high doses of simethicone through the water pump and after injecting through the working channel. The clinical relevance of this residue is debatable, and no link with increased infection has been established. Most recent outbreaks of endoscopy-related infection are linked with the difficulty in cleaning the elevator mechanism in duodenoscopes. Major endoscope manufacturers recommend either avoiding simethicone use altogether during endoscopy, or using



**Figure 2** Risk of bias summary for randomized clinical trials.

the lowest concentration necessary<sup>[24]</sup>. The European Society of Gastrointestinal Endoscopy recommends using simethicone in bowel preparation instead of through the endoscope, with the thought that it is less likely to persist in the endoscope<sup>[25]</sup>. However, this area requires further study.

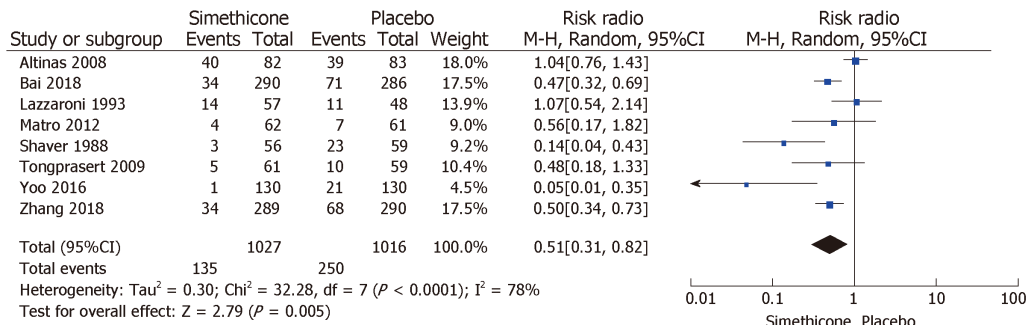
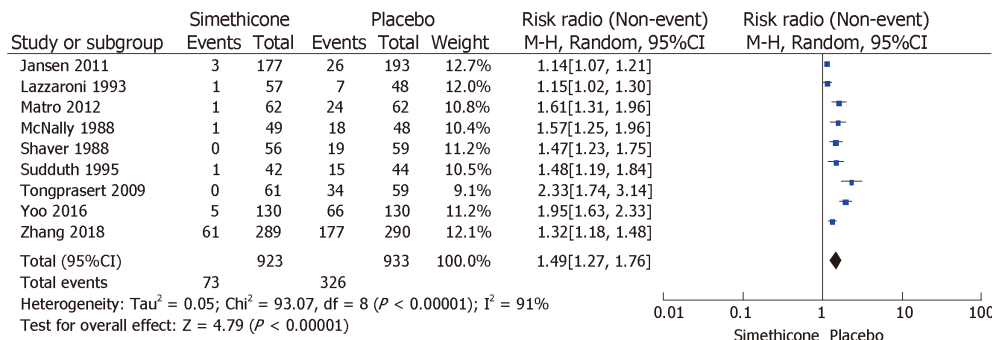
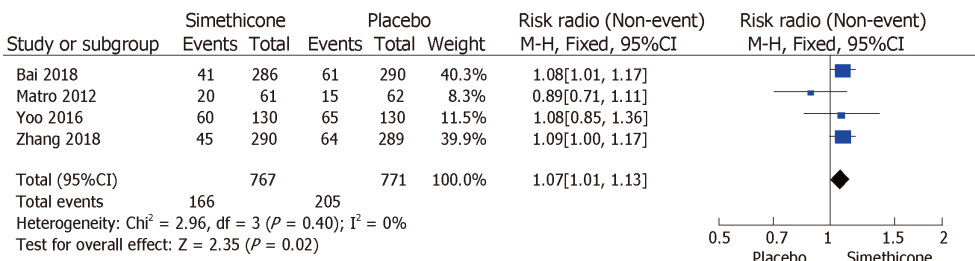
The results bring us to the question; should simethicone be routinely utilized in addition to standard bowel preparation? Although simethicone is already suggested by American<sup>[26]</sup> and European<sup>[25]</sup> clinical guidelines to reduce foaming and to improve tolerability, we feel that simethicone as a colonoscopy adjuvant is currently underutilized by gastroenterologists worldwide. By mixing simethicone with bowel preparation, the need for injection through the endoscope during the procedure would be reduced, allaying concerns about infection.

In conclusion, the addition of medium doses of simethicone to colonoscopy bowel preparation improves the tolerability and quality of bowel preparation and promises improvements in ADR. Simethicone appears to be safe, with a low incidence of adverse events in this pooled analysis.

**Table 2 Sensitivity analyses**

	PEG purgatives	NaP purgatives	Simethicone dose above the mean (478 mg)	Simethicone dose below the mean(478 mg)
Inadequate bowel preparation	0.44 (0.26-0.73), $P = 0.002$ , $n = 6$	0.82 (0.41-1.67), $P = 0.59$ , $n = 2$	0.61 (0.38-0.98), $P = 0.04$ , $n = 4$	0.29 (0.07-1.18), $P = 0.08$ , $n = 4$
Significant bubbles	1.42 (1.2-1.7), $P < 0.0001$ , $n = 7$	1.84 (1.1-2.9), $P = 0.01$ , $n = 2$	1.79 (1.26-2.53), $P = 0.001$ , $n = 3$	1.37 (1.17-1.59), $P < 0.0001$ , $n = 6$
ADR	NA	NA	1.08 (1.03-1.15), $P = 0.003$ , $n = 2$	1.0 (0.85-1.19), $P = 0.98$ , $n = 2$

PEG: Polyethylene glycol; NaP: Sodium phosphate; N/A: Not available;  $n$ : Number of studies; ADR: Adenoma detection rate.


**Figure 3 Forrest plot of the pooled risk ratio of the effect of simethicone on quality of bowel preparation and  $I^2$  statistic for heterogeneity.**

**Figure 4 Forrest plot of the pooled risk ratio of the effect of simethicone on the quality of colonic bubbles and  $I^2$  statistic for heterogeneity.**

**Figure 5 Forrest plot of the pooled risk ratio of the effect of simethicone on adenoma detection rate and  $I^2$  statistic for heterogeneity.**

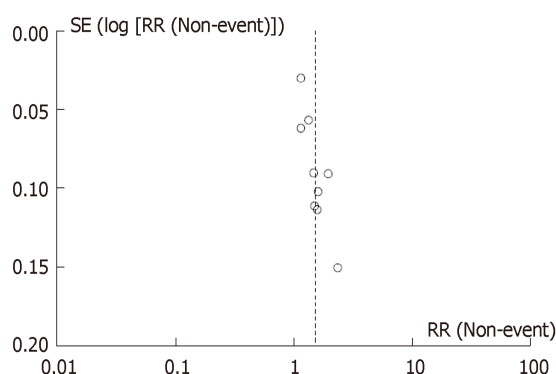


Figure 6 Funnel plot.

## ARTICLE HIGHLIGHTS

### Research background

Colon cancer is the second most common cause of cancer related deaths in both men and women across the world. Colonoscopy is an essential tool that can help screen and prevent it. However, inadequate bowel preparation decreases rate of adenoma detection, increases procedure time; decreasing overall quality of colonoscopy. Antifoaming agents, such as simethicone, may help improve adequate preparation if added to bowel preparation. However, data regarding this is unclear. There is also upcoming data that injection of simethicone through the endoscopy channel may be associated with particle deposition and lead to scope reprocessing infection outbreaks.

### Research motivation

So far, it is unclear whether simethicone is effective in increasing adenoma detection rates (ADRs) in different bowel preparation and there is no data on what dose should be used in bowel preparation.

### Research objectives

To conduct a meta-analysis to help summarize available data for simethicone use during various bowel preparations, confirm the effect on ADR, bowel prep tolerability and investigate an optimal dose.

### Research methods

Studies related to this topic were searched for in multiple databases. Only 11 studies met the strict inclusion criteria. Two reviewers independently scored the identified studies for methodology and abstracted pertinent data. Review Manager 5 was used to analyze the data.

### Research results

We were able to show that addition of simethicone to bowel preparation lead to a significant decrease in inadequate bowel preparation and number of colonic bubbles. This resulted in a significant increase in the ADR as well. We also noted higher doses of simethicone (approximately 500 mg) were more effective.

### Research conclusions

Our study confirms the effectiveness of simethicone use in bowel preparations in helping improve quality of colonoscopies.

### Research perspectives

Whilst searching for literature, we realized few meta-analyses have effectively analyzed simethicone effectiveness in different bowel preparations and looked at optimal dosage. More studies are needed to investigate an association of simethicone use with bowel preparation with scope reprocessing infection.

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## Endoscopic management of biliary strictures post-liver transplantation

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### Abstract

Biliary complications play a significant role in morbidity of liver transplant recipients. Biliary strictures occur between 10%-25% of patients with a higher incidence in living donor recipients compared to deceased donors. Strictures can be classified as either anastomotic or non-anastomotic and may be related to ischemic events. Endoscopic management of biliary strictures in the post-transplant setting has become the preferred initial approach due to adequate rates of resolution of anastomotic and non-anastomotic strictures (NAS). However, several factors may increase complexity of the endoscopic approach including surgical anatomy, location, number, and severity of bile duct strictures. Many endoscopic tools are available, however, the approach to management of anastomotic and NAS has not been standardized. Multi-disciplinary techniques may be necessary to achieve optimal outcomes in select patients. We will review the risk factors associated with the development of bile duct strictures in the post-transplant setting along with the efficacy and complications of current endoscopic approaches available for the management of bile duct strictures.

**Key words:** Liver transplantation; Endoscopic management; Anastomosis; Biliary strictures; Biliary balloon dilation; Biliary stents

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**Core tip:** Biliary strictures occur between 10%-25% of patients with a higher incidence in living donor recipients compared to deceased donors. Strictures can be classified as either anastomotic or non-anastomotic and may be related to ischemic events. Many endoscopic tools are available, however, the approach to management of anastomotic and non-anastomotic strictures has not been standardized. We will review the risk factors associated with the development of bile duct strictures in the post-transplant setting

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## INTRODUCTION

Biliary complications after liver transplantation (LT) is a known and significant cause of morbidity in LT recipients. The incidence of post-LT biliary complications is increasing due to increased volume of transplants and longer survival of LT recipients<sup>[1]</sup>. It is estimated between 5%-35% of LT recipients have biliary complications<sup>[2,3]</sup>. The incidence of complications can be attributed to various techniques of LT including the use of living and deceased cardiac donors, number of donor bile ducts used, and type of surgical anastomosis<sup>[4]</sup>. Most often the donor liver and residual native bile duct are established in continuity with the creation of a choledochocholedochostomy<sup>[5,6]</sup>. However, the presence of primary sclerosing cholangitis (PSC) results in the creation of a hepaticojejunostomy. A roux limb is created and adds to the complexity of endoscopic management of biliary complications and may require the aid of a balloon assisted enteroscope for technical success<sup>[7]</sup>.

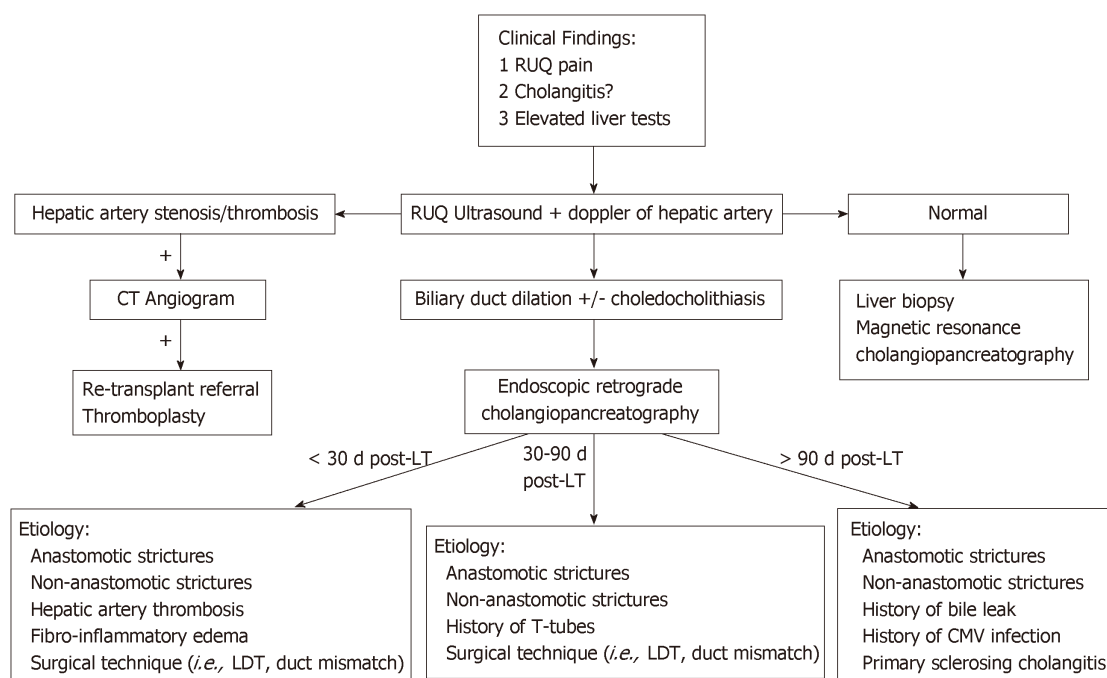
There are a variety of biliary complications that can arise which include the development of anastomotic and non-anastomotic strictures (NAS), bile duct leaks, papillary stenosis, and presence of bile duct stones/casts. Diagnosis is usually made with a combination of non-invasive tests including liver chemistries, abdominal ultrasound, and cross-sectional imaging (computed tomography and/or magnetic resonance cholangiopancreatography). It is important to consider non-obstructive causes of cholestasis including cellular rejection, drug induced cholestasis, or recurrence of primary disease as this may prevent a delay in therapeutic intervention. Advancements in endoscopic techniques and tools have allowed endoscopic management to be the preferred method to manage most biliary complications<sup>[8,9]</sup>. Our review will focus on endoscopic management of biliary strictures that can arise after LT.

## BILE DUCT STRICTURES

There are several risk factors that predispose to the development of bile duct strictures including hepatic artery thrombosis, donor after cardiac death, ABO incompatibility, preservation injury (cold and warm ischemia time), cytomegalovirus infection, duct mismatch between donor and recipient, presence of PSC, bile duct leaks, placement of T-tubes, and living donor transplantation (LDT)<sup>[10-15]</sup>. Bile duct strictures can be noted early (< 30 d), delayed (30-90 d), or late (> 90 d) after LT<sup>[11,16]</sup> (**Figure 1**). Early complications include hepatic artery thrombosis which can result in ductal stenosis and strictures as well as hepatic ischemia<sup>[5]</sup>. Post-operative edema can also result in early ductal stenosis. Delayed and late complications can involve biliary obstruction at the anastomotic site or intrahepatic ducts due to ischemia<sup>[17]</sup>. Bile leaks and recurrence of PSC are risk factors for the development of delayed/late bile duct strictures. T-tubes were previously used more frequently after LT to help maintain the reconstruction of the bile duct anastomosis. However, recent studies have found they may increase the risk of biliary complications including biliary strictures and may be more beneficial for select patients such as those who have a donor-recipient duct mismatch or a bile duct diameter < 7 mm<sup>[18,19]</sup>.

LDT was first performed successfully in 1994 and has been steadily increasing due to limited supply of deceased donors<sup>[20]</sup>. LDT has advantages over deceased donor transplantation (DDT) including reduction of cold ischemia time and improved graft viability<sup>[21,22]</sup>. Nonetheless, there is a higher risk of biliary complications and specifically biliary strictures in LDT *vs* DDT (13%-32% *vs* 5%-15%)<sup>[23-25]</sup>. Incidence of biliary strictures in living donors' range between 0.5%-4%<sup>[26,27]</sup>. LDT is presumed to carry a higher risk of biliary strictures due to the anastomosis of low-caliber and small





**Figure 1 Evaluation of suspected bile duct strictures post-liver transplantation.** LDT: Living donor transplantation; LT: Liver transplantation; RUQ: Right upper quadrant.

ducts as well as increased number of donor ducts needed to establish biliary continuity<sup>[23]</sup>. Bile duct strictures can be categorized as anastomotic or non-anastomotic with differences in endoscopic management and outcomes.

## ANASTOMOTIC BILE DUCT STRICTURES

Anastomotic bile duct strictures (AS) occur in 5%-10% of patients within the first 12 mo of transplantation<sup>[28,29]</sup>. However, they should always be considered in the setting of a cholestatic pattern of liver injury in LT recipients. As opposed to NAS, AS are segmental, shorter, and localized to the site of anastomosis<sup>[23,30]</sup>. Bile leaks may be an independent risk factor for the development of an AS. An AS may form within 60 d after LT due to post-operative edema and fibro-inflammatory response along with transient ischemia<sup>[1,31,32]</sup>. Strictures that form within the first 60 d respond well to 1-2 sessions of endoscopic dilation and plastic stent placement<sup>[1]</sup>.

However, biliary strictures that form after 3 mo have a protracted course and require prolonged endoscopic sessions for adequate response. Endoscopic approaches for anastomotic strictures include balloon dilation, passage dilation with a Soehendra biliary dilation catheter, plastic biliary stents, and self-expandable metal stents (SEMS). A guidewire is used to cross the stricture and balloon dilators from 4-10 mm are used to dilate the anastomosis along with placement of 7 Fr to 11.5 Fr plastic stents bridging the anastomosis. The balloon size used to dilate is predicated upon the diameter of the donor bile duct. Soehendra dilators are useful in patients whom the anastomosis is severely stenosed and can be dilated from 4-10 Fr. In addition, balloon dilation is generally avoided in early strictures (< 3 mo) to avoid perforation or leaks of a recently constructed anastomosis. Most patients with an AS and those who present after 3 mo of LT, require several endoscopic sessions (3-5) for long-term success<sup>[28,33]</sup>. The patency of most plastic biliary stents is 3 mo and thus, endoscopic sessions are performed at 8-12-wk intervals to prevent biliary obstruction<sup>[16]</sup>. The pre-existing stent is removed using a snare or forceps and a cholangiogram is performed to evaluate the patency of the anastomosis. There is no standardized bile duct diameter that corresponds to a clinically significant bile duct stenosis. However, cholangiogram features of a thin focal narrowing with proximal bile duct dilation along with evaluating the resistance encountered with antegrade and/or retrograde biliary balloon sweeps with an 8.5 mm or 11.5 mm biliary balloon across the anastomosis can help determine the patency of the anastomosis. In general, the goal is to dilate the anastomosis with larger sized dilators and in combination with increasing size or number of plastic biliary stents until patency is achieved and a waist

is no longer seen (Figure 2). Combination of balloon dilation and biliary stenting have shown to be more effective than balloon dilation alone<sup>[34,35]</sup>. Balloon dilation alone has a high recurrence rate of stricture formation when compared to balloon dilation and biliary stenting (62% *vs* 31%)<sup>[35]</sup>. LDT has lower success rates of stricture resolution compared to DDT despite similar techniques of balloon dilation plus plastic biliary stents (37%-71% *vs* 75%-91%)<sup>[36-39]</sup>. This may in part be explained due to the use of peripheral ducts and presence of smaller multiple anastomotic strictures<sup>[4]</sup>. Resolution of anastomotic strictures are improved with multiple and maximum number of plastic biliary stents. Several studies evaluating anastomotic stricture resolution in LT recipients found resolution rates to range between 87%-100% with recurrence in 0%-18% of patients<sup>[32,40-43]</sup>. Number of endoscopic sessions to achieve stricture resolution ranged between 3-4 with a complication rate of 1.5%-5%. Complications were primarily related to pancreatitis and cholangitis.

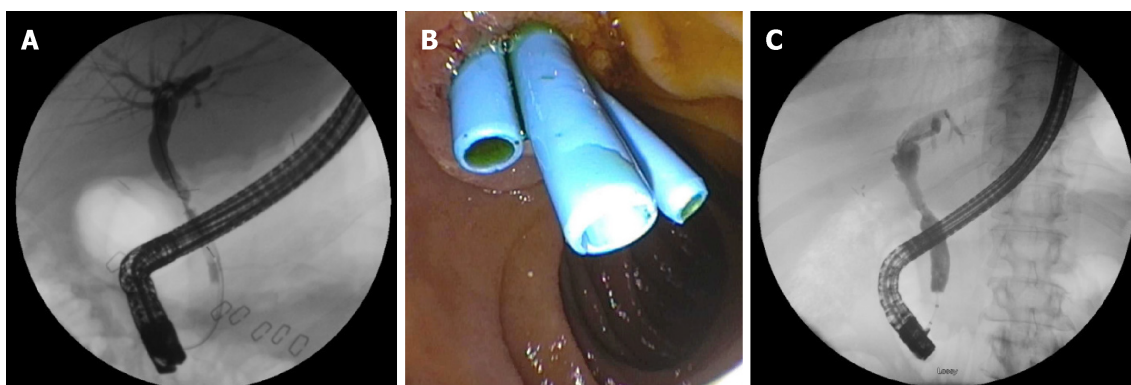
An alternative strategy is to place a SEMS to prevent or reduce the need for frequent ERCPs that is necessary in the setting of plastic biliary stenting. Covered metallic stents have been used as uncovered SEMS may not be able to be removed and may preclude surgical bile duct intervention. In addition, a metallic stent may lead to hyperplasia leading to the formation of sludge/stone formation proximal to the stent<sup>[4]</sup>. The role of covered SEMS has yet to be precisely defined but can be useful because of their larger diameter (10 mm), longer patency, and ability to be removed. However, they are limited because of rates of stent migration (4%-38%). Several studies examining the utility of covered SEMS after LT found resolution rates of anastomotic strictures between 61%-83%<sup>[44-48]</sup>. Recurrence rates were higher in those who received SEMS ranging between 7%-32%<sup>[44-49]</sup>. A randomized trial evaluating covered SEMS and plastic biliary stents found in sub-group analysis of post-transplant patients resolution rates of 89% *vs* 86% with 158 to 194 d till resolution respectively. Stricture recurrence was higher in the covered SEMS group and stent migration occurred more frequently in post-transplant AS compared to all other cases<sup>[50]</sup>. To mitigate the risks of stent migration an alternative is to use partially covered SEMS or stents with special anchoring flanges and anti-migration waists<sup>[51]</sup>. A systematic review of case series including 446 patients by Kao *et al*<sup>[52]</sup> did not find SEMS to have a clear advantage over multiple plastic biliary stents in LT recipients but found stricture resolution was improved in those patients whom the stent duration was longer than 3 mo. A recent meta-analysis of four randomized controlled trials comparing plastic stents to fully covered SEMS found no difference between stricture resolution, stricture recurrence, and adverse events. However, those who received a metal stent did have fewer ERCPs performed as compared to those who had plastic stents<sup>[53]</sup>. Currently, there is no standardized approach for endoscopic management of AS. The use of multiple plastic biliary stents with balloon dilation and fully covered SEMS can provide similar resolution rates of AS after LT with overall low risk of adverse events.

## NAS

NAS of the bile ducts have an incidence of 5%-10% after LT<sup>[33,54,55]</sup>. The definition of a NAS is the presence of stenosis > 5 mm away from the anastomosis and may be located within the intrahepatics, hilum, or anywhere else along the bile duct (including the recipient duct). In contrast to AS, NAS may be multiple and longer in length. Recurrent PSC in the allograft or vascular insufficiency may result in the development of NAS. Vascular ischemia secondary to hepatic artery thrombosis results in biliary destruction and warm and cold ischemia, donation after cardiac death, ABO incompatibility, and chronic rejection are also risk factors for the development of NAS<sup>[1,30]</sup>. NAS tend to occur 3-6 mo after LT though as many as 50% of patients may develop NAS after the first-year post-transplant<sup>[54,56,57]</sup>.

The principles regarding the management of NAS are similar to anastomotic strictures, however, the optimal protocol has not been established. Balloon dilation with placement of plastic biliary stents have shown to be helpful though with less success and longer time to resolution as compared to anastomotic strictures<sup>[33,58]</sup>. Balloon dilation is often not as aggressive as in AS with 4-6 mm biliary balloons commonly used. Overall, resolution rates of NAS range between 50%-75% and are associated with worse graft survival<sup>[30,59]</sup>. However, a study of 48 patients comparing balloon dilation alone *vs* balloon dilation and plastic biliary stents found a significant difference and improvement in stricture resolution in those who only underwent balloon dilation (91% *vs* 31%)<sup>[60]</sup>. This may in part be explained by most of these strictures being located extra-hepatic.

Bile duct strictures involving the hilum and intrahepatics may be more challenging



**Figure 2** Anastomotic bile duct stricture managed with biliary stenting and balloon dilation. A: A patient less than 60 d post-liver transplantation who presented with elevated liver tests and found to have an anastomotic bile duct stricture; B: The patient was managed with serial balloon dilation and multiple biliary stents; C: Approximately 9 mo post-liver transplantation the anastomotic stricture had resolved and required no further intervention.

due to difficulty with traversing the stricture secondary to the small caliber of these ducts as well as tortuosity that may be encountered. Longer, fenestrated stents (Johlin), are flexible and can be used for intrahepatic strictures and allow for adequate drainage *via* multiple side holes and interstent space<sup>[61]</sup>. Covered metal stents have not been readily used as they may impede flow from surrounding bile ducts and potentially increase risk of cholangitis.

NAS may progress despite improvement in liver enzymes in up to two-third of patients<sup>[40,54]</sup>. Progression of NAS is more common in patients who develop NAS within the first year after transplantation or who have recurrent cholangitis<sup>[57]</sup>. Like AS endoscopic resolution rates for NAS in LDT is lower than in DDT 25%-33% *vs* 50%-60% respectively<sup>[39,62]</sup>. Currently there is no standard protocol for management of NAS. NAS are of varying complexity with intrahepatic and hilar strictures providing an especially unique challenge to the endoscopist which may require alternative approaches for access and therapeutic interventions to the bile duct.

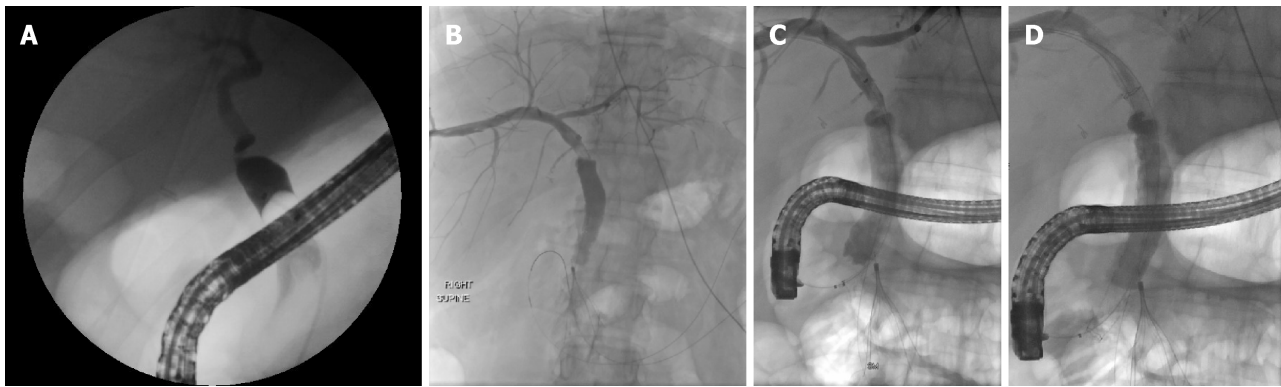
## ALTERNATIVE APPROACHES

Endoscopic methods may not be feasible due to surgical anatomy (bilio-enteric anastomosis), tortuosity and angulation of the bile duct, or severity and location of the stricture which prevents a guidewire or dilation devices to traverse the stricture. Roux-en-Y hepaticojejunostomy or roux-en-Y gastric bypass require deep ERCP methods such as balloon-assisted enteroscopy, endoscopic ultrasonography-directed transgastric ERCP, or percutaneous transhepatic cholangiography (PTC). A multi-center trial showed balloon assisted enteroscopy to be successful in two thirds of cases and in 88% of patients in whom the papilla is reached. Single or double balloon assisted enteroscopy may be an alternative before pursuing PTC or surgical alternatives<sup>[63]</sup>.

A rendezvous technique may also be used which combines PTC and an endoscopic transpapillary approach to access the bile duct and traverse the stricture that otherwise may have failed with conventional endoscopy (Figure 3). PTC in cases of benign bilio-enteric anastomotic strictures are reported to have an overall success rate of 80%<sup>[64]</sup>. It is also especially helpful in those with intractable or multiple intrahepatic strictures as internal-external stents can be placed and relieve the obstruction. In addition, the potential of swing-tip cannulas in accessing tight intrahepatic strictures have been reported and may also help achieve faster cannulation of the bile duct<sup>[65,66]</sup>.

Single-operator peroral cholangioscopy can also be used in the treatment of bile duct strictures by providing direct visualization of the lumen of the bile ducts. Direct visualization of the inside of the bile duct may help predict outcomes of endoscopic therapy based upon the pattern and severity of edema and inflammation seen<sup>[67]</sup>. In addition, direct visualization can also be used in conjunction with the rendezvous technique to puncture the bile duct and safely traverse a completely obstructed duct<sup>[68,69]</sup>.

Magnetic compression anastomosis (MCA) is a rescue technique used in the setting of complete biliary obstruction. A magnet is advanced to the site of the stricture *via* ERCP and another magnet is advanced percutaneously *via* PTC. Fluoroscopy is used to properly align the magnets and a hole in the center of the magnets allow a guidewire to be advanced. Recanalization can be achieved *via* PTC and serial biliary



**Figure 3 Anastomotic bile duct stricture treated with rendezvous technique.** A: A 56-year-old patient who presented two years after transplantation with jaundice and found to have a severe anastomotic stricture which was not able to be traversed with a guidewire; B: Percutaneous transhepatic cholangiogram showing a stricture at the anastomosis with the guidewire inserted through the transhepatic tract; C: The rendezvous technique was used to advance the endoscopic catheter over the transhepatic guidewire and proximal to the anastomotic stricture; D: A fully covered metal biliary stent was placed traversing the anastomosis.

stenting can be performed. Magnet approximation and recanalization have been reported to be successful in 84% and 77% of patients respectively. MCA has been shown to be effective for short strictures (< 1 cm) with a low stricture recurrence rate<sup>[70,71]</sup>.

## CONCLUSION

Biliary strictures play a significant role in morbidity of LT recipients. There exists a variety of techniques to approach anastomotic and NAS. However, despite the use of balloon and passage dilators along with plastic and metal stents, there is no standardized method to approach intrahepatic, hilar, or extra-hepatic bile duct strictures. In addition, patients with altered surgical anatomy and increasing use of LDT add to the complexity of providing successful outcomes. Nonetheless, endoscopic management of anastomotic and NAS are predominantly successful with relatively low complication rates. Further larger and comparative trials along with the advent of more endoscopic tools may allow for increasing rates of success and improved times till resolution of biliary strictures.

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## Anti-inflammatory properties of antidiabetic agents

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### Abstract

The reciprocal relationship between hyperglycemia and inflammation in the setting of diabetes mellitus has been the subject of extensive research. Insulin resistance, the hallmark of diabetic metabolic dysregulation, has been linked to the inflammatory cascade occurring mainly in adipose tissue. The main pathophysiologic processes facilitating the aforementioned interplay, is a phenotype switch of macrophages to the M1 class following gluco- and lipotoxicity and gut microbial remodeling. Given the correlation between inflammation and metabolic abnormalities, the elucidation of the exact mechanisms linking the two along with exploring the possible role of modulation of one in order to alter the other, could open up the possibility of novel therapeutic approaches for diabetes mellitus and its complications. Therefore, the aim of this review is to summarize the growing body of evidence concerning the molecular basis and results of pro-inflammatory processes in diabetic subjects along with the effect of current antidiabetic treatment options on tissue inflammation.

**Key words:** Inflammation; Adipose tissue; Anti-inflammatory; Type 2 diabetes mellitus; Antidiabetic drugs

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**Core tip:** In this review, we aim to create a concise overview of the interplay between hyperglycemia and inflammation, while describing the immunomodulatory potential of each antidiabetic drug and its effects exerted in the inflammatory cascade in subjects with type 2 diabetes.

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## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is currently considered a worldwide epidemic. It is regarded as one of the most important chronic conditions because of the high disease prevalence and its debilitating chronic complications, responsible for elevated indexes of morbidity and mortality. According to World Health Organization, the number of people affected by diabetes in 2014 has approximately quadrupled since 1980, worldwide. In detail, the age-standardized prevalence of diabetes in adults has nearly doubled since 1980, reaching 8.5%<sup>[1]</sup>.

While the development of diabetes and its complications is a multifactorial process, the interplay between innate and acquired immunity in the pathogenesis of metabolic diseases has been attracting increasing research interest, mainly in the context of seeking novel treatment approaches and, ultimately, a causative therapy. Inflammation has been speculated to play an important role, central to the pathophysiologic dysregulation of the pancreatic islet in type 1 diabetics. Furthermore, growing evidence suggests that inflammation also affects the pathogenetic process of T2DM, modulating processes like obesity-related insulin resistance, impaired insulin secretion, and diabetes-related vascular dysfunction<sup>[2]</sup>. Furthermore, it is now understood that inflammation plays a major role in the pathogenesis of cardiovascular disease with ongoing research in the field of prevention of coronary artery disease by use of anti-inflammatory drugs<sup>[3-6]</sup>. Therefore, the purpose of this review is to discuss the potential anti-inflammatory effects of currently available antidiabetic medications in relation to the disruption of metabolic homeostasis.

## TYPE 2 DIABETES AND INFLAMMATION

Adipocytes are the main site of interplay between inflammation and insulin resistance in T2DM. The immunomodulatory role of adipose tissue has now been well-described, as adipocytes not only produce various adipocytokines that can interfere with insulin production and sensitivity but interact in close communication with the immune cells surrounding them<sup>[7]</sup>. Adipose tissue macrophages affect tissue remodeling and metabolic balance through presenting with an M2 phenotype in lean fat<sup>[8]</sup>. The activity and expression patterns of M2 macrophages depend heavily on cytokine signaling cascades, namely those including interleukin (IL)-4 and IL-13. Macrophages shifted to the M2 phenotype produce arginase and IL-10<sup>[9]</sup>. In obesity, macrophages proliferate and shift to the M1 phenotype, activated by pro-inflammatory cytokines. M1 macrophages express CD11c and produce tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6, and reactive oxygen species (ROS)<sup>[9]</sup>. The accumulation of M1 macrophages is incremental in the development of insulin resistance<sup>[7]</sup>. Adipose tissue inflammation can also be induced by localized decreased oxygen perfusion in tissues rapidly expanding with disproportional to the proliferation vascular adaptation<sup>[10]</sup>.

In diabetes, hyperglycemia and elevated levels of free fatty acids (FFAs) may act as proinflammatory stimulants through the induction of glucose utilization and modulating the process of oxidative phosphorylation<sup>[11,12]</sup>. Such metabolic dysregulation has been shown to induce a proinflammatory shift in adipose, islet and vascular tissue-related anti-inflammatory cells<sup>[7-9]</sup>. Glucotoxicity and lipotoxicity fuel processes induce oxidative and endoplasmic reticulum stress, further initiating inflammation by activation of thioredoxin-interacting protein and the NLR family, pyrin domain containing 3 (NLRP3) inflammasome, which increase the release of active IL-1 $\beta$ <sup>[11-14]</sup>. IL-1 $\beta$  plays an important initiator role in the inflammatory cascade, recruiting macrophages and other cells of the immune response ("auto-stimulation")<sup>[14]</sup>. The same interplay between metabolic dysregulation and inflammation occur between other tissue and cell types in the pancreas and circulatory system<sup>[13,14]</sup>. In T2DM, amyloid depositions in pancreatic islets induce inflammation through NLRP3 inflammasome formation and the production of IL-1 $\beta$ <sup>[15]</sup>. Increasing stress and inflammation, instigated in a positive-feedback manner, trigger cellular pro-apoptotic cascades and  $\beta$ -cell impairment, insulin resistance, and arterial atheromatosis.

Additionally, obesity is associated with alterations in the gut microbiome leading to

functional changes of inherent gut homeostasis, with bacterial wall lipopolysaccharides (endotoxins) further promoting tissue inflammation<sup>[16]</sup>. Endotoxins, FFAs and cholesterol have a pro-inflammatory capacity through the activation of Toll-like receptor (TLR) pathways and, subsequently, nuclear factor- $\kappa$ B (NF- $\kappa$ B)-mediated cytokine and chemokine signaling including TNF- $\alpha$ , IL-1 $\beta$ , IL-8, and monocytes chemoattractant protein-1 (MCP-1) promoting immune cell attraction in several tissue types<sup>[17]</sup>. It has recently been reported that in obesity, gut microbiota aberrant growth patterns might affect the innate and acquired immune system responses, thereby promoting insulin resistance<sup>[18]</sup>.

The interplay of metabolism and inflammation could justify the theory that metabolic dysfunction amelioration through lifestyle modification and pharmaceutical intervention could attenuate inflammation. Current antidiabetic treatments induce normoglycemia by acting on several different pathways. Many of these treatments also exert anti-inflammatory effects that might be mediated *via* their hypoglycemic and hypolipidemic capacities or by directly modulating the immune system. Below, we gather and discuss the current data on the anti-inflammatory properties of antidiabetic medications.

## ANTIDIABETIC TREATMENT AND INFLAMMATION

### Metformin

Metformin is considered a first-line treatment for T2DM in almost all guidelines and expert recommendations issued worldwide. The molecular mechanisms behind the pharmacologic activity of metformin appear to be rather complex and remain controversial. However, it is universally accepted that metformin phosphorylates and activates AMP-activated protein kinase (AMPK)<sup>[19]</sup>. In the liver, the AMPK cascade activates fatty acid oxidation with inhibition of cholesterol and triglyceride synthesis<sup>[19]</sup>. Peripheral effects include the activation of fatty acid oxidation and glucose uptake in skeletal muscle as well as a systemic increase in insulin sensitivity<sup>[19]</sup>.

It has been reported that metformin increased nitric oxide (NO) synthesis via activation of AMPK<sup>[20]</sup> and decreased ROS production through inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the respiratory mitochondrial chain<sup>[21]</sup>. Another study showed that metformin inhibited NF $\kappa$ B activation in the vessel wall and decreased serum C-reactive protein (CRP) level in high-fat-fed atherogenic rabbits<sup>[22]</sup>. Furthermore, Isoda *et al*<sup>[23]</sup> have reported that metformin inhibited NF $\kappa$ B activation through blockade of the phosphoinositide 3-kinase (PI3K)-Akt pathway in human vascular wall cells. Also, in lipopolysaccharide-activated macrophages, metformin inhibited production of the IL-1 $\beta$  precursor molecule and other pro-inflammatory cytokines, while it boosted induction of the anti-inflammatory cytokine, IL-10<sup>[24]</sup>. Another possible mechanism of the anti-inflammatory action of metformin is inhibition of advanced glycation end products (AGEs) formation. Metformin inhibits the formation of AGEs which promote inflammation and ROS (glycoxidation)<sup>[25]</sup>.

Apart from the studies where the molecular effects of metformin were examined *in vitro*, there are also clinical studies. In the Diabetes Prevention Program (DPP) study, metformin slightly reduced the levels of CRP compared with placebo<sup>[26]</sup>. Similar results were provided by the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial, in which metformin and/or a thiazolidinedione (TZD) led to lower plasma insulin, lower plasminogen activator inhibitor type 1 (PAI-1) antigen, lower CRP and lower fibrinogen levels compared with a sulphonylurea (SU) or meglitinide in a population of diabetic patients with coronary artery disease<sup>[27]</sup>. Krysiak *et al*<sup>[28]</sup> have reported that metformin reduced monocyte release of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, MCP-1 and IL-8, as well as plasma CRP level in patients with impaired fasting glucose. On the other hand, in the LANCET Trial: A Trial of Long-acting Insulin Injection to Reduce C-reactive Protein in Patients with Type 2 Diabetes, metformin did not alter inflammatory biomarkers in patients with a short T2DM duration, in spite of glucose regulation<sup>[29]</sup>. In addition, it has been reported that metformin treatment did not change CRP or 8-iso-prostaglandin F2 $\alpha$ (8-iso-PGF2 $\alpha$ ) level in subjects with normal glucose tolerance<sup>[30]</sup>.

Overall, the various results are conflicting and even though metformin seems to have several anti-inflammatory pharmacologic properties *in vitro*, those are not always observed *in vivo*. Therefore, it remains uncertain whether the anti-inflammatory effect of metformin is due to its direct tissue-action or, induced indirectly, through the improvement of insulin sensitivity and hyperglycemia.

### Sulphonylureas

Apart from the potent hypoglycemic effect of SUs, many studies have shown that they may have additional anti-inflammatory potential. Glyburide has been shown to inhibit the NLRP3 inflammasome and subsequent IL-1 $\beta$  activation in macrophages<sup>[31]</sup> while gliclazide, as compared with glibenclamide, decreased the serum levels of soluble intercellular adhesion molecule-1 (sICAM-1), sE-selectin and high sensitive CRP (hsCRP), in a population of diabetic patients<sup>[32]</sup>. Mu-Huo *et al*<sup>[33]</sup> showed that in an animal model of sepsis, glibenclamide pretreatment exerted protective properties on the lung parenchyma by inhibiting both the inflammatory responses and oxidative stress. In addition, glibenclamide reduces pro-inflammatory cytokine production by neutrophils in patients with diabetes in response to bacterial infection<sup>[34]</sup>. Mavridis *et al*<sup>[35]</sup> reported that T2DM patients treated with SU had significantly lower cytokine levels than the insulin-treated.

By contrast, in various head-to-head clinical trials, no significant changes in CRP and other inflammatory markers were observed with SU therapy, whereas significant reductions were found with TZD, pioglitazone and the glucagon-like peptide 1 (GLP-1) receptor agonist (GLP-1 RA) exenatide<sup>[36-38]</sup>. Also, in a recent 52-wk head-to-head study between metformin, gliclazide, and pioglitazone on pro-inflammatory biomarkers, coagulation, and endothelial function, no improvements were seen in the circulating levels of inflammatory markers selected (IL-1, IL-6, and TNF- $\alpha$ ) with SU therapy when compared to the other treatment types, while glycemic control was comparable among all treatment groups<sup>[39]</sup>.

It is notable that while SU appears to have some effect in the expression of various inflammatory cytokines, its anti-inflammatory effect is less potent when compared to metformin or pioglitazone.

### Alpha-glucosidase inhibitors

Alpha-glucosidase ( $\alpha$ -glucosidase) inhibitors are a unique class of antidiabetic medications which, by competitive and reversible inhibition of intestinal alpha-glucosidases, delay carbohydrate digestion and thereby extend the total time of glucose absorption<sup>[40]</sup>. Given the research data suggesting that postprandial glucose load results in a biomarker profile consistent with systemic low-grade inflammation and endothelial dysfunction, with increased levels of hsCRP, IL-6, TNF- $\alpha$ , sICAM-1, soluble vascular cell adhesion molecule 1 (sVCAM-1), E-selectin, and metalloproteinases (MMPs) 2 and 9 in patients with T2DM compared to healthy patients<sup>[41]</sup>,  $\alpha$ -glucosidase inhibitors are expected to have anti-inflammatory potential, justified by their mechanism of action.

Osonoi *et al*<sup>[42]</sup> suggested that miglitol depresses the production and release of inflammatory cytokines/cytokine-like factors in peripheral leukocytes by flattening glucose level fluctuation curves in Japanese patients with T2DM, incrementally more than other  $\alpha$ -glucosidase inhibitors. Emoto *et al*<sup>[43]</sup> studied patients with T2DM and coronary artery disease on a 3-mo regimen of miglitol and demonstrated an improvement in both the insulin resistance index and CRP. Derosa *et al*<sup>[44]</sup> evaluated effects of acarbose in patients with T2DM and found it to be effective in reducing the post-oral-fat-load peaks of various parameters including inflammatory markers such as hsCRP, after 7 mo of therapy.

In a randomized double-blind, placebo-controlled crossover study, acarbose-induced normoglycemia did not affect adiponectin, insulin sensitivity, or pro-inflammatory circulating biomarkers (MCP-1, IL-6, and IL-1 $\beta$ )<sup>[45]</sup>. Similarly, a comparison of pioglitazone *vs* voglibose by Fujitaka *et al*<sup>[46]</sup> showed an improvement in serum adiponectin, hsCRP levels and insulin resistance assessment through the homeostatic model only in the pioglitazone group.

While,  $\alpha$ -glucosidase inhibitors may have some indirect anti-inflammatory properties mainly *via* lowering the post prandial glucose levels, they do not seem to have further immunomodulatory potential.

### Thiazolidinedione

Rosiglitazone and pioglitazone, also known as TZDs, are selective agonists of nuclear transcription factor peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ).

There is plenty of scientific evidence that TZDs act not only as hypoglycemic medications but as anti-inflammatory agents as well. Specifically, PPAR- $\gamma$  is mainly expressed in adipocytes and appears to attenuate pro-inflammatory biomarkers in visceral adipose tissue (VAT) deposits, steatotic liver, atherosclerotic plaques and plasma. Furthermore, *in vitro* results demonstrate that the anti-inflammatory activity of TZDs is partially resulting from their modulatory properties in glucocorticoid nuclear translocation activation, in a PPAR- $\gamma$ -independent manner<sup>[47]</sup>.

TZDs have been shown to decrease inflammatory markers in visceral adipose tissue, liver, atherosclerotic plaques, and circulating plasma<sup>[48]</sup>. Pioglitazone treatment



decreased invasion of adipose tissue by proinflammatory macrophages and increased hepatic and peripheral insulin sensitivity in obese subjects<sup>[49]</sup>. Patients with insulin resistance had a decreased total adipose macrophage population, with a decrease in M1 macrophages and an increase in M2 macrophages with pioglitazone treatment<sup>[50]</sup>. Also, treatment with TZDs attenuated inflammation in nonalcoholic steatohepatitis and in atherosclerotic lesions<sup>[51,52]</sup>.

The notion that PPAR- $\gamma$  activation by TZDs can modulate monocyte and macrophage activity and have an impact on the inflammatory process is supported by *in vitro* research data. Further evidence of this is provided by studies *in vivo*, both in animal models and in humans. Haffner *et al*<sup>[53]</sup> in their study of approximately 300 T2DM patients on a 26-wk rosiglitazone treatment regimen, reported a reduction of at least 20% in plasma CRP levels and MMP-9 and approximately 12% in total white blood cell count. A reduction in MMP-9 was also observed by Marx *et al*<sup>[54]</sup> along with a concomitant decrease in plasma sCD40 levels (another emerging marker of inflammation and cardiovascular risk) in T2DM patients with established cardiovascular disease, following rosiglitazone treatment.

In addition, a recent meta-analysis of 27 randomized controlled trials, found that circulating levels of hsCRP, monocyte chemoattractant protein-1, von Willebrand factor, fibrinogen, and E-selectin were significantly decreased after TZD therapy. However, IL-6, MMP-9, sCD40 ligand, PAI-1 and ICAM-1 were not significantly affected<sup>[55]</sup>. In the PERISCOPE trial, treatment of diabetic patients with known coronary artery disease with pioglitazone resulted in a significantly lower rate of progression of coronary atherosclerosis and a decrease in hsCRP levels, compared with glimepiride<sup>[56]</sup>.

Nonetheless, one could argue that all the aforementioned anti-inflammatory actions of TZDs can be attributed to their effect on glucose lowering. Satoh *et al*<sup>[57]</sup>, following pioglitazone treatment in T2DM, observed a decrease in CRP levels of the same magnitude both in patients who responded to therapy (defined as an improvement in glucose control) and in non-responders. Also, Nitta *et al*<sup>[58]</sup> showed that, compared with glimepiride, pioglitazone reduced coronary arterial inflammation in patients with T2DM or impaired glucose tolerance, even though both agents decreased glucose-control related parameters such as HbA1c and fasting plasma glucose.

TZDs appear to be potent modulators of the inflammatory cascade, independently of their glucose lowering effect. Currently, various studies examine their potential use as immunomodulators outside the setting of diabetes, in normoglycemic subjects with rheumatic and other auto-immune diseases<sup>[59,60]</sup>.

### SGLT-2 inhibitors

Sodium-glucose cotransporter (SGLT) 2 inhibitors improve glycemia by inhibiting reabsorption of glucose in the proximal tubule of the kidney, inducing glucosuria and lowering plasma glucose levels, without inducing hypoglycemia.

In T2DM mice, the SGLT-2 inhibitor ipragliflozin was shown to improve hyperglycemia, insulin secretion, hyperlipidemia, and liver levels of oxidative stress biomarkers and reduce markers of inflammation including IL-6, TNF- $\alpha$ , MCP-1, and CRP levels<sup>[61]</sup>. In another study, short-term luseogliflozin treatment normalized the expression of inflammation-related genes such as F4/80, TNF $\alpha$ , IL-1 $\beta$ , IL-6, ICAM-1, platelet endothelial cell adhesion molecule-1 (PECAM-1), MMP2 and MMP9 in apolipoprotein-E deficient knockout (ApoE KO) mice, while markedly attenuating the progression of atherosclerosis<sup>[62]</sup>. Another study in mice treated with empagliflozin provided similar results<sup>[63]</sup>. Furthermore, empagliflozin reduced M1-polarized macrophage accumulation while inducing the anti-inflammatory M2 phenotype of macrophages within adipose tissue and liver, lowering plasma TNF $\alpha$  levels and attenuating obesity-related chronic inflammation in diet-induced obese mice<sup>[64]</sup>. Also, empagliflozin, alone or in combination with linagliptin, attenuated (nonalcoholic steatohepatitis) NASH development in diabetic mice, through reducing hepatic expression of inflammatory genes (TNF- $\alpha$ , IL-6, and MCP-1)<sup>[65]</sup>. Dapagliflozin also reduced mRNA levels of various cytokines and attenuated the development of diabetic cardiomyopathy in diabetic mice<sup>[66]</sup>.

Moreover, there is evidence that the important renoprotective effect of SGLT-2 inhibitors is partly due to their anti-inflammatory properties. Vallon *et al*<sup>[67]</sup> showed that administration of empagliflozin in diabetic mice not only attenuated glomerular hyperfiltration, albuminuria, but also inhibited diabetes-induced renal expression of inflammation markers, such as NF- $\kappa$ B and IL-6. Hatanaka *et al*<sup>[68]</sup> reported that the administration of dapagliflozin to Akita mice induced an incremental renal macrophage tissue accumulation and attenuated interstitial fibrosis when compared with insulin, despite glycemic control being equally efficient in the two groups, indicating that dapagliflozin exerts renoprotective effects beyond glucose reduction. In addition, studies using cultured proximal tubular cells support the notion that a



decrease in the expression and circulation of pro-inflammatory molecules, such as transforming growth factor- $\beta$ , MCP-1, osteopontin, and ICAM-1, oxidative stress, NADPH oxidase 4 (Nox4) expression and ROS production underlie the major actions of dapagliflozin<sup>[69]</sup>.

In a small study with 32 male diabetic patients empagliflozin and canagliflozin lowered interferon- $\lambda$ , TNF- $\alpha$ , IL-6<sup>[70]</sup>. Sato *et al*<sup>[71]</sup> studied the effect of dapagliflozin on epicardial adipose tissue and observed that treatment with dapagliflozin resulted in a slight reduction of serum PAI and a greater reduction of serum TNF- $\alpha$  in T2DM patients with coronary artery disease. Okamoto *et al*<sup>[72]</sup> studied the effects of dapagliflozin on several biomarkers using a population of 27 obese T2DM patients and showed that dapagliflozin treatment led to a significant increase in serum adiponectin and a mild decrease in CRP. A small decrease in CRP with dapagliflozin treatment was also observed in another study<sup>[73]</sup>. In a post-hoc exploratory analysis of the CANTATA-SU study, changes from baseline in serum leptin, adiponectin, IL-26, TNF- $\alpha$ , CRP, PAI-1, VCAM-1 and MCP-1 were measured in T2DM patients taking metformin, but also receiving either canagliflozin or glimepiride. Canagliflozin shifted the balance of appetite-related hormones, significantly decreasing median serum leptin and increasing median serum adiponectin when compared to glimepiride. Median serum IL-6 was accordingly decreased as well accompanied by a trend towards a slight reduction in hsCRP which, however, contrasted with a modest increase in median serum TNF- $\alpha$  in the canagliflozin group over glimepiride. Despite changes in serum leptin being associated with changes in body weight, there were no notable correlations between changes in adiponectin, IL-6, TNF- $\alpha$  and CRP levels and alterations in body weight and HbA1c<sup>[74]</sup>.

The majority of studies that have been discussed above used animal models. Evidence from clinical trials in human subjects is limited and as a result it is not safe to reach a conclusion regarding the anti-inflammatory effect of this particular category.

#### **DPP-4 inhibitors**

Dipeptidyl peptidase (DPP)-4 inhibitors reduce DPP-4 activity in peripheral plasma, preventing the inactivation of the incretin hormone GLP-1<sup>[75]</sup>. The ubiquitous tissue localization of DPP-4 (monocytes, natural killer cells, macrophages, epithelial and endothelial cells, lung, spleen, pancreas, kidney, liver and intestinal cells) could play a role in explaining the immunomodulatory role of this enzyme. DPP-4 hormone production in macrophages, especially in visceral adipose tissue depots, binds to adenosine deaminase, facilitating, *via* nonenzymatic function, T-cell proliferation and activation. Also, CD26, which can partially act as an *in vivo* DPP-4 mimic, serves as a signaling molecule in T-cell activation and immunoregulation<sup>[76]</sup>.

The DPP-4 inhibitor alogliptin can attenuate TLR-4 mediated extracellular signal regulated kinase (ERK) activation and ERK-dependent expression of MMPs in histiocytes, and inhibit TLR4-mediated IL-6 and IL-1-beta production<sup>[77]</sup>. In human macrophages cultured *in vitro*, the DPP-4 inhibitor sitagliptin significantly increased GLP-1 induced the levels of cyclic adenosine monophosphate (cAMP) in the cytosol, resulting in hindering of NF- $\kappa$ B p65 nuclear translocation and suppression of pro-inflammatory mediator production in response to lipopolysaccharide (LPS)<sup>[78]</sup>. Treatment with linagliptin notably suppressed the activation of the fibrotic process in an experimental model of autoimmune myocarditis mice and was associated with reduced inflammatory cytokine (IL-2, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) gene expression<sup>[79]</sup>. Another study indicated that sitagliptin treatment of obese insulin-resistant mice was associated with an improved metabolic phenotype and concurrent reduction of inflammation in pancreatic islets and adipose tissue<sup>[80]</sup>. In diabetic rats, sitagliptin decreased circulating levels of CRP, MCP-1, TNF- $\alpha$ , IL-6, PAI-1, and suppressed vascular smooth muscle cells proliferation<sup>[81]</sup>.

Surface expression of CD26 on CD4+ and CD8+ T-cells was found to be higher in T2DM patients when compared to healthy controls<sup>[82]</sup>. In a recent study concerning the production of inflammatory mediators, treatment with sitagliptin for 12 wk reduced mRNA expression of CD26, TNF- $\alpha$ , TLR2, TLR4, proinflammatory kinases c-Jun N-terminal kinase-1 and inhibitory  $\kappa$ B kinase, and inhibitor of chemokine receptor CCR-2 in mononuclear cells, as well as of plasma CRP, IL-6, and FFAs<sup>[83]</sup>. Similarly, another study showed that sitagliptin reduced the expression of inflammatory cytokines and improved the unfavorable M1/M2 phenotypes of peripheral blood monocytes in Japanese diabetic patients<sup>[84]</sup>. Treatment with sitagliptin or vildagliptin lowered plasma IL-6, IL-18, TNF- $\alpha$  and nitrotyrosine levels compared with baseline in T2DM patients<sup>[85]</sup>. Furthermore, in a study of subjects with coronary artery disease and uncontrolled T2DM, sitagliptin significantly improved endothelial function and inflammatory state beyond its hypoglycemic action<sup>[86]</sup>. In hemodialysis patients with T2DM, linagliptin decreased levels of prostaglandin E2, IL-6, hsCRP, glycated

albumin, and blood glucose which was associated with an increase in active GLP-1<sup>[87]</sup>. However, on a model of sitagliptin or metformin as add-on therapy to a pioglitazone regimen in patients with poorly controlled T2DM demonstrated that only metformin led to a decrease of body weight and to a faster and superior improvement of insulin resistance and inflammatory parameters, such as adiponectin and TNF- $\alpha$ <sup>[88]</sup>.

In summary, research suggests that all currently available DPP-4 inhibitors have multiple immunomodulatory effects, in a way that is independent of their glucose lowering effect.

### **GLP-1 receptor agonists**

GLP-1 receptor agonists have been shown to activate GLP-1 receptor to increase the intracellular concentration of cAMP in acinar cells of the pancreas, resulting in an increased insulin secretion and decreased glucagon secretion.

In patients with T2DM, treatment with GLP-1 analogs appears to modulate the pro-inflammatory activity of the innate immune system, leading to reduced pro-inflammatory activation of macrophages and consequently the expression and secretion of proinflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 and increased adiponectin. This effect is not dependent on the glycemic or body weight effects of GLP-1<sup>[89]</sup>. With regard to the effects of GLP-1 analogs on CRP, a small placebo-controlled study demonstrated a significant reduction in CRP levels with exenatide<sup>[90]</sup>. Also, in a 12-mo comparative study, exenatide demonstrated a significant decrease in hsCRP compared with SU<sup>[97]</sup>.

Treatment of cultured human islets with exendin-4, a GLP-1 RA, suppressed the expression of inflammatory genes such as NF- $\kappa$ B1(p105), NF- $\kappa$ B2(p100), RelA (also termed p65), TNF receptor superfamily member 1A, and receptor-interacting serine/threonine kinase 2<sup>[91]</sup>. In addition, administration of a recombinant adenovirus producing GLP-1 to ob/ob mice reduced the macrophage population and production of TNF- $\alpha$ , MCP-1, and IL-6 in adipose tissue via inhibition of NF- $\kappa$ B activation and phosphorylation of ERK1/2 and c-Jun N-terminal kinases<sup>[92]</sup>. Also, Arakawa *et al*<sup>[93]</sup> observed that GLP-1 receptor agonists reduced monocyte/macrophage accumulation in the arterial wall by inhibiting the inflammatory response in macrophages, in C57BL/6 or apolipoprotein E-deficient mice apoE(-/-). In another study, exenatide significantly increased the level of IL-10 and decreased both TNF- $\alpha$  and IL-1 $\beta$  in LPS-treated monocytes/macrophages, via activation of protein kinase A<sup>[94]</sup>.

Exendin-4 also prevented macrophage infiltration, and decreased protein levels of ICAM-1 and type IV collagen, as well as decreasing oxidative stress and NF- $\kappa$ B activation in kidney tissue, in a rat model of type 1 diabetes<sup>[95]</sup>. Furthermore, Kim *et al*<sup>[96]</sup>, showed that exendin-4 had an anti-inflammatory, neuroprotective effect in mice after a stroke, through inhibition of COX-2 through modulating JNK signaling-mediated stimulation of islet brain 1. Moreover, exendin-4 treatment reduced hepatic expression of the inflammatory markers TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 and macrophage markers, cluster of differentiation 68 (CD68), and F4/80 in the liver of mice fed a western-type diet<sup>[97,98]</sup>.

In addition, exenatide plus metformin resulted in a significant reduction in CRP and TNF- $\alpha$  compared with baseline<sup>[99]</sup>. In another study, treatment of diabetic patients with exenatide for 1 year significantly reduced increased total adiponectin by 12% and reduced hsCRP by 61% and these changes were statistically independent of the change in total body fat mass and body weight<sup>[100]</sup>. Moreover, Daousi *et al*<sup>[101]</sup> showed that GLP-1 continuous infusion in patients with T2DM was associated with a significant reduction in circulating IL-6 at 120 and 180 min post-administration. In a retrospective analysis of 110 obese patients with T2DM treated with liraglutide, the mean concentration of CRP declined after treatment with liraglutide for a mean duration of 7.5 mo<sup>[102]</sup>.

Overall, the results of a number of studies all agree that GLP-1 RAs present many anti-inflammatory properties via multiple molecular pathways. It is also important to underscore that these immunomodulatory effects seem to be independent of their metabolic effects in weight and glucose lowering.

### **Insulin**

Insulin induces an attenuation of inflammatory processes through several mechanisms, including increased endothelial nitric oxide release and decreased expression of proinflammatory cytokines and immune mediators, such as NF- $\kappa$ B, ICAM-1, and MCP-1, as well as several TLRs<sup>[103]</sup>.

In a randomized parallel-group study in patients with T2DM, serum concentrations of hsCRP and IL-6 were markedly reduced in insulin-treated patients compared with metformin, despite the achievement of similar glycemic control<sup>[104]</sup>. This may suggest that insulin reduces inflammation, irrespectively of its effects on glycemia. In another study, treatment of insulin in patients with poorly controlled T2DM reduced serum

hsCRP levels, without affecting plasma fibrinogen or serum MCP-1 levels<sup>[105]</sup>. In contrast, in the LANCET trial, treatment with insulin compared with a placebo or metformin did not reduce inflammatory biomarker levels despite improving glucose control<sup>[29]</sup>. Also, Jansen *et al*<sup>[106]</sup> observed that patients characterized by a pronounced insulin-associated weight gain had an influx of macrophages into the adipose tissue and higher protein levels of MCP-1, TNF- $\alpha$  and IL-1 $\beta$  after 6 mo of insulin therapy compared with those who had not gained weight.

Overall, the results of the various studies concerning insulin are rather conflicting. It is unclear both whether insulin has notable anti-inflammatory properties and whether or not they correlate with its hypoglycemic effect. The lack of large, randomized, double-blind, controlled trials on the subject, or head-to-head studies with other antidiabetic agents, is a major limitation in drawing safe conclusions.

## CONCLUSION

The inflammatory process and its causal relationship with the pathophysiology of diabetes mellitus and its complications remains a rather complex matter due to the numerous intertwining pathways involved in various tissue types, along with the interpersonal multifactorial variation of the inflammatory response. While the antidiabetic agents and their indications in the treatment algorithm are mainly evaluated based on their glucose lowering attributes, their immuno-modulatory potential, most importantly on M1 macrophages could carry great therapeutic benefit, especially in highly insulin resistant patients. Another point of great interest when discussing the aforementioned attributes of these agents is whether the attenuation of the inflammatory cascade activation is secondary to normoglycemia achievement or independent to glycemic regulation, a differentiation that significantly alters the appropriate setting in which they could be successfully introduced to a particular anti-inflammatory-oriented treatment regimen. Moreover, most of the research data on the subject derives from studies on animal subjects, with large, randomized, double-blind studies lacking at the moment, a fact that does not allow for safe conclusions to be drawn as far as clinical correlation of molecular changes is concerned. In conclusion, there is need for further research quantifying the immunomodulatory capacity of antidiabetic agents, elucidating the mechanisms by which those effects are induced and exploring whether those theoretical alterations in circulatory and tissue cytokine and cell-phenotype patterns can be translated into clinical benefit for diabetes and its complications.

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## Subcellular expression of maspin – from normal tissue to tumor cells

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### Abstract

Maspin or SerpinB5, a member of the serine protease inhibitor family, was shown to function as a tumor suppressor, especially in carcinomas. It seems to inhibit invasion, tumor cells motility and angiogenesis, and promotes apoptosis. Maspin can also induce epigenetic changes such as cytosine methylation, de-acetylation, chromatin condensation, and histone modulation. In this review, a comprehensive synthesis of the literature was done to present maspin function from normal tissues to pathologic conditions. Data was sourced from MEDLINE and PubMed. Study eligibility criteria included: Published in English, between 1994 and 2019, specific to humans, and with full-text availability. Most of the 118 studies included in the present review focused on maspin immunostaining and mRNA levels. It was shown that maspin function is organ-related and depends on its subcellular localization. In malignant tumors, it might be downregulated or negative (e.g., carcinoma of prostate, stomach, and breast) or upregulated (e.g., colorectal and pancreatic tumors). Its subcellular localization (nuclear *vs* cytoplasm), which can be proved using immunohistochemical methods, was shown to influence both tumor behavior and response to chemotherapy. Although the number of maspin-related papers increased, the exact role of this protein remains unknown, and its interpretation should be done with extremely high caution.

**Key words:** Maspin; SerpinB5; Prognosis; Cancer; Tumor suppressor

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**Core tip:** The present paper concentrated on showing different patterns of immunohistochemical expression and mRNA levels of maspin, as presented in published

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studies from 1994 until the beginning of 2019 that were included in the PubMed database. It was shown that maspin, a member of the serine protease inhibitor family, functions as a tumor suppressor or tumor promoter. Its function is organ-related and depends on its subcellular localization. In colorectal cancer specimens, maspin was a helpful marker of budding assessment. In most of the malignant tumors, it was demonstrated to be an independent prognostic and predictive factor.

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## INTRODUCTION

Maspin, also known as SerpinB5, is a member of the serine protease inhibitor family, which was identified by Zou *et al*<sup>[1]</sup> in 1994<sup>[2-4]</sup>. In most of the studies, it acted as a tumor suppressor through inhibitory effects on invasion, motility, and angiogenesis and through stimulation of a mitochondrial apoptosis pathway<sup>[1-4]</sup>. This negative impact on tumor cells is supposed to be p53-linked<sup>[5]</sup>.

Maspin can also induce epigenetic changes like cytosine methylation, deacetylation, chromatin condensation, or histone modulation<sup>[3]</sup>. Recent *in vitro* studies focused on maspin secretion<sup>[6,7]</sup>. These studies tried to prove that maspin is a soluble free or an exosome cargo protein, which might be chemically synthesized and used as a future medical drug<sup>[6,7]</sup>. *In vitro*, maspin influenced the peritumoral micro-environment by enhancing macrophage secretion of inflammatory cytokines<sup>[6,7]</sup>.

In the human body, maspin is expressed in many tissues or organs and is down or upregulated in malignant tumors. As maspin shows different subcellular localizations (cytoplasmic and nuclear), in both normal and tumor tissues, it is difficult to appreciate its exact role in tumorigenesis, tumor invasion, or progression<sup>[8]</sup>. The aim of this review was to perform a complex synthesis regarding maspin expression in different organs, from normal tissue to non-tumor disorders and malignant transformation. The organ-related subcellular expression was also emphasized.

## METHODOLOGY

The present paper represents a narrative review of the literature on the serine protease inhibitor maspin, focusing mainly on its immunohistochemical (IHC) expression in different tissues and pathologic processes.

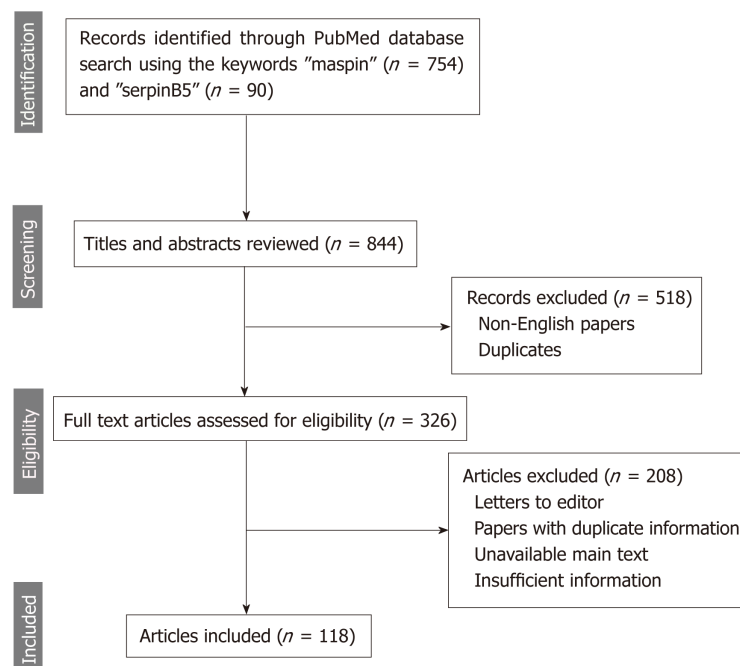
The online search consisted of browsing the PubMed/MEDLINE database using the MeSH terms and keywords “maspin” and “serpinB5” to identify articles published between 1994 and the beginning of 2019. Eligible for inclusion were only publications written in English, studies for human species, and with full-text availability (Figure 1).

Besides the detailed presentation of data, summary tables regarding maspin immunoexpression in different organs in various conditions were constructed based on the data published in the included articles (Tables 1-3).

## TISSUE- AND ORGAN-RELATED MASPIN EXPRESSION, IN NORMAL AND PATHOLOGIC CONDITIONS

### Placenta

Dokras *et al*<sup>[9]</sup> first evaluated IHC expression and mRNA levels of placenta maspin, in 2002. Placentas obtained after first and second trimester pregnancy and after caesarian deliveries at term were included in their observations. The maximum values of maspin mRNA level were detected in the third trimester of pregnancy. On the other hand, negative expression was observed in the immortalized first trimester cytotrophoblasts and choriocarcinoma cell lines with high invasive ability. Similar to the mRNA levels, IHC expression showed patchy staining of the cytotrophoblastic layer in the first trimester, uniform cyto- and syncytiotrophoblastic layers in the



**Figure 1** The methodology (PRISMA flow diagram) used for this review.

second trimester, and more intense expression in the third trimester<sup>[9]</sup> (Table 1).

In preeclamptic (PE) placentas, both mRNA and protein levels were upregulated (Table 1) and correlated with modifications observed with Hematoxylin and Eosin stain<sup>[10]</sup>. It was intimal enlargement of the vessel wall, thickening of the syncytiotrophoblast membranes, and increased number of syncytial knots. It was concluded that hypomethylation of the maspin promoter might be the causal factor of the increased expression of maspin in PE placentas<sup>[10]</sup>.

Qi *et al.*<sup>[11]</sup> evaluated the plasmatic level of unmethylated maspin DNA in a population consisting of women with normal pregnancies, PE, and gestational trophoblastic disease. Unmethylated maspin DNA was not detected in healthy nonpregnant women and in those with the trophoblastic gestational disease. The level was higher in women with severe PE than in those with normal third trimester pregnancies and presented a gradual increase with the gestational age<sup>[11]</sup>.

Methylated and unmethylated maspin DNA blood concentrations may be useful for identification of noninvasive fetal trisomy 18 beginning in the first trimester<sup>[12]</sup>. Methylation of the maspin gene induces downregulation of maspin protein expression and subsequently inhibits migration and invasion of the first trimester extravillous trophoblast cell line through interaction with the proangiogenic factors such as mismatch repair proteins (e.g., MMP2) or vascular endothelial growth factors (VEGF-A and VEGF-C)<sup>[13]</sup>. This interaction might lead to the occurrence of PE<sup>[13]</sup>.

Regarding maspin subcellular localization, nuclear expression was limited to the chorionic plate with significant downregulation in the extravillous trophoblasts<sup>[14]</sup>. Cytoplasmic positivity can be seen in endothelial cells and trophoblasts<sup>[9,14]</sup> (Table 1).

### Mammary gland

Maspin expression was evaluated in both normal tissues, especially during pregnancy and carcinomas of the mammary gland<sup>[15-20]</sup>. In late pregnancy, a peak of expression is seen during lactation and the level decreases and remains constant after the lactation period<sup>[20]</sup>. Almost all cells presented cytoplasmic staining with infrequent nuclear positivity (Table 1), which can be an indicator of epithelial growth factor induced maspin phosphorylation<sup>[20]</sup>.

In breast carcinomas, there are several maspin-related studies, but in most of them no data about the subcellular localization of staining were included. In these tumors, maspin IHC positivity (independently by the localization) was directly correlated with larger tumor size, younger age, high histologic grade, negative expression of estrogen receptor and/or progesterone receptor, positivity for p53, and a lymphocyte-rich stroma<sup>[15-18]</sup>. In other studies, it was hypothesized that maspin is not involved in breast cancer histogenesis, at least in those carcinomas with extremely aggressive behavior<sup>[21]</sup>.



**Table 1** Maspin expression in placenta, mammary gland, and urogenital organs

Organ/ tissue	Subcellular expression in normal tissue	Subcellular expression in pathologic conditions
Placenta	Cytoplasm: Syncytio- and cytotrophoblasts, and endothelial cells; Nucleus: Chorionic plate	Preeclampsia: Upregulation
Mammary gland	Cytoplasm: Myoepithelial cells (intense in pregnancy and lactation); Nucleus: Myoepithelial cells	Invasive breast cancer: Maspin positivity is more frequent in ductal than lobular carcinomas; Cytoplasm only: Negative prognostic indicator, ER and PgR negativity; Nucleus: Better prognosis, ER and PgR positivity; Negativity: Loss or cytoplasm to nuclear translocation in metastatic tissue
Ovary	Negative	Benign tumors: Negative or infrequent nuclear; Ovarian carcinomas: Cytoplasm only: Cisplatin sensitivity; Mixed expression (cytoplasm and nucleus): Indicator of low malignant potential
Uterine cervix	Squamous epithelium: Cytoplasmic and nuclear staining	CIN3: Cytoplasm: Down regulation; Nucleus: Upregulation; Squamous cell carcinoma: Cytoplasm: Tumor suppressor role; Adenocarcinoma: Cytoplasm: Aggressive behavior
Uterine body	Negative or positive (mostly nuclear) staining in normal endometrial glands; Low intensity in atrophic endometrium	Endometrial hyperplasia: Nucleus: Indicator of atypia; Endometrioid endometrial adenocarcinoma: Cytoplasm: Aggressive behavior; Nucleus: Better prognosis
Prostate	Basal cells: Positive; Secretory cells: Negative	HGPIN: Basal cells: Positive (same intensity as normal); Secretory cells: Positive; Adenocarcinoma: Low-grade carcinoma: Reduced expression compared with HGPIN; High-grade carcinoma: Low or no expression
Urinary bladder	Positive in epithelial cells	Urothelial carcinoma: Nucleus: Better prognosis

CIN: Cervical intraepithelial neoplasia; ER: Estrogen receptor; HGPIN: High-grade prostate intraepithelial neoplasia; PgR: Progesterone receptor.

Examination of the subcellular localization (Table 1) revealed that maspin cytoplasmic positivity is observed in 36% of invasive ductal carcinomas and 7% of lobular carcinomas, the latter being mostly maspin-negative<sup>[18,21]</sup>. The nuclear expression is related with estrogen receptor and progesterone receptor positivity, while the cytoplasmic location is an indicator of negativity for hormone receptors, high S-phase fraction, and aneuploidy<sup>[19]</sup>. Maspin cytoplasmic positivity was suggested to be a poor prognostic indicator of invasive breast cancer, independently of the histologic subtype<sup>[19]</sup>.

Machowska *et al*<sup>[22]</sup> presented nuclear location as a better prognostic factor in cases with invasive ductal carcinoma. Nuclear positivity was an indicator of Ki-67 negativity or low expression<sup>[22]</sup>. In a study by Strien *et al*<sup>[23]</sup>, which compares luminal subtype A and B breast cancers, it was shown that maspin expression was lost in metastases, in the majority of maspin-positive primary tumors, or presented translocation from cytoplasmic to nuclear positivity. No differences between subtype A and B were noted.

Wakahara *et al*<sup>[24]</sup>, examining four categories of maspin expression [cytoplasmic only, nuclear only, mixed (cytoplasm + nuclei), and negative] and their correlations with histone deacetylase 1, showed that maspin cytoplasmic only represents an independent negative prognostic factor, thus being an indicator of higher histological grade, negative progesterone receptor expression, shorter disease-free survival, and higher histone deacetylase 1 compared with the mixed expression group. They suggested that inhibition of histone deacetylase 1 could represent an inhibitory mechanism for maspin<sup>[24]</sup>.

Recently, Umekita *et al*<sup>[25]</sup> demonstrated that maspin mRNA expression in sentinel lymph nodes represents an independent factor of nonsentinel lymph node metastasis. Maspin was shown to act upon peritumoral stroma and to increase collagen production as a cause for doxorubicin resistance<sup>[26]</sup>.

### Urogenital system

**Ovary:** Expression of maspin was not present in the normal ovary<sup>[27-30]</sup>. In ovarian carcinomas, maspin expression in over 50% of the tumor cells was associated with higher tumor grade, positive peritoneal effusion cytology, lower survival rate, and positivity for the proangiogenic factors VEGF-A, -C, and -D<sup>[27,28]</sup>. Most of the

**Table 2 Maspin expression in organs of the respiratory and gastroenteropancreatic system**

Organ/ tissue	Subcellular expression in normal tissue	Subcellular expression in pathologic conditions
Lung	Bronchial basal cells: Nuclear staining; Alveolocytes: Negative	Non-small cell carcinomas: Cytoplasm only; Negative prognostic factor; Nucleus only: Low aggressivity
Esophagus	Squamous epithelium: Negative or weak cytoplasm	SCC: Nucleus: Low pTNM stage; Cytoplasm: Risk for lymph node metastases
Stomach	Foveolar and glandular cells: Cytoplasm or negative	Dysplasia: Nucleus: High-grade dysplasia; Carcinomas: Cytoplasm: Better prognosis; Nuclear: Local aggressive behavior; Negative: Risk for distant metastases or neuroendocrine component
Colon and rectum	Normal mucosa: Cytoplasm or negative	Dysplasia: Nucleus: High-grade dysplasia; Adenocarcinoma: Cytoplasm only: Low-grade tumor, low risk for metastases, high chance for MSI-H status; Nuclear only: High pTNM stage, high-grade budding; Negative: Risk for distant metastases or neuroendocrine component
Liver and intrahepatic biliary ducts	Negative in most of the normal hepatocytes and in normal biliary ducts	Carcinoma: Positive (cytoplasmic, nuclear or mixed cyto-nuclear expression), with unknown significance
Pancreas	Negative in exo- and endocrine pancreas	PanIN grade 1 and grade 2: Negative; PanIN grade 3 and PDAC: Positive (cytoplasmic and nuclear staining); Endocrine tumors: Negative; Ductal adenocarcinoma: Nuclear
Gallbladder	Negative or positive (cyto-nuclear staining)	Dysplasia: Negative or weak staining; BillIN, carcinoma: Cyto-nuclear expression gradually increases from normal epithelium to BillIN and carcinoma

BillIN: Biliary intraepithelial neoplasia; MSI-H: High microsatellite instability; PanIN: Pancreatic intraepithelial neoplasia; SCC: Squamous cell carcinoma; PDAC: Pancreatic ductal adenocarcinoma

malignant tumors presented with cytoplasmic only expression, but those with low malignant potential showed mixed positivity (cytoplasm and nucleus)<sup>[29]</sup>. The localization of maspin expression might have therapeutic importance because the cytoplasmic positivity associates with cisplatin sensitivity<sup>[30]</sup> (Table 1).

**Uterine cervix:** Maspin is expressed both in the cytoplasm and nucleus of the normal squamous cervical epithelium<sup>[31,32]</sup>. The cytoplasmic expression is downregulated in premalignant disorders such as cervical intraepithelial neoplasia grade 3 and even more downregulated from microinvasive to invasive squamous cell carcinoma (SCC)<sup>[31,32]</sup>.

In SCC, cytoplasmic maspin can be colocalized with cytoplasmic testisin, a serine protease normally found in testicular germ cells, which inhibits the tumor suppressor activity of maspin<sup>[33]</sup>. Maspin positivity is correlated with advanced stage, increased lymphatic microvessel density, and the presence of lymph node metastases<sup>[31,32]</sup>. Nuclear expression increases in cervical intraepithelial neoplasia grade 3 but significantly decreases in SCC cells<sup>[31,32]</sup>. Maspin expression is decreased or lost in intravascular emboli from SCCs<sup>[31,32]</sup>. In adenocarcinomas of the uterine cervix, cytoplasmic expression of maspin was found to be an indicator of aggressive behavior<sup>[34]</sup> (Table 1).

**Uterine body:** The normal endometrium is maspin negative or localizes to the nucleus<sup>[35-37]</sup>. Maspin is positive in most of the cases diagnosed as atypical hyperplasia or endometrioid endometrial adenocarcinoma (nuclear and/or cytoplasmic staining). Maspin expression is also correlated with lymph node metastases and FIGO stage in endometrioid endometrial adenocarcinoma<sup>[35-37]</sup>. Nuclear subcellular localization was correlated with squamous cell differentiation of endometrioid endometrial adenocarcinoma and with better prognosis, while concurrent cytoplasmic positivity represents an indicator of a more aggressive tumor<sup>[36,38]</sup> (Table 1).

**Prostate gland:** In normal prostate, maspin marks basal but not secretory cells<sup>[39-43]</sup>. Its expression is upregulated in high-grade prostatic intraepithelial neoplasia and downregulated during progression to invasive carcinoma<sup>[41]</sup> (Table 1). In prostate carcinomas, maspin exerts a tumor suppressing role<sup>[39,40]</sup>. Negative or decreased IHC

**Table 3** Maspin expression in brain, organs of the head and neck area, skin and soft tissues

Organ/ tissue	Subcellular expression in normal tissue	Subcellular expression in pathologic conditions
Brain	Positive in nucleus and cytoplasm	Decreased expression in parallel with the advancing glioma stage
Head and neck	Cytoplasm: Oral cavity epithelium and temporal bone; Nucleus: Salivary glands: Myoepithelial cells, basal cells of the ducts and some luminal cells; Negative: Salivary glands: Secretory cells	Oral SCC: Cytoplasm (better prognosis); Temporal bone SCC: Negative, cytoplasm-only or cyto-nuclear; Salivary glands: Pleomorphic adenoma (cyto-nuclear or cytoplasmic only), Warthin's tumor (cyto-nuclear or cytoplasmic only, weaker than in pleomorphic adenoma), adenoid cystic carcinoma, mucoepidermoid carcinoma (cytoplasmic only, negative in an anaplastic variant of adenoid cystic carcinoma); Laryngeal SCC: Cytoplasm and nucleus
Thyroid	Negative	Negative follicular adenoma, follicular carcinomas, poorly and undifferentiated carcinomas; Cytoplasm: Papillary thyroid carcinoma
Skin	Cytoplasmic: Normal epidermis; Nuclear: Myoepithelial cells of the sweat glands and mature sebaceous glands	SCC: Cytoplasm expression in low stages and nuclear staining in dedifferentiated tumors; Basal cell carcinoma: Cytoplasm and nucleus; Melanoma: Nuclear expression is an indicator of aggressiveness
Soft tissue	Negative	Inflammation: Negative; Lipoma, atypical lipomatous tumor: Cytoplasm; Sarcomas: Cytoplasm or nucleus, as indicators of aggressive behavior

SCC: Squamous cell carcinoma.

expression was correlated with p53 positivity and a higher tumor grade and stage<sup>[39,40]</sup> (Table 1).

Positive immunostaining was noted in tumors that showed a histological response to therapy administered before prostatectomy<sup>[39,40]</sup>. Maspin also proved its ability to enhance the sensitivity of hormone-resistant prostate cancer cells to curcumin treatment by modulating levels of proapoptotic proteins Bad and Bax<sup>[42]</sup>. The experimental studies proved that maspin can influence prostate carcinoma host immune response through stimulation of neutrophil maturation at both the systemic and intratumoral levels along with antibody-dependent cytotoxicity and decreased lymphatic vessels formation<sup>[43]</sup>.

**Urinary bladder:** In normal bladder, maspin expression can be seen in epithelial cells<sup>[44-47]</sup>. Maspin downregulation in bladder carcinoma cells has been shown to be significantly associated with a lower progression-free survival rate<sup>[44-46]</sup> (Table 1). Elevated levels inhibited proangiogenic factors such as insulin-like growth factor binding protein-2 or VEGF-C and upregulated the apoptosis rate of cancer cells<sup>[44-46]</sup>. Maspin increased the sensitivity of bladder cancer cells to cisplatin therapy by enhancing its inhibitory effect on tumor cell proliferation<sup>[44-46]</sup>.

Induction of maspin was suggested to be the mechanism through which Prostate-derived E-twenty six factor (decreased in tumor cells compared with the normal bladder) inhibit tumor development and invasion along with repressing epithelial-mesenchymal transition by upregulating E-cadherin expression and downregulating vimentin, SNAIL, SLUG, and N-cadherin<sup>[47]</sup>.

Studies of IHC expression observed contradictory results. In some studies, maspin was mostly positive in low-grade tumors and associated with better survival. Others showed an important increase in maspin expression in high-grade bladder tumors<sup>[8]</sup>.

### Lung

In normal bronchial cells, maspin expression can be seen in the nuclei of basal cells<sup>[48-52]</sup>. In non-small cell carcinomas, both SCC and adenocarcinomas, subcellular localization of maspin proved to be correlated with some clinicopathological parameters (Table 2).

Cytoplasmic expression was an independent negative prognostic indicator and was correlated with the micropapillary component, higher pTNM stage, shorter disease-free survival, and low disease-specific survival<sup>[48-51]</sup>. On the other hand, nuclear only staining (without synchronous cytoplasm positivity) was correlated with earlier

pathological stage, absence of aggressive invasion, and negative p53<sup>[48-51]</sup>. Maspin mRNA expression appeared to be upregulated in adenocarcinoma cells compared to the adjacent normal lung with higher levels of mRNA in advanced stages<sup>[52]</sup>.

### Gastrointestinal tract

**Esophagus:** Maspin can show infrequent cytoplasmic positivity in squamous cell epithelium<sup>[53-55]</sup> (Table 2). In SCC cells, downregulation of maspin was noted compared with the adjacent normal epithelium. Strong nuclear staining is associated with favorable prognosis, increased patient survival, and a lower pTN stage while high cytoplasmic staining correlates with the presence of lymph node metastases<sup>[53,54]</sup>. Based on an *in vitro* study, which used esophageal SCC cell lines, it was hypothesized that the inhibitory effect of maspin is based on switching the metabolic phenotype to low glycolysis through disrupting the hypoxia inducible factor 1 $\alpha$ <sup>[55]</sup>.

**Stomach:** Maspin expression can be absent or in the cytoplasm of normal epithelium and increases in gastric epithelial cells with intestinal metaplasia likely as a result of demethylation of the maspin gene promoter<sup>[56-62]</sup>. Nuclear maspin marks cells with high-grade intraepithelial neoplasia and is one of the factors that plays a role in the progression of intramucosal clusters of signet ring cells to signet ring cell carcinoma, especially multifocal carcinomas<sup>[57,58]</sup> (Table 2).

In gastric adenocarcinoma cells, maspin expression can be lost, which is an indication of a high risk for distant metastases<sup>[21,59]</sup>. Complete loss of maspin was also observed more frequently in elderly patients, poorly cohesive carcinomas, and poorly differentiated adenocarcinomas located in the distal part of the stomach<sup>[58-61]</sup>. Maspin is negative in neuroendocrine components of adenocarcinomas and is not involved in tumorigenesis of gastric neuroendocrine tumors<sup>[62]</sup>.

Cytoplasm only staining is rarely seen in clinical practice in poorly cohesive carcinomas<sup>[58]</sup>. In adenocarcinomas, cytoplasm expression is as an indicator of lower pTNM stage and high angiogenic phenotype and correlates with positivity for p53, Bax, Ki-67 and E-cadherin<sup>[58]</sup>.

Nuclear positivity (with or without associated cytoplasm expression) is predominant in undifferentiated intestinal type carcinoma and poorly cohesive carcinoma<sup>[58]</sup>. Nuclear positivity is associated with locally aggressive behavior and high risk for lymph node metastases and is more frequent in young patients<sup>[21,58,59,62]</sup>. It is associated with Bax, p53, and Ki-67 negativity and lower angiogenesis<sup>[58]</sup>. In daily practice, we use nuclear maspin for a better approach of the depth of invasion (pT stage) of poorly cohesive carcinomas (personal unpublished observations).

**Colon and rectum:** Similar to the gastric epithelium, in colorectal segments maspin expression can be absent or present in the cytoplasm of normal epithelium and increases in epithelial cells with high-grade dysplasia<sup>[62-73]</sup>. Maspin serum levels are increased in patients with high-grade dysplasia and carcinomas and might be used as an indicator for colonoscopy<sup>[69,70]</sup>.

In colorectal segments, maspin does not mark neuroendocrine tumors<sup>[62]</sup>, but its subcellular expression has a great value in the assessment of adenocarcinomas<sup>[72,73]</sup>. Although there are studies that proved that maspin expression is correlated with carcinoembryonic antigen serum levels, infiltrative borders, and high histological grade and stage in colorectal adenocarcinomas<sup>[65]</sup>, few articles regard maspin subcellular expression. We use this marker for daily diagnosis and data showed in this review are based on personal observations (over 200 cases were revised) and literature data (Table 2).

Maspin cytoplasmic only positivity is correlated with the absence or a low number of lymph node metastases, low-grade buddings, and absence of p53 positivity<sup>[72,73]</sup>. It is important to consider a case with cytoplasmic only staining is necessary to have no nuclear positivity (in both tumor center and invasion front).

Maspin nuclear staining is an indicator of aggressive tumor behavior, high tumor grade, high budding grade, high pTNM stage, high risk of local recurrence, or lymph node metastases and absence of peritumoral lymphoid reaction and p53 and VEGF-A positivity<sup>[63,64,72,73]</sup>. As the maspin nuclear expression is characteristic for tumor buds<sup>[73]</sup>, we use this marker to diagnose patients because maspin is more efficient than cytokeratins (personal observations). For stage II and III colon cancer patients, nuclear maspin staining is an independent predictor of sensitivity for adjuvant chemotherapy with 5-fluorouracil and levamisole<sup>[67,68,74]</sup>.

Although it was postulated that elevated nuclear maspin is associated with microsatellite instability<sup>[63]</sup>, we observed that it is associated with low microsatellite instability. The high microsatellite instability (MSI-H) cases usually show cytoplasmic or mixed (cyto-nuclear) maspin positivity<sup>[72]</sup>. In a few cases, nuclear predominance can be seen in MSI-H cases, but this pattern is observed in p53 negative carcinomas

only<sup>[74]</sup>. It was even suggested that MSI-H carcinomas with nuclear maspin might respond to 5-fluorouracil-based therapy<sup>[74]</sup>. For patients who received preoperative neoadjuvant chemoradiotherapy, maspin should be downregulated as a result of chemotherapeutic influence<sup>[66]</sup>.

Combining the microsatellite status with BRAF mutation and IHC expression of p53 and maspin, a classification of colorectal cancer was proposed with the best prognosis attributed to MSI-H/BRAF mutated/p53 negative cases with a high number of tumor infiltrating lymphocytes and cytoplasmic maspin expression<sup>[71]</sup>. Cases with the worst prognosis were described as those being MSS/BRAF mutated/p53 positive with low tumor infiltrating lymphocytes and nuclear maspin staining predominance<sup>[71]</sup>. Similar to gastric carcinomas, loss of maspin expression is an indicator of a neuroendocrine component or high risk for distant metastases<sup>[72]</sup>.

### **Hepatic and pancreatic system**

**Liver and intrahepatic bile ducts:** Maspin infrequently marks hepatocytes and biliary epithelium<sup>[75-78]</sup>. It is downregulated in hepatocellular carcinoma cells (Table 2), but the exact mechanism is still unknown. Maspin downregulation can be the result of activation of inhibitor  $\kappa$ B kinase  $\alpha$  by HBx protein and can induce chemoresistance<sup>[75]</sup>. Decreased maspin expression along with increased VEGF-A expression may be induced by overexpression of chloride intracellular channel 1<sup>[76]</sup>. Yang *et al*<sup>[77]</sup> identified a correlation between patients with a C allele polymorphism of maspin rs2289520 and high Child-Pugh grade (B/C)<sup>[77]</sup>.

In intrahepatic cholangiocarcinomas, a delayed progression was shown to be the benefit of maspin and Bax coexpression<sup>[78]</sup>.

**Pancreas:** Normal pancreatic ducts, Langerhans islets, endocrine tumors, and low-grade lesions of the pancreas do not express maspin<sup>[79-82]</sup>. Maspin is localized in the cytoplasm and nucleus of the premalignant lesions such as pancreatic intraepithelial neoplasia (PanIN) grade 3 and also in pancreatic ductal adenocarcinoma (with/without cystic changes; with/without mucinous component) (Table 2) along with strong expression of carcinoembryonic antigen and p53<sup>[79-82]</sup>.

In chronic pancreatitis, which causes diagnostic problems, the presence of an unmethylated maspin promoter can be used to differentiate this lesion from pancreatic ductal adenocarcinoma (PDAC)<sup>[83]</sup>. In a recent meta-analysis, maspin and trefoil factor 1 were found to display significantly higher blood plasma levels in PDAC compared to normal tissue<sup>[84]</sup>. Overexpression of maspin was confirmed by RT-PCR in PDAC and normal adjacent pancreatic tissue<sup>[85]</sup>.

**Gallbladder:** Although maspin is mostly negative in normal epithelium, it might be helpful for differentiating a malignant tumor from atypical reactive changes of the bile ducts (Table 2) in combination with p53<sup>[86-91]</sup>. Maspin shows gradually increasing cyto-nuclear expression from regenerative atypia to biliary intraepithelial neoplasia with significant upregulation in carcinomas<sup>[86]</sup>. The stepwise rise in maspin level from normal epithelium to gallbladder carcinoma is also reflected in its mRNA level<sup>[87]</sup>.

For bile duct biopsy specimens, the use of an immunomarkers complex was also proposed consisting of maspin, insulin-like growth factor-II mRNA binding protein-3, S100P, and von Hippel-Lindau gene product. Positive reactions for maspin, S100P, insulin-like growth factor-II mRNA binding protein-3 along with negativity for von Hippel-Lindau gene product was suggested as a specific staining pattern for bile duct adenocarcinoma<sup>[88,89]</sup>. Double IHC expressions for maspin (nuclear and cytoplasmic) and claudin-18 (membrane) may improve the diagnostic sensitivity to differentiate a bile duct carcinoma from a ductal adenocarcinoma<sup>[90]</sup>. This combination was also proposed for distinguishing biliary intraepithelial neoplasia from non-neoplastic changes<sup>[91]</sup>.

### **Brain**

Normal brain tissue strongly expresses maspin in the cytoplasm and nucleus and is downregulated in parallel with increasing glioma grade (Table 3) possibly by maspin promoter methylation<sup>[92,93]</sup>.

### **Head and neck**

Maspin expression is observed in the cytoplasm or nuclei of salivary glands (myo-epithelial cells) and also in oral cavity epithelium<sup>[94-105]</sup> (Table 3). Maspin mRNA was identified in the corneal layers and stroma where it may exert adhesion regulatory functions between the cells and matrix molecules and where it may play a role in wound healing through regulation of the activated fibroblasts migration<sup>[105]</sup>. In inflammation, maspin was hypothesized to be an indicator of invasive fungal rhinosinusitis. It was downregulated in comparison with the noninvasive type and



with chronic rhinosinusitis for both cyto-plasm and nucleus<sup>[104]</sup>. Nuclear reaction and a higher intensity of maspin staining were associated with benign lesions of the salivary glands<sup>[102,103]</sup>.

No significant differences in maspin expression were discovered between recurrent and nonrecurrent ameloblastoma and/or ameloblastic carcinoma<sup>[94]</sup>. After studying the maspin gene in a large number of participants, it was found that heterozygous T-C of rs17071138 polymorphism and G-G homozygotes or heterozygotes of rs2289520 increase the susceptibility to oral cancer development<sup>[95,96]</sup>. IHC-based studies on SCC of the oral cavity and tongue emphasized an association between high maspin expression and better overall survival, while the absence of maspin was correlated with high pT stage and presence of lymph node metastases<sup>[97-99]</sup>.

In the temporal bone SCC cases, cytoplasmic subcellular localization of maspin expression was significantly higher in the recurrence-free group, thus representing a potential prognostic marker<sup>[100]</sup>. For laryngeal SCC, a separate evaluation of cytoplasmic and nuclear immunostaining has led to an association of the nuclear positivity with a longer disease-free interval after surgery<sup>[101]</sup>.

**Thyroid:** Maspin is one of the six gene panel proposed for distinguishing normal thyroid from papillary thyroid carcinoma, along with TIMP3, RARB2, RASSF1, TPO, and TSHR<sup>[106]</sup>. In a study by Boltze *et al*<sup>[107]</sup>, positive maspin immunoreaction (cytoplasm and nucleus) was observed in papillary thyroid carcinomas, while the normal thyroid tissue, follicular adenomas, follicular carcinomas, and poorly and undifferentiated carcinomas were negative (Table 3). The study also presented maspin promoter methylation as a factor of the silencing mechanism of the dedifferentiation degree<sup>[107]</sup>.

### Skin and soft tissues

**Skin:** Normal epidermis and sweat or sebaceous glands are maspin positive<sup>[108-111]</sup>. In SCCs, translocation of maspin immunoexpression from the cytoplasm to the nucleus in the front of invasion was seen as an indicator of tumor dedifferentiation<sup>[108,111]</sup> (Table 3). All well-differentiated tumors and all cases diagnosed in pT1 stage presented cytoplasmic maspin expression only<sup>[108]</sup>.

The PCR-related studies showed maspin downregulation in tumor tissues compared with the normal adjacent cutis showing the potential role of maspin in tumor development inhibition<sup>[109]</sup>. Basal cell carcinoma cells variably express maspin at the cytoplasm and nucleus in the center of the nodules especially in nodular basal cell carcinoma<sup>[110]</sup>. Although infrequently observed, nuclear maspin can be seen in Merkel carcinoma cells, especially in sun-exposed areas<sup>[111]</sup>. A sun-activated maspin-induced DNA damage was hypothesized<sup>[111]</sup>.

In melanoma cases, a significant association of nuclear maspin staining with aggressive tumor behavior and shorter disease-free survival was shown, while cytoplasmic predominance was present in superficial spreading melanoma<sup>[111,112]</sup>. High maspin intensity in the invasive margins of primary melanomas was correlated with an unfavorable prognosis<sup>[113]</sup>.

**Soft tissues and joints:** Although it can act as a proangiogenic marker, maspin expression is negative in soft tissue structures and does not mediate osteoarthritis<sup>[114]</sup>. For malignant soft tissue tumors, cytoplasmic expression of maspin was correlated with higher histological grade and risk for distant metastasis<sup>[115]</sup>. In liposarcomas, maspin and VEGF-A seem to be angiogenic promoters<sup>[116]</sup>. Negative staining was observed for most soft tissue tumors such as granular cell (Abrikossoff) tumor<sup>[117]</sup>, but also for other mesenchymal tumors such as gastrointestinal stromal tumors<sup>[118]</sup>.

## CONCLUSION

Although several studies tried to elucidate parts of the molecular journey in which maspin influences the transformation of epithelial cells and tumor behavior, the maspin-related processes are yet to be elucidated. Experimental studies are needed before chemical synthesis of a maspin-based agent can begin. Despite several unknown areas of the effects of maspin, several aspects have been confirmed by us and others. These aspects include: maspin is a good marker of budding quantification in colorectal carcinomas; it can be used for identification of intragastric mucosa signet ring cells (in biopsic specimens) or a proper evaluation of poorly cohesive gastric carcinoma invasion; and it is a useful marker for differential diagnosis of PanIN from a ductal adenocarcinoma of pancreas. The other aspects should be elucidated by further studies.

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## Drug interactions of dipeptidyl peptidase 4 inhibitors involving CYP enzymes and P-gp efflux pump

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### Abstract

Dipeptidyl peptidase 4 (DPP4) inhibitors are oral antidiabetic drugs approved to manage type 2 diabetes mellitus. Saxagliptin is a substrate of CYP3A4/5 enzymes while other DPP4 inhibitors such as sitagliptin, linagliptin, gemigliptin and teneligliptin are weak substrates of CYP3A4. DPP4 inhibitors have also been identified as substrates of P-gp. Hence, the drugs inhibiting or inducing CYP3A4/5 enzymes and/or P-gp can alter the pharmacokinetics of DPP4 inhibitors. This review is aimed to identify the drugs interacting with DPP4 inhibitors. The plasma concentrations of saxagliptin have been reported to be increased significantly by the concomitant administration of ketoconazole or diltiazem while no significant interactions between various DPP4 inhibitors and drugs like warfarin, digoxin or cyclosporine have been identified.

**Key words:** Drug interactions; Sitagliptin; Saxagliptin; Linagliptin; Gemigliptin; Teneligliptin; Vildagliptin; Anagliptin; CYP3A4; P-gp efflux pump

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**Core tip:** The probability of adverse drug interactions is higher among diabetic patients due to the concomitant administration of antidiabetic drugs with multiple medications to treat comorbidities such as hypertension, dyslipidemia, other cardiovascular problems, infections, depression, and others. Dipeptidyl peptidase 4 (DPP4) inhibitors are oral antidiabetic drugs approved to manage type 2 diabetes mellitus. Some of the DPP4 inhibitors have been identified as substrates of CYP3A4/5 enzymes and P-gp efflux pump. The drugs inhibiting or inducing CYP3A4/5 enzymes and/or P-gp can alter the pharmacokinetics of DPP4 inhibitors. The prescribers and the pharmacists are required to be aware of the drugs altering the pharmacokinetics of DPP4 inhibitors significantly to prevent adverse drug interactions.



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## INTRODUCTION

Dipeptidyl peptidase 4 (DPP4) inhibitors are oral antidiabetic drugs approved to manage type 2 diabetes mellitus. The members of this class include sitagliptin, vildagliptin, saxagliptin, linagliptin, gemigliptin, anagliptin, teneligliptin and alogliptin. DPP4 enzyme is involved in the biodegradation of incretins such as glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide. DPP4 inhibitors help to increase the postprandial insulin secretion and inhibit glucagon secretion through the inhibition of inactivation of glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide<sup>[1]</sup>.

Diabetes is a group of metabolic disorders occurring due to the defects in insulin secretion and insulin action. It has been estimated that more than 500 million people around the globe were living with diabetes in 2018 and the numbers are increasing daily<sup>[2]</sup>.

Inappropriate use of multiple medications or polypharmacy is more common among diabetic patients as they may receive many medications to manage comorbid conditions such as hypertension, dyslipidemia, other cardiovascular problems, infections, depression, and others along with their antidiabetic medications. The risk of drug interactions increases with the number of comedications. Drug interaction is defined as the interference of effects of a drug by the concomitantly administered other drug(s), herbs, minerals, vitamins, food, fruit juices, tobacco smoke or alcohol, and the drug interaction resulting in increased unintended effects or decreased intended effects is termed adverse drug interaction<sup>[3,4]</sup>.

The cytochrome P450 (CYP) enzymes are involved in the phase 1 metabolism of drugs and they consist of 57 different CYP forms. Almost 90% of drugs are metabolized by seven CYP enzymes including CYP3A4 and others<sup>[5]</sup>. Saxagliptin is a substrate of CYP enzymes, and it is primarily metabolized by CYP3A4/5 to form the active metabolite, 5-hydroxy saxagliptin through hydroxylation<sup>[6]</sup>. Moreover, other DPP4 inhibitors such as sitagliptin<sup>[7]</sup>, linagliptin<sup>[8]</sup>, gemigliptin<sup>[9]</sup> and teneligliptin<sup>[10]</sup> are weak substrates of the CYP3A4 enzyme. They are metabolized incompletely by CYP3A4, and major parts of the drugs are excreted as unchanged drug through urine except linagliptin, which is excreted through feces. Vildagliptin<sup>[11]</sup> and anagliptin<sup>[12]</sup> are metabolized by cyano group hydrolysis and about 50% of the administered dose is excreted as unchanged drug. The drugs inhibiting or inducing the CYP3A4 enzyme may interact with DPP4 inhibitors as some of them are substrates of the CYP3A4 enzyme.

P-glycoprotein (P-gp) is an efflux transporter and it is also known as multidrug resistance protein 1 as it is overexpressed in tumor cells causing resistance to different anticancer drugs. P-gp is involved in the absorption and excretion of drugs as it is also found in various tissues like small intestine, liver and kidney. P-gp pumps the orally administered drugs back in to lumen and limit their bioavailability<sup>[13]</sup>. DPP4 inhibitors have been identified as substrates of P-gp<sup>[14]</sup> and the drugs inducing or inhibiting P-gp transporters may also affect the pharmacokinetics of DPP4 inhibitors.

## LITERATURE REVIEW

As the DPP4 inhibitors are the substrates of both CYP3A4 enzymes and the P-gp transporter, the present review is focused on the possible drug-drug interactions of them. The literature review was done in databases such as MEDLINE/PubMed/PMC, ScienceDirect, Google scholar, Cochrane Library and reference lists using the keywords such as drug interactions, sitagliptin, saxagliptin, linagliptin, gemigliptin, teneligliptin, vildagliptin, anagliptin, CYP3A4 and P-gp efflux pump.

## LITERATURE REVIEW RESULTS

Most of the drug-drug interactions of DPP4 inhibitors involve mainly saxagliptin as it

is metabolized extensively by the CYP3A4 enzyme. The plasma concentrations of saxagliptin increased by the concomitant administration of CYP3A4 and P-gp inhibitors such as ketoconazole and diltiazem and future studies are required to confirm the possibility of drug-drug interactions with other CYP3A4 inhibitors. In addition, other DPP4 inhibitors such as sitagliptin, linagliptin, gemigliptin and teneligliptin interact with CYP3A4 inhibitors insignificantly as they are weak substrates of CYP3A4 enzyme. The prescribers and the pharmacists are required to be aware of the drug-drug interactions of saxagliptin to prevent adverse complications.

### **Ketoconazole**

Ketoconazole is an antifungal agent and it is a known potent inhibitor of CYP3A4 enzyme and P-gp transporter<sup>[15]</sup>. It has been observed that the plasma exposure of saxagliptin was increased by the concurrent administration of ketoconazole due to the inhibition of CYP3A4 enzyme-mediated metabolism of saxagliptin and a weak inhibition of P-gp mediated transport. Hence, it has been suggested to use the lowest therapeutic dose (2.5 mg) of saxagliptin when concomitant use of ketoconazole and saxagliptin is necessary<sup>[16]</sup>. Significant elevation of plasma concentrations of gemigliptin was observed in healthy male Korean volunteers who took ketoconazole along with gemigliptin<sup>[17]</sup> while there was no significant interaction reported with the concomitant use of ketoconazole and teneligliptin<sup>[18]</sup>.

### **Diltiazem**

Diltiazem is a calcium channel blocker and it is indicated in the management of hypertension, angina and certain cardiac arrhythmias. Diltiazem is a moderate inhibitor of CYP3A4 enzyme and P-gp transporter<sup>[19]</sup> and its coadministration with saxagliptin resulted in a significant increase in plasma exposure of saxagliptin<sup>[16]</sup>.

### **Other CYP3A4 inhibitors**

The plasma concentrations of saxagliptin might be elevated by its coadministration with strong CYP3A4 inhibitors including macrolide antibiotics like clarithromycin and antiretroviral drugs (protease inhibitors) such as ritonavir, atazanavir, and others<sup>[20]</sup>. Future studies are required to confirm the interaction of macrolide antibiotics, antiretroviral drugs and other potent CYP3A4 inhibitors with saxagliptin and other DPP4 inhibitors.

### **3-Hydroxy-3-Methyl-Glutaryl-CoA reductase inhibitors (statins)**

3-Hydroxy-3-Methyl-Glutaryl-CoA reductase inhibitors or statins are used to lower the risk of acute cardiovascular events by controlling dyslipidemia<sup>[21]</sup>. Statins include lovastatin, simvastatin, atorvastatin, cerivastatin, fluvastatin, pravastatin, rosuvastatin and pitavastatin<sup>[22]</sup>. The statins such as lovastatin, simvastatin, atorvastatin and cerivastatin are reported to be substrates of CYP3A4 enzyme and P-gp transporter<sup>[23]</sup>.

The exposure of saxagliptin was slightly increased by the concomitant use of simvastatin<sup>[16]</sup>, and no clinically significant changes in pharmacokinetics of simvastatin and sitagliptin<sup>[24]</sup> or vildagliptin<sup>[25]</sup> was observed when they were used concomitantly.

Although the initiation of sitagliptin in a patient with chronic renal insufficiency and receiving simvastatin resulted in developing the symptoms of rhabdomyolysis such as leg pain, weakness and tenderness<sup>[26]</sup> the efficacy and safety of the fixed dose combination of sitagliptin and simvastatin was found to be acceptable<sup>[7]</sup>. However, the pharmacokinetics of either gemigliptin or rosuvastatin was not altered during their concurrent use<sup>[27]</sup>.

Furthermore, it has been reported that a patient taking sitagliptin and lovastatin<sup>[28]</sup> and the patients taking sitagliptin and atorvastatin<sup>[29,30]</sup> developed rhabdomyolysis. The patients taking sitagliptin along with statins like atorvastatin and lovastatin are required to be monitored for the symptoms of muscle toxicity.

### **Warfarin**

Warfarin is an oral anticoagulant agent, and R-warfarin is a substrate of CYP1A2 and CYP3A4 enzymes<sup>[31]</sup>. The pharmacokinetics of warfarin and sitagliptin<sup>[32]</sup>, linagliptin<sup>[33]</sup>, or vildagliptin<sup>[34]</sup> did not significantly get altered during their concomitant use, and it has been reported that no dosage adjustments of either drugs are required.

### **Digoxin**

Digoxin is a cardio tonic agent, and it is approved to treat patients with heart failure and arrhythmias including atrial fibrillation<sup>[35]</sup>. Digoxin is a substrate of P-gp and its co-administration with linagliptin<sup>[36]</sup> or vildagliptin<sup>[37]</sup> did not lead to significant alterations in pharmacokinetic parameters of digoxin. Moreover, no dosage

adjustment of either drugs are required when digoxin and linagliptin or vildagliptin are used concomitantly.

### Cyclosporine

Cyclosporine is an immunosuppressant, and it is an inhibitor of CYP3A4 enzymes<sup>[38]</sup> and P-gp transporter<sup>[39]</sup>. The Pgp-mediated transport of sitagliptin was reported to be inhibited significantly by the coadministration of cyclosporine<sup>[40]</sup>. The magnitude of this interaction is considered low as sitagliptin has a high safety margin<sup>[41]</sup>.

### Rifampicin

Rifampicin is an antitubercular antibiotic, and it is a potent inducer of CYP3A4 enzymes and P-gp transporter<sup>[42]</sup>. Clinically insignificant reduction of systemic exposure of saxagliptin was observed when it was coadministered with rifampicin and no dosage adjustment of saxagliptin is required<sup>[43]</sup>. However, the concomitant use of gemigliptin and rifampicin in Korean volunteers resulted in significant reduction of systemic exposure of gemigliptin. The dose of gemigliptin may need to be adjusted when concurrent use is necessary<sup>[17]</sup>.

## CONCLUSION

Saxagliptin is a substrate of CYP3A4/5 enzymes and other DPP4 inhibitors such as sitagliptin, linagliptin, gemigliptin and teneligliptin are metabolized incompletely by CYP3A4 enzymes as they are weak substrates of CYP3A4. The plasma concentrations of saxagliptin have been reported to be increased significantly by the concomitant administration of ketoconazole or diltiazem while no significant interactions between various DPP4 inhibitors and drugs like warfarin, digoxin or cyclosporine have been identified.

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# Safety and efficacy of percutaneous transhepatic balloon dilation in removing common bile duct stones: A systematic review

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## Abstract

### BACKGROUND

Endoscopic sphincterotomy (EST) is widely regarded as the first choice in the management of common bile duct (CBD) stones. However, for some patients, this treatment is not possible. The percutaneous transhepatic balloon dilation (PTBD) technique has been suggested as an alternative but has yet to gain wide acceptance.

### AIM

To review cases of PTBD for removing CBD stones and explore the safety and efficacy of this treatment.

### METHODS

We conducted a systematic review using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. We searched EMBASE, PubMed, and Web of Science for cases of PTBD that underwent CBD stone removal from 1981 to January 2019. We analyzed all relevant articles available in full text. We extracted data on patient's age, gender, overall technique success rate, reasons for technique failure, and the presence and type of major and minor complications. We analyzed the data and reported the results in a table and text. Altogether, we retrieved 12 case series and 6 case reports, for a total of 1347 patients. Thirty cases were excluded due to a lack of patient data.

### RESULTS

The overall technique success rate for removing a CBD stone was 98.5% (1327/1347) and 98.1% (109/111) for removing concurrent CBD and gallbladder stones. Based on available data ( $n = 1312$ ), mean age of all patients (687 males and 625 females) was 68.9 years. The total number of procedures in the remaining 1317 patients (after exclusion) was 3237 (average 2.4 procedures per patient). The total number of failures for eliminating a CBD stone was 20, and the reasons for failure included: Stone impaction ( $n = 10$ ), intrahepatic bile duct stricture ( $n = 5$ ),

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large stone ( $n = 2$ ), severe CBD dilation ( $n = 1$ ), multiple stones ( $n = 1$ ), and duodenal perforation ( $n = 1$ ). Various major complications related to the procedure were reported, but the incidence rate was low (1.4%). No pancreatitis or procedure related mortality was reported. Minor complications including transient hyperamylasemia, nausea, vomiting, abdominal pain, fever, and mild hemobilia were reported. For 218 patients (88 patients with unsuccessful endoscopic removal due to anatomical change and large or impacted stone and 130 cases who refused endoscopic procedure due to poor general condition or other additional disease), the CBD stones were successfully pushed into the duodenum by performing the PTBD procedure.

## CONCLUSION

PTBD is a safe and effective approach in the nonoperative management of CBD stones. PTBD provides an alternative treatment when endoscopic procedures fail or are unsuitable for the patient.

**Key words:** Common bile duct stone; Percutaneous transhepatic approach; Balloon dilation; Interventional procedures; Papilla; Endoscopic sphincterotomy

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**Core tip:** Endoscopic treatment for common bile duct (CBD) stones has been widely accepted. However, for specific patients, such as those with gastrointestinal anatomical changes, duodenal diverticulum, esophageal varices, or other conditions, endoscopic treatment is unsuitable and difficult to perform. Under these circumstances, it has been shown that percutaneous transhepatic balloon dilation (PTBD) can remove CBD stones via a percutaneous transhepatic route after papilla dilation. However, no review on this technique has been published. Therefore, we performed a systematic review to confirm the safety and efficacy of PTBD in removing CBD stones in terms of the key outcomes, success rate, reasons for failure, and procedure-related complications.

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## INTRODUCTION

Since 1974 when Kawai first described endoscopic sphincterotomy (EST), this treatment has been widely accepted and regarded as the first choice in the management of common bile duct (CBD) stones<sup>[1]</sup>. Indeed, endoscopic therapies have initiated a great revolution in the treatment of choledocholithiasis<sup>[2-4]</sup>. However, for specific patients, such as those with gastrointestinal anatomical changes, duodenal diverticulum, esophageal varices, or poor general condition, endoscopic treatment can be difficult to perform, and it has been deemed unsuitable in these particular cases<sup>[5-7]</sup>.

In cases that preclude EST, percutaneous transhepatic stone removal through the papilla into the duodenum without balloon dilation was first reported as an alternative in 1979 by Dotter *et al*<sup>[8]</sup> and Perez *et al*<sup>[9]</sup>. Further, in 1981 Centola *et al*<sup>[10]</sup> first introduced transpapillary elimination of a stone by dilating the papilla with a 6-mm balloon, and since then, this technique has been implemented as a standard percutaneous stone removal procedure. This technique has increased efficacy, with a high success rate and low incidence of complications. Despite these reports, the percutaneous transhepatic balloon dilation (PTBD) technique has still not gained wide acceptance. This is mainly due to a lack of awareness and evaluation of the safety, efficacy, and risk of complications associated with this procedure.

Individual studies alone may not provide strong and sufficient evidence to help PTBD gain greater acceptance, and to the best of our knowledge, no review on the use of PTBD in removing CBD stones has previously been published. In the current review, we aim to objectively evaluate the potential role of PTBD in the management of CBD stones, as an alternative to EST. We performed a systematic review of the

currently available literature for success rate, reasons for failure, and procedure-related complications associated with the implementation of the PTBD procedure. This review was conducted in an effort to clarify the safety and efficacy of the procedure.

## MATERIALS AND METHODS

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines<sup>[11]</sup>. We searched Embase, PubMed, and Web of Science for relevant studies involving the use of PTBD for removal of CBD stones. Our search covered studies conducted during the period from 1981 to January 2019. We used the following Medical Subject Headings (MeSH): “gallstone” and “dilation” and “percutaneous” and “transhepatic” and “balloon”. The complete terms used for the PubMed search were: (dilation [Title/Abstract]) OR dilations [Title/Abstract] OR dilatations [Title/Abstract] OR dilatation [Title/Abstract] AND (transhepatic [Title/Abstract] OR interventional radiography [Title/Abstract] AND (percutaneous [Title/Abstract] OR radiography, interventional [Title/Abstract] AND (balloon [Title/Abstract] AND (gallstones [Mesh] OR gallstone [Title/Abstract] OR gall Stones [Title/Abstract] OR biliary calculi [Title/Abstract] OR calculi, biliary [Title/Abstract] OR gall Stone [Title/Abstract] OR common bile duct calculi [Title/Abstract] OR gallstones, common bile duct [Title/Abstract] OR common bile duct gallstones [Title/Abstract] OR gall Stones, common bile duct [Title/Abstract] OR biliary calculi, common bile duct [Title/Abstract] OR common bile duct gall stones [Title/Abstract])).

We regarded studies as available for inclusion if they applied a percutaneous transhepatic route, applied a balloon dilation technique, and involved treatment of CBD stones or concurrent CBD stones in addition to gallbladder stones. Case reports and case series were both included. We excluded non-English published studies and studies for which the full text article was unavailable. The studies were reviewed by two individual researchers (DL and BL) and data analysis and extraction were done by the same two researchers (DL and BL). After screening the full text, we extracted the following data from each study for inclusion in our review: Age, gender, number of procedures, overall technique success rate, reasons for failure, and various major and minor complications. Using descriptive statistical analysis, the variables were described as number, proportion, and mean (Table 1).

## RESULTS

The search results and flow diagram are shown in Figure 1. We retrieved 12 case series and 6 case reports, for a total of 1347 cases treated by percutaneous transhepatic papilla balloon dilation<sup>[10,12-28]</sup>.

According to our findings, 7 studies were published before the year 2000 and 11 studies were published after the year 2000. Centola *et al*<sup>[10]</sup> from England was the first to report a case in which a balloon was used to dilate the papilla and remove a stone in the duodenum in 1981. Among those case series which applied PPBD, the largest included 916 cases and was reported by Shin *et al*<sup>[14]</sup> in South Korea in 2017. In our review, 1050 cases were published from Asia, with 297 cases published from Europe and North America.

As for the patient characteristics, not all the studies reported age and sex ( $n = 35$ )<sup>[16,20,27]</sup>. Based on the available data ( $n = 1312$ ), the average age of patients was 66.89 years and there were 687 males and 625 females. All patients were treated by PTBD for CBD stone removal, and 111 patients who had CBD stones and gallbladder stones concurrently were treated by the combination of PTBD and an additional procedure. Indications cited in these studies for the use of the PTBD procedure to remove stones were: unsuitable for endoscopic procedure due to the poor condition or other additional disease ( $n = 130$ ), which included coronary artery disease, emphysema, pulmonary insufficiency, cardiac insufficiency, multiple sclerosis, and other diseases, unsuccessful endoscopic removal due to the anatomical change and large or impacted stone ( $n = 88$ ), and unsuccessful basket extraction ( $n = 2$ ). Determination of the number of patients treated by an unsuccessful endoscopic procedure or who were unsuitable for an endoscopic procedure was low (16.1%), as the largest case series ( $n = 916$ ) did not mention the other forms of treatment or the patients' additional diseases.

The overall PTBD technique success rate for removing a CBD stone was 98.5% (1327/1347), and 98.1% (109/111) for removing concurrent CBD and gallbladder



**Table 1 Characteristics of the patients and procedure**

Characteristic	Value
No. of patients	1347
Gender	1312
Female	625 (47.64)
Male	687 (52.36)
Average age	66.89
Overall technique success rate	98.51
Average number of procedure	2.46
Reasons of failure	
Severe CBD dilation	1 (0.07)
Multiple stones	1 (0.07)
Large stone	2 (0.15)
Stone impaction	10 (0.74)
Intrahepatic bile duct stricture	5 (0.37)
Duodenal perforation	1 (0.07)
Major complications	
Cholangitis	11 (0.82)
Bile duct hemorrhage	1 (0.07)
Subcapsular biloma	1 (0.07)
Subcapsular hematoma	1 (0.07)
Subcapsular abscess	1 (0.07)
Bile peritonitis	1 (0.07)
Duodenal perforation	1 (0.07)
CBD perforation	1 (0.07)
Gastroduodenal artery pseudoaneurysm	1 (0.07)
Right hepatic artery transection	1 (0.07)

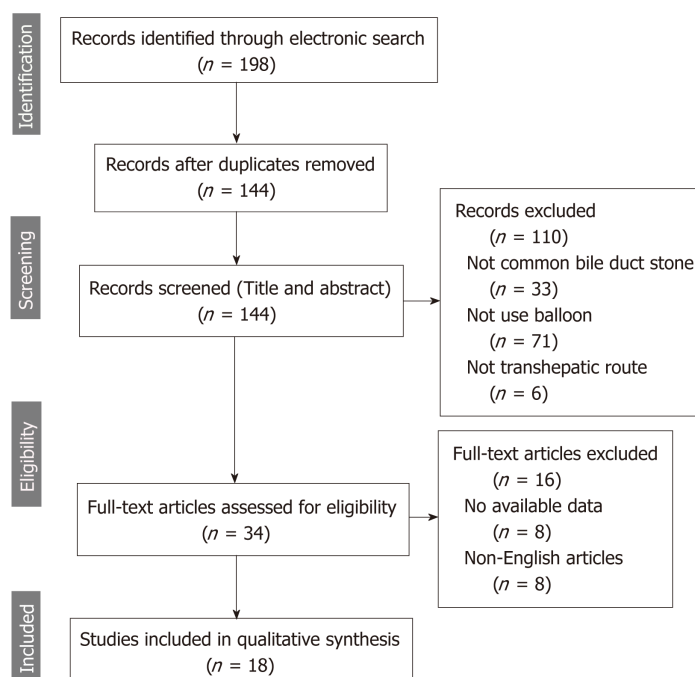
CBD: Common bile duct.

stones. The largest diameter of CBD stone was 25 mm reported by Chang *et al.*<sup>[13]</sup> in 2018, and the CBD stone was successfully removed through dilated papilla by using a 24 mm balloon. Before stone removal, percutaneous transhepatic biliary drainage was conducted on 1024 patients, which is performed to relieve clinical symptoms and build the approach for the stone removal procedure that follows. Based on the available data, the total number of procedures in 1317 patients was 3237, with an average of 2.4 procedures per patient (30 cases did not have this information). The total number of failures in eliminating a CBD stone was 20, and there were multiple reasons for failure, including severe CBD dilation ( $n = 1$ ), large stone ( $n = 2$ ), multiple stones ( $n = 1$ ), stone impaction ( $n = 10$ ), bile duct stricture ( $n = 5$ ), and duodenal perforation ( $n = 1$ ).

Major complications related to the procedure were reported, but the incidence rate for these complications was low (1.4%). Among the included studies, the incidence rate of major complications varied from 0%-6.8%. Major complications included cholangitis ( $n = 11$ ), bile duct hemorrhage ( $n = 1$ ), subcapsular biloma ( $n = 1$ ), subcapsular hematoma ( $n = 1$ ), subcapsular abscess ( $n = 1$ ), bile peritonitis ( $n = 1$ ), duodenal perforation ( $n = 1$ ), CBD perforation ( $n = 1$ ), gastroduodenal artery pseudoaneurysm ( $n = 1$ ), and right hepatic artery transection ( $n = 1$ ). No pancreatitis or procedure related mortality was reported. Minor complications, such as hyperamylasemia, nausea, vomiting, abdominal pain, fever, and mild hemobilia, were reported. The complete data for these complications were not provided in many case series as most of the minor complications had transient adverse effects and did not require any treatment.

## DISCUSSION

The results of this review show that the use of the PTBD technique in removing CBD stones can yield a high success rate and a low incidence of complications.



**Figure 1** Flow diagram of study selection.

Furthermore, our findings suggest that PTBD offers a safe and effective choice for removing CBD stones in those patients with a prior failed endoscopic treatment or who are unsuitable for an endoscopic procedure. These data demonstrate and support the PTBD technique as an effective and safe therapeutic management tool, which can be implemented as an alternative, and supplement, to endoscopic therapies.

The overall technique success rate for removing a CBD stone by performing PTBD was 98.5%. The success rate of endoscopic treatment of CBD stones is compromised by several limitations, including gastrointestinal anatomical changes (*e.g.*, Billroth II surgery and duodenal diverticulum) and a limited application in those with a poor general condition. Under these circumstances, endoscopic treatment is unsuitable and difficult to perform. In the current study, the results show that among 218 patients, 88 had unsuccessful endoscopic removal and 130 were unfit for an endoscopic procedure, and the CBD stone was successfully pushed into the duodenum by performing the PTBD procedure. Compared to endoscopic procedures, PTBD uses percutaneous transhepatic and transpapillary routes which could avoid the effects of anatomical changes and is easier to complete the procedure through the papilla. The overall technique success rate for removing concurrent CBD and gallbladder stones was 98.1% (109/111) when performing a combination of the PTBD procedure and another treatment such as laparoscopic choledochotomy (LC) and percutaneous transcystic procedure. Interestingly, in the studies included in our review, there is data suggesting that PTBD + LC is more effective and safe in patients with both CBD and gallbladder stones when compared to the endoscopic papillary balloon dilatation + LC technique. Based on these findings, we postulate that PTBD is an alternative technique that can potentially mitigate the limitations of endoscopic treatment.

Although the success rate of PTBD was quite high, there were a few failed cases. Our results show that the reasons some cases failed were related to the presence of a large stone and duodenal perforation. The large stone is difficult to push through the papilla, which needs use of stone basket or other lithotripsy. And a larger balloon may be used to dilate the papilla, which could cause more abdominal pain and overexpansion of the papilla. For patients with a history of gastrointestinal surgery, it should be performed gently when the guide wire pass through the papilla and the stones were pushed into the duodenum with a balloon. In our systematic review, we conclude several procedure details or key points, which could help surgeons improve their performance with this technique. We suggest the following: (1) In the supine position, puncturing the bile duct in the right anterior lobe under the guidance of B-type ultrasound to make the angle between the bile duct and the CBD as large as possible; (2) After passing through the Oddi sphincter, the stiff guide wire is introduced for greater support; (3) When dilating the Oddi sphincter, the balloon catheter should be accurately positioned and fully dilated. The preferred diameter of

the balloon is 8 mm. If the expansion is unsatisfactory, it can be increased by 2 mm successively, with a maximum of 20 mm; (4) Intermittent expansion should be used to avoid tearing of sphincter fibers. We found that the duodenal papilla can be expanded at multiple angles for a duration of 15 s; (5) When the diameter of the stone is > 10 mm, transpapillary stone removal can be achieved by performing lithotripsy first, and then pushing the stone into the duodenum with a balloon; (6) Multiple stones should be rolled out one by one to avoid pancreatitis caused by stone debris reflux to the pancreatic duct. Alternatively, the clinician can leave an external drainage tube for the second stage of stone removal; and (7) Routine placement of internal and external biliary drainage tubes can effectively reduce the incidence of pancreatitis by reducing the intrabiliary pressure. We believe that these suggestions will result in increased efficacy and a further reduction in complications due to the PTBD procedure.

For the 1347 cases we retrieved in this study, major complications related to the PTBD procedure were reported, but the incidence rate was low (1.4%). The most common major complication was cholangitis, at a rate of less than 1% of all cases included in our review. The incidence of other major complications was even lower. Further, we found no reported procedure related mortality. Importantly, the minor complications noted in the results from the included studies were easily controlled by conservative treatment. Several case series ( $n = 4, 26$  patients) reported transient hyperamylasemia after the procedure; however, the level of amylase was decreased to normal after a few days of recovery. These data suggest that PTBD is a safe procedure with a low incidence of complications.

To the best of our knowledge, no review on the effectiveness of the PTBD procedure in removing CBD stones has previously been published. Therefore, we performed this systematic review to confirm the safety and efficacy of PTBD procedure in removing CBD stones by analyzing key outcomes such as success rate, reasons for failure, and procedure-related complications. However, our study had several limitations. First, there are no randomized clinical trials currently published that compare endoscopic treatment and the PTBD procedure. These trials would provide stronger evidence in proving the safety and efficacy of the PTBD procedure as an alternative to endoscopic treatment. However, even given this lack of data, we believe our review fills in some of the blanks that currently exist pertaining to the safety and efficacy of PTBD. Second, long-term effectiveness of this procedure is unknown. There are no long-term follow-up studies published currently, and as such there is no data on any long-term complications, such as stone recurrence and reflux cholangitis. Moreover, there remain no high quality, rigorous manuscripts published on the PTBD procedure. This has resulted in a lack of patient characteristics and incomplete procedure details, which may cause bias. Although further research is required to investigate better application of this treatment, our limited evidence clearly demonstrates that PTBD is a safe and effective approach in the nonoperative management of the CBD stones. This technique provides an alternative treatment when endoscopic procedures fail or are unsuitable for specific patients.

## ARTICLE HIGHLIGHTS

### Research background

Endoscopic sphincterotomy (EST) is widely regarded as the first choice in the management of common bile duct (CBD) stones. However, for some patients, this treatment is not possible.

### Research motivation

The percutaneous transhepatic balloon dilation (PTBD) technique has been suggested as an alternative but has yet to gain wide acceptance.

### Research objectives

This review was conducted in an effort to clarify the safety and efficacy of the procedure *via* reviewing cases of PTBD for removing CBD stones. We conducted a systematic review using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

### Research methods

We searched EMBASE, PubMed, and Web of Science for cases of PTBD that underwent CBD stone removal from 1981 to January 2019. We analyzed all relevant articles available in full text. We extracted data on patient's age, gender, overall technique success rate, reasons for technique failure, and the presence and type of major and minor complications.

### Research results

The overall technique success rate for removing a CBD stone was 98.5% (1327/1347) and 98.1% (109/111) for removing concurrent CBD and gallbladder stones. The total number of failures for eliminating a CBD stone was 20, and the reasons for failure included: Stone impaction ( $n = 10$ ),

intrahepatic bile duct stricture ( $n = 5$ ), large stone ( $n = 2$ ), severe CBD dilation ( $n = 1$ ), multiple stones ( $n = 1$ ), and duodenal perforation ( $n = 1$ ).

### Research conclusions

Various major complications related to the procedure were reported, but the incidence rate was low (1.4%). No pancreatitis or procedure related mortality was reported. Minor complications including transient hyperamylasemia, nausea, vomiting, abdominal pain, fever, and mild hemobilia were reported. For 218 patients (88 patients with unsuccessful endoscopic removal due to anatomical change and large or impacted stone and 130 cases who refused endoscopic procedure due to poor general condition or other additional disease), the CBD stones were successfully pushed into the duodenum by performing the PTBD procedure.

### Research perspectives

PTBD is a safe and effective approach in the nonoperative management of CBD stones. PTBD provides an alternative treatment when endoscopic procedures fail or are unsuitable for the patient.

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## Effectiveness of taxanes over anthracyclines in neoadjuvant setting: A systematic-review and meta-analysis

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### Abstract

#### BACKGROUND

Anthracyclines and taxanes are more active group of chemotherapy regimen. Randomized controlled trials (RCTs) reported variable evidences regarding efficacy of taxanes over anthracyclines for tumor response and survival outcomes. The present study compares the relative efficacy of taxanes over anthracyclines using pathological complete response (pCR), clinical responses, breast-conserving surgeries and survival outcomes in female breast cancer patients by systematic review and meta-analysis of available RCTs.

#### AIM

To assess the effectiveness of taxanes over anthracyclines in neoadjuvant setting in terms of tumor response and survival outcomes.

#### METHODS

All RCTs assessing efficacy of taxanes over anthracyclines in neoadjuvant setting for management of breast cancer searched through PubMed and Cochrane register of controlled trials on 28 April 2017 and published in English language were considered. Following PRISMA guideline, retrieved records were screened and data were extracted by two independent reviewers. Meta-analysis was performed using fixed effect or random effect method depending on heterogeneity assessed using  $I^2$  statistic. Subgroup meta-analyses on the basis of taxane alone or taxane along with anthracycline in comparison to anthracycline alone were also performed for each considered outcomes.

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## RESULTS

A total of 16 RCTs involving 6752 breast cancer patients were found eligible. Taxanes based chemotherapy significantly improved pCR ( $n = 7$ ,  $RR = 1.48$ ,  $95\% CI: 1.04-2.12$ ), disease free survival [ $n = 6$ ,  $RR = 0.89$  ( $0.80-0.99$ )] and loco-regional recurrence free survival [ $n = 4$ ,  $RR = 0.74$  ( $0.59-0.94$ )]. Interestingly in subgroup analysis, addition of taxane to anthracyclines showed better effectiveness regarding these survivals over anthracyclines than taxane alone over anthracycline.

## CONCLUSION

Addition of taxanes to anthracyclines based chemotherapy significantly improves pCR, disease free survival and loco-regional recurrence free survival but with no significant impact on breast conservation rates.

**Key words:** Docetaxel; Paclitaxel; Epirubicin; Doxorubicin; Pathological complete response; Breast conserving surgery

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**Core tip:** There is contradictory reporting through randomized controlled trials regarding relative efficacy of taxanes over anthracyclines which are used in neo-adjuvant setting for treatment of breast cancer patients. As a first systematic review and Meta analysis on the topic, present study is to assess the relative efficacy of taxanes (docetaxel and paclitaxel) alone or their addition to anthracyclines over anthracyclines alone in terms of pathological complete response, clinical response, breast conserving surgery, survival outcomes and toxicity.

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## INTRODUCTION

Neoadjuvant chemotherapy, given prior to loco-regional treatment (surgery/radiotherapy) is standard of care for locally advanced breast cancer but now became popular for early breast cancer as well<sup>[1]</sup>. The response to chemotherapy depends on the used regimen. Anthracyclines and taxanes are more active group of chemotherapy regimen used for breast cancer<sup>[2]</sup>. These regimens are usually administered with other chemotherapy drugs like cyclophosphamide, flurouracil. Anthracyclines based drugs include doxorubicin, epirubicin and mitoxantrone. On the other hand, widely used taxanes, originally identified from plant of genus *Taxus*, are docetaxel and paclitaxel<sup>[3]</sup>. Neoadjuvant chemotherapy increases the chance of breast conserving surgery (BCS) but there is no consensus regarding role of chemotherapy drugs in further increasing BCS rate<sup>[4,5]</sup>. Further, pathological complete response (pCR) to neoadjuvant chemotherapy predict long term survival outcomes as the breast cancer patients achieving pCR have better survival than the patients who do not<sup>[6]</sup>. The reported results from randomized controlled trials (RCTs) were contradictory as some favored taxanes based chemotherapy over anthracyclines based chemotherapy<sup>[7,8]</sup> regarding pCR, while some showed the other way<sup>[9,10]</sup>. The efficacy of taxanes over anthracyclines has been examined and found to be associated with increased overall survival in adjuvant setting<sup>[11]</sup>. Two reviews have discussed about the relative effectiveness of taxanes but could not synthesize the results for response because of very few RCTs at that point of time<sup>[12,13]</sup>. Further, these reviews could not comment on the effect of taxanes on long term outcomes. To the best of our knowledge, the relative efficacy of taxanes over anthracyclines has not been synthesized in neoadjuvant setting. Accordingly, present study aims to assess the effectiveness of taxanes based Neoadjuvant chemotherapy in comparison to anthracyclines based neoadjuvant chemotherapy on the basis of pCR, clinical responses, breast conserving surgeries and survival outcomes in female breast cancer patients by systematic review and meta-

analysis of RCTs.

## MATERIALS AND METHODS

The present systematic review is designed as per the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)<sup>[14-16]</sup>. This study has been registered with International prospective register of systematic reviews and the registration Number is CRD42016027236.

### Eligibility criteria

All RCTs assessing efficacy of taxanes based Neoadjuvant Chemotherapy (NACT) in comparison to anthracyclines based NACT in the management of breast cancer, published in English language were considered. There was no restriction regarding the regimens used in the chemotherapy. The Population, Intervention, Comparator, Outcome and Time considered in the present systematic review is given below: (1) Population: Non-metastatic Female Breast Cancer Patients; (2) Intervention: Taxanes (Docetaxel or Paclitaxel); (3) Comparator: Anthracyclines; (4) Outcomes: PCR, overall response (OR) and BCS; (5) Design: RCTs; and (6) Time: Assessed on and up to 28 April 2017.

### Outcome definitions

**pCR:** pCR was reported under three definitions as follows: (1) pCR1: pCR was defined as complete response of primary as well as axilla; (2) pCR2: pCR was defined as complete response of primary regardless of axilla; and (3) pCR3: pCR was defined as complete response of primary allowing for ductal carcinoma *in situ* (DCIS).

Considering variability in the definitions, results were synthesized separately under these three definitions.

**OR:** OR was defined as complete disappearance of clinically palpable tumor or more than 50% reduction in tumor volume.

**BCS:** BCS rate was defined as rate of breast conserving surgery, *i.e.*, removal of lump only or removal of partial breast including tumor as well as some normal tissues.

Long term outcomes, *i.e.*, overall survival, disease free survival, loco-regional recurrence free survival and metastasis free survival were also considered as secondary outcomes.

### Information sources and study selection

Details of search strategies development as well as electronic search strategies for PubMed and Cochrane register of controlled trials along with methodologies for study selection are available in the published protocol<sup>[17]</sup>. Data collection process, data extraction tool and method for risk of bias assessment are also available under published protocol. There was no deviation from the published protocol<sup>[17]</sup>.

### Summary measures

Effect sizes under consideration were “risk ratio” for all response outcomes and BCS. However, long term outcomes including, overall survival, disease free survival, loco-regional recurrence and distant metastasis, it was “hazards ratio”.

### Data synthesis and analysis

Statistical heterogeneity was examined by  $I^2$  statistics<sup>[18]</sup>. Publication bias assessment was performed using Eggers test and visualized using funnel plot<sup>[19]</sup>. In case of very low extent of heterogeneity (*i.e.*,  $I^2 = 0-25\%$ ), fixed effect method of synthesizing the effect size was used. However, for moderate to large extent of heterogeneity random effect method of meta-analysis was used. All analyses were performed using Stata 14 (StataCorp, Texas, United States) and RevMan 5.3.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

### Additional analysis

To derive additional inferences, Subgroup analyses were performed for the RCTs comparing taxanes versus anthracycline; and, addition of taxanes to anthracyclines versus anthracyclines under neoadjuvant setting.

## RESULTS

### Study selection

A total of 16 RCTs comparing effectiveness of taxanes versus anthracyclines involving 6752 breast cancer patients and measuring atleast one of the considered outcomes were found eligible out of 1286 searched records. These details are presented using PRISMA flow chart (Figure 1).

### Study characteristics

RCTs assessing the effectiveness of taxanes were sub-divided in two groups, *i.e.*, RCTs comparing taxanes alone to anthracyclines alone<sup>[20-23]</sup> ( $n = 5$ ); and RCTs comparing taxanes and anthracyclines together to anthracyclines alone<sup>[4-6,9,24-30]</sup> ( $n = 11$ ). Out of these 16 RCTs, 10 RCTs assessed the effectiveness of docetaxel (3 RCT assessed the effectiveness of docetaxel *vs* doxorubicin along with other chemotherapy drugs however 7 RCTs assessed effectiveness of addition of docetaxel to anthracyclines based chemotherapy). However, six RCTs assessed effectiveness of paclitaxel, more precisely two RCT assessed paclitaxel and four RCT assessed addition of paclitaxel to anthracyclines based chemotherapy. The details of Population, intervention and outcome are presented in Table 1.

### Risk of bias within studies

Risk of bias was assessed for each individual study using Cochrane bias assessment tool Figure 2. However, overall summary of risk of bias for all considered studies is presented in Figure 3. In summary, there were around 30% of the studies, which did not perform blinding of patients and/or outcome assessment. In addition, a large proportion of the trials did not report sufficient details to judge blinding. However, it is worthwhile to mention here that objective measurement of pCR and BCS was not affected by non-blinding of outcome assessment. But clinical responses might get affected by non-blinding of outcome assessment because of obvious subjectivity.

### Publication bias

There was no publication bias for any of the outcomes except OR while assessing the effectiveness of taxane based chemotherapy tested using Egger's test (Table 2) and visualized using Funnel Plots.

### Meta-analysis

**Pathological complete response:** As mentioned earlier, effect sizes were synthesized separately under three definitions of pCR. Considering pCR to breast as well axilla reported under eight RCTs randomizing 1442 patients, 127 (16.8%) in anthracycline arm and 127 (18.5%) in taxane arm achieved pCR. But this increase in pCR with taxane (especially with addition of taxane to anthracycline) was not statistically significant ( $n = 8$ ,  $I^2 = 34.4\%$ ,  $RR = 1.14$ , 95%CI: 0.84-1.55). Further, subgroup analyses also revealed the similar results for anthracycline versus taxane ( $n = 2$ ,  $I^2 = 38.3\%$ ,  $RR=0.74$ , 95%CI: 0.23-2.39) as well as anthracycline versus taxane along with anthracycline ( $n = 6$ ,  $I^2 = 41.9\%$ ,  $RR = 1.23$ , 95%CI: 0.86-1.76). Although some of the RCTs on which evidence is based were non-blinded but objective measurement of pCR would not change drawn evidences which were graded as high (Table 2).

PCR of breast regardless of axilla was reported by seven RCTs randomizing 4007 patients due to inclusion of two large RCTs<sup>[26,31]</sup> which contribute around 50% in the pooled effect estimates. Out of these seven RCTs, six RCTs assessed the effectiveness of addition of taxanes. pCR by this definition was observed to be 16.8% with anthracycline group and 23.0% in taxane group which was found to be statistically significant because of significant results under two big RCTs involving 390 patients ( $I^2 = 72.6\%$ ,  $RR = 1.48$ , 95%CI: 1.04-2.12). Similarly, pCR of breast with DCIS was found to be significantly higher in taxanes group ( $I^2 = 69.6\%$ ,  $RR = 1.54$ , 95%CI: 1.11-2.15). Apart from inclusion of some non-blinded trials for the objectively measured outcomes, evidences were downgraded one label to moderate due to high heterogeneity. The third definition of pCR, *i.e.*, allowing for DCIS revealed beneficial effect of addition of taxanes to anthracycline based chemotherapy.

**Clinical response:** OR measured clinically was higher with taxane based chemotherapy (79.0%) in comparison to anthracycline based chemotherapy (73.5%) ( $I^2 = 69.1\%$ ,  $RR = 1.13$ , 95%CI: 1.04-1.24). Similarly, addition of taxane based chemotherapy also improved clinical complete response in comparison to anthracyclines alone ( $I^2 = 49.5\%$ ,  $RR = 1.18$ , 95%CI: 0.97-1.44). Subjective measurement of these outcomes was based on few non-blinded trials which downgraded the evidence one level. Further, due to high heterogeneity and presence of publication bias, evidence for OR further downgraded to Low but that for complete clinical response remains at moderate level.

**Breast conserving surgery:** Taxane based chemotherapy could not further improve BCS, weather it was given with or without anthracycline in comparison to

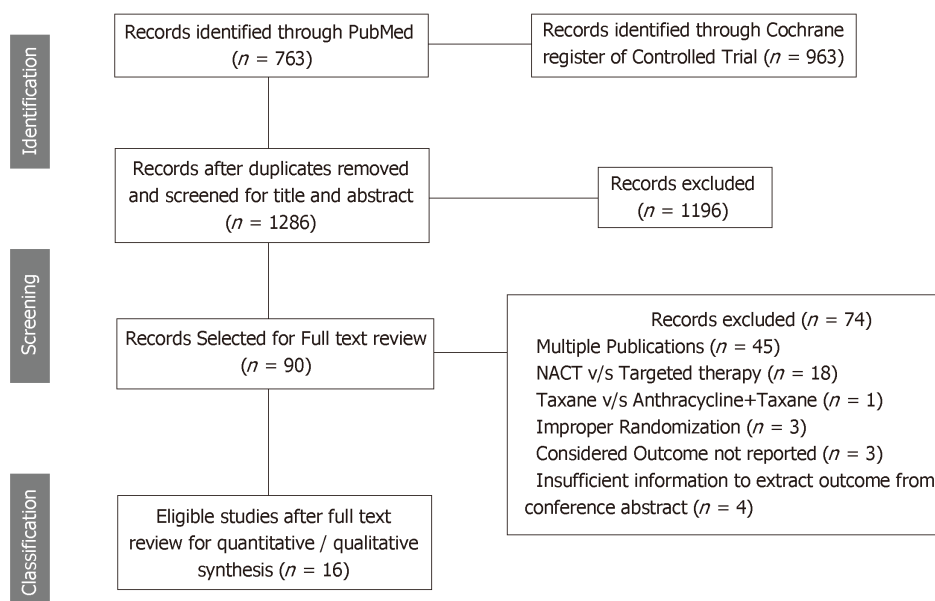


Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analysis flow chart for inclusion of studies.

anthracyclines ( $I^2 = 1\%$ ,  $RR = 1.04$ , 95%CI: 0.98-1.10) with high grade of evidence.

**Survival and recurrences:** Long term outcomes like overall survival outcomes, disease free survival and recurrences were reported by very few RCTs. A total of seven RCTs (Three compared taxane v/s anthracycline and four RCTs compared anthracycline + taxane v/s anthracycline) reported overall survival (Table 2). Overall survival was relatively better with taxane based NACT in comparison to anthracyclines but due to few RCTs, it could not reach at significance level [ $n = 7$ ,  $RR = 0.86$  (0.70-1.05)]. However, taxanes based NACT especially addition of taxanes to anthracycline based NACT significantly improved disease-free survival [ $n = 6$ ,  $RR = 0.89$  (0.80-0.99)]. Recurrences, *i.e.*, loco-regional recurrence and distant metastasis were reported by only four RCTs comparing addition of taxanes to anthracycline based NACT in comparison to anthracycline alone. Taxanes played a significant role in combating loco-regional recurrence [ $RR=0.74$  (0.59-0.94)] but not distant metastasis [ $RR=0.94$  (0.82-1.07)].

**Toxicities:** Meta-analysis of toxicities involve both group of RCTs comparing taxane alone *vs* anthracycline alone as well as taxane and anthracycline combination *vs* anthracycline alone. In comparison to anthracycline based chemotherapy, taxane based chemotherapy was found to lower down the risk for nausea ( $n=7$ ,  $RR = 0.33$ , 95%CI: 0.24-0.44) and vomiting ( $n = 4$ ,  $RR = 0.23$ , 95%CI: 0.16-0.33) (Table 3). In contrary, it was found to raise the chance of febrile Neutropenia [ $n = 4$ ,  $RR = 2.67$  (2.33-3.07)] and infection [ $n = 4$ ,  $RR = 2.53$  (2.00-3.19)] significantly. However, due to lack of sufficient sample size/event rate, stable results could not be obtained for allergic reaction, hand foot syndrome, sensory neuropathy, gastrointestinal problems and dermatological problems as the confidence interval of the related risk ratio was observed to be very wide while comparing taxanes and anthracyclines.

## DISCUSSION

A total of 16 RCTs assessing efficacy of taxanes based NACT (taxanes alone or addition of taxanes) in comparison to anthracyclines based NACT in the treatment of breast cancer, reporting at least one of the considered outcomes were included for this systematic review and meta analyses. Out of these trials, most of the big RCTs compared addition of taxanes to anthracyclines over anthracyclines (having 86% of the total randomized patients among all 16 RCTs). Meta-analysis sample size varied for outcome-wise synthesis. It was highest with 11 RCTs reporting clinical responses, *i.e.*, OR and clinical complete response. Further, pCR was reported by six to eight RCTs with varying definitions and BCS rates were reported by 9 RCTs. Survival outcomes (overall survival and disease free survival) were reported by only seven RCTs and recurrences (loco-regional recurrences and distant metastasis) were



**Table 1 Population, intervention, comparison and outcome characteristics of included studies**

Study	Accrual	Population	Regimen Comparison	Outcomes
ABDREEN <sup>[9]</sup> , 2002	104	Locally advanced breast cancer patients in which only responders of previous four cycles of CVAP	Anthracycline Arm: CVAP chemotherapy, comprised of cyclophosphamide (C) 1000 mg/m <sup>2</sup> , doxorubicin (A) 50 mg/m <sup>2</sup> , vincristine (V) 1.5 mg/m <sup>2</sup> (I.V.), and prednisone 40 mg/d p.o. for 5 d; Taxane Arm: 4 cycles of Docetaxel (D) 100 mg/m <sup>2</sup> was given as an I.V. infusion over 1 h and repeated at 21-d intervals. In addition, these patients received prednisone 100 mg for 5 d, beginning 24 h prior to docetaxel administration.	pCR1, pCR2, OR, cCR, OS
ACCOG <sup>[24]</sup> , 2010	363	Patients with primary tumour >3 cm, inflammatory or locally advanced non-metastatic breast cancer patients	Anthracycline Arm: Six cycles of doxorubicin (60 mg/m <sup>2</sup> ) and cyclophosphamide (600 mg/m <sup>2</sup> ) both administered every 3 wk (6xAC); Taxane Arm: Six cycles of doxorubicin (50 mg/m <sup>2</sup> ) and docetaxel (75 mg/m <sup>2</sup> ) administered as a 1-h I.V., with both drugs being given every 3 wk (6xAD).	pCR1, pCR2, pCR3, OR, cCR, BCS; Toxicity, OS, DFS, LRR, DM
Amsterdam trial <sup>[25]</sup> , 2005	57	Invasive breast cancer greater than 3 cm and/or at least one tumor-positive auxiliary lymph node	Anthracycline Arm: Six cycles of doxorubicin 60 mg/m <sup>2</sup> and cyclophosphamide 600 mg/m <sup>2</sup> administered every 3 ws (6xAC); Taxane Arm: Six cycles of doxorubicin 50 mg/m <sup>2</sup> and docetaxel 75 mg/m <sup>2</sup> (6xAD) 3 wk.	pCR2,
EORCT BIG-01 <sup>[26]</sup> , 2011	1856	Invasive breast cancer <71 years with large operable/inflammatory breast cancer patients suitable for neoadjuvant chemotherapy	Anthracycline Arm: Six cycles of iv FEC (fluorouracil 500 mg/m <sup>2</sup> , epirubicin 100 mg/m <sup>2</sup> , and cyclophosphamide 500 mg/m <sup>2</sup> ) or tailored FEC (F600, E75, C900) starting on day 1 and then every 21 d with GCF (6xFEC); Taxane Arm: Three cycles of docetaxel 100 mg/m <sup>2</sup> iv, followed by 3 cycles of epirubicin 90 mg/m <sup>2</sup> and docetaxel 75 mg/m <sup>2</sup> on day 1 every 21 d, without GCF.	pCR2, cCR, BCS; Toxicity
NSABP FB-9 <sup>[8]</sup> , 2015	50	HER2 negative breast cancer patients with palpable mass of ≥ 2cm in breast or axilla or inflammatory breast cancer patients	Anthracycline Arm: 4 cycles of Eribuline 1.4 mg/m <sup>2</sup> on days 1 and 8 of a 21-d cycle followed by A60 C600, every 21 d for 4 cycles; Taxane Arm: Weekly Paclitaxel 80 mg/m <sup>2</sup> for 12 doses followed by standard A60C600 every 21 d for 4 cycles.	pCR1, OR, cCR, BCS, Toxicity
Madrid trial <sup>[20]</sup> , 2011	211	Female breast cancer patients aged 18-78 years of clinical stage IIB, IIIA or IIIB and with palpable breast cancer not amenable to BCS	Anthracycline Arm: Four cycles of doxorubicin (75 mg/m <sup>2</sup> body surface area); Taxane Arm: Four cycles docetaxel 100 mg/m <sup>2</sup> with G-CSF support every 3 wk.	pCR1

Saura <i>et al</i> <sup>[4]</sup> , 2013	295	Breast cancer patients of stage T2-3N0-3M0	Pretreatment: patients received four cycles of doxorubicin (60mg/m <sup>2</sup> iv) and cyclophosphamide (600 mg/m <sup>2</sup> iv) every 3 wk; Anthracycline Arm: Ixabepilone (40 mg/m <sup>2</sup> , 3-h infusion) every 3 wk for 4 cycles; Taxane Arm: paclitaxel (80 mg/m <sup>2</sup> , 1-h infusion) weekly for 12 wk.	pCR1, pCR3, OR, cCR, BCS, Toxicity
NCC Korea <sup>[21]</sup> , 2008	209	Previously untreated stage II/III breast cancer patients with auxiliary lymph node involvement of age ≥ 18 years, ECOG performance status ≤ 1	Anthracycline Arm: doxorubicin 60 mg/m <sup>2</sup> IV on day 1 plus cyclophosphamide 600 mg/m <sup>2</sup> IV on day 1 every 3 wk for four cycles; Taxane Arm: docetaxel 75 mg/m <sup>2</sup> 1-h infusion on day 1 plus capecitabine 1000 mg/m <sup>2</sup> orally twice daily on days 1-14 every 3 wk for four cycles.	pCR3, OR, cCR, Toxicity, OS, DFS
Norwegian trial <sup>[22]</sup> , 2012	223	Primary stage III breast cancer patients	Anthracycline Arm: 4x Epirubicin 90 mg/m <sup>2</sup> administered at 3 wk interval; Taxane Arm: four cycles of paclitaxel 200 mg/m <sup>2</sup> administered at 3 wk intervals.	OR, cCR, BCS, OS
Learn <i>et al</i> <sup>[27]</sup> , 2005	144	Invasive breast carcinoma with clinical staging T1c-T3, N0M0 or T1-3, N1M0	Anthracycline Arm: 4 cycles of doxorubicin and cyclophosphamide (A60 C600) every 21 as well as tamoxifen 20 mg per day for 5 yr as NACT; Taxane Arm: 4 cycles of A60 C600 every 21 d further 4 cycles of docetaxel at 100 mg/m <sup>2</sup> every 21 d as NACT; Arm 3 (Docetaxel as ACT): 4x AC as ACT (not part of the current study).	pCR1; OR
Diéras <i>et al</i> <sup>[5]</sup> , 2004	240	Breast cancer patients of stage T2-3N0-1M0, who were not assessable for breast conserving surgery	Anthracycline Arm: 4 cycles of A60 C600 i.v. every 21 d; Taxane Arm: doxorubicin 60 mg/m <sup>2</sup> as (IV) bolus during 5 to 15 min immediately followed by paclitaxel 200 mg/m <sup>2</sup> as a 3-h infusion every 21 d for 4 cycles.	pCR3, OR, cCR, cPR, BCS; Toxicity, OS, DFS, LRR, DM
Tabchy <i>et al</i> <sup>[28]</sup> , 2010	273	Breast cancer patients with clinical stage I to III	Anthracycline Arm: six courses of 5-fluorouracil (500 mg/m <sup>2</sup> ), doxorubicin 50/epirubicin 100, and cyclophosphamide (500 mg/m <sup>2</sup> ) all on day 1 repeated in 21-d cycles; Taxane Arm: 12 courses of weekly paclitaxel (80 mg/m <sup>2</sup> /wk) followed four cycles of anthracycline chemotherapy all on day 1 repeated in 21-d cycles.	pCR1; BCS
NSABP-27 <sup>[6]</sup> , 2006	2411	Primary operable breast cancer patients with palpable tumor of stage T1c-3, N0-1 M0.	Arm1- 4 cycles of Doxorubicin 60 mg/m <sup>2</sup> Cyclophosphamide 600 mg/m <sup>2</sup> every 3 wk; Arm 2- Doxorubicin 60 mg/m <sup>2</sup> Cyclophosphamide 600 mg/m <sup>2</sup> every 3 wk × 4 followed by Docetaxel 100 mg/m <sup>2</sup> every 3 wk × 4 followed by surgery; Arm3 (ACT arm)- Doxorubicin 60 mg/m <sup>2</sup> Cyclophosphamide 600 mg/m <sup>2</sup> every 3 wk × 4 followed by surgery--> Docetaxel 100 mg/m <sup>2</sup> every 3 wk × 4	pCR2, pCR3, OR, cCR, BCS; Toxicity, OPS, DFS, LRR, DM

Buzdar <i>et al</i> <sup>[23]</sup> , 1999	174	Invasive, but non-inflammatory, breast cancer with stage II to IIIA disease	Anthracycline Arm: 4 × FAC (fluorouracil 500, cyclophosphamide 500 mg/m <sup>2</sup> , doxorubicin 50 mg/m <sup>2</sup> ) every 3 wk interval; Taxane Arm: Paclitaxel 250 mg/m <sup>2</sup> as a 24-h continuous infusion at 3-wk intervals for four cycles.	pCR3, OR, cCR, BCS; Toxicity, DFS
Cortés-Flores <i>et al</i> <sup>[30]</sup> , 2008	41	Stage IIB and IIIA, locally advanced breast cancer patients	Anthracycline Arm: 5-fluorouracil epirubicine cyclophosphamide; Taxane Arm: docetaxel and epirubicine.	pCR2
Sivasanker <i>et al</i> <sup>[29]</sup> , 2017	101	Locally advanced breast cancer patients' candidates for NACT	Anthracycline Arm: Cyclophosphamide 500 mg/m <sup>2</sup> , Doxorubicin 50 mg/m <sup>2</sup> and 5-FU 500/m <sup>2</sup> as IV infusion repeated every 21 d; Taxane Group: Paclitaxel 175 mg/m <sup>2</sup> as a 3 h IV infusion, Doxorubicin 50 mg/m <sup>2</sup> as IV infusion.	pCR1, pCR2, OR, cCR, BCS

pCR1: Pathological complete response to breast as well as axilla; pCR2: Pathological complete response to breast regardless of axilla; pCR3: Pathological complete response to breast allowing for ductal carcinoma *in situ*; NACT: Neoadjuvant Chemotherapy; OR: Overall response; cCR: Clinical complete response; BCS: Breast conserving surgery; OS: Overall survival; DFS: Disease free survival; LRR: Loco-regional recurrence; DM: Distant metastasis.

reported by only four RCTs assessing the effectiveness of addition of taxanes over anthracyclines alone. Pattern of reported toxicities were also varied among RCTs. Most of the trials ( $n = 7$ ) reported Neutropenia. All of the included RCTs used proper method for randomization but enough information to assess concealment was not reported by many RCTs. Further, six out of 16 RCTs were open label RCTs and may have an obvious impact on subjectively measured outcomes like OR and clinically complete response. Most of the RCTs have reported results based on intention to treat analysis. Overall, quality of included RCTs can be treated as adequate for objectively measured outcomes. Further, quantity of RCTs was sufficient to assess the relative effectiveness of addition of taxanes to anthracyclines over anthracyclines alone but not for subgroup assessing efficacy of taxanes alone versus anthracyclines alone.

The effectiveness of neoadjuvant chemotherapy depends on the used regimens. Response to neoadjuvant chemotherapy predict the prognosis and recurrence in breast cancer patients regardless of the type of surgery performed<sup>[32]</sup>. Patients having pCR have prolonged disease-free survival and overall survival. NSABP B-18 and B-27 trials revealed significantly better disease-free survival and overall survival among patients achieving pCR in comparison to the patients who could not<sup>[6]</sup>. It revealed that pCR is valid surrogate point for long term outcomes. This may be the reason; most of the trials comparing two neoadjuvant chemotherapy regimens reported tumor response instead of survival outcomes. Anthracyclines and taxanes are most active groups of chemotherapy regimens. Taxanes are standard of care for metastatic breast cancer patients because they are significantly more effective than anthracyclines based regimens<sup>[33]</sup>. Systematic review to assess the role of taxanes was performed a long back in 2004 and 2005 by Nowak *et al*<sup>[12]</sup> and Trudeau *et al*<sup>[13]</sup>. But most of the RCTs assessing effectiveness of taxanes over anthracyclines were reported after publication of these two reviews. Further, these two reviews included abstracts of ongoing trials which were not complete at that point of time. Since trials were not mature to report survival outcomes, these two studies could not comment on efficacy of taxanes for long term outcomes like survival and recurrences<sup>[13]</sup>. Further, Due to availability of very few trials, these two reviews could not perform meta-analysis for response outcomes and limit their finding with qualitative synthesis of these trials. As a matter of fact, present study could be able to quantitatively synthesize the results of tumor responses as well as for long term outcomes like overall survival, disease free survival, loco-regional recurrence and distant metastasis.

Addition of taxanes to anthracyclines based chemotherapy was found to be associated with higher pCR, better disease-free survival and decreased loco-regional recurrence. It was also found beneficial for clinical responses like OR and clinical complete response. But evidences on clinical responses have limited use due to downgrading of their quality because of involvement of some non-blinded trials. Also, clinical response to NACT also guides for further systemic therapy<sup>[34,35]</sup>. NACT increases the chance of BCS<sup>[36]</sup> but taxanes could not further improve the conservation

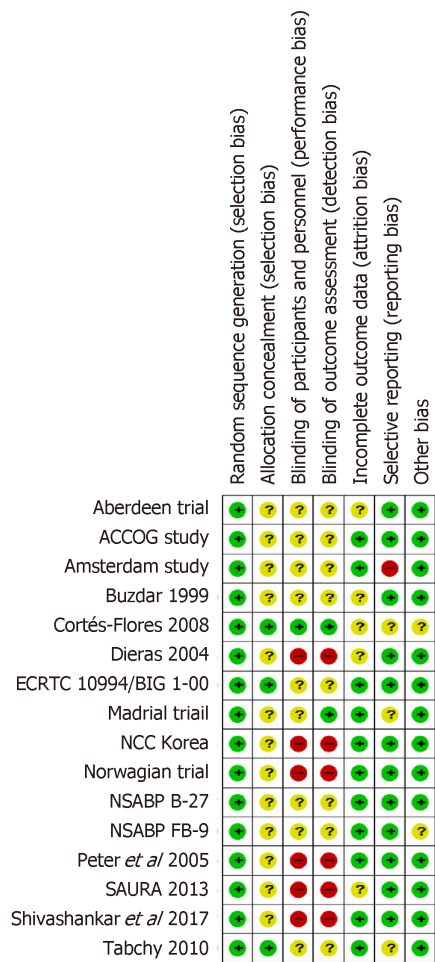


Figure 2 Risk of bias assessed for each individual study using Cochrane bias assessment tool.

surgery rate over anthracyclines alone. Evidences for the subgroup assessing effectiveness of addition of taxanes to anthracyclines over anthracyclines were rated as of high grade and stable due to availability of large RCTs with adequate number. On the other hand, synthesized results for the subgroup comparing taxanes alone to anthracyclines alone involved few RCTs that too of small sample size. Further, the conclusive results for toxicities cannot be summarized because these were reported by very few RCTs and reporting of toxicities also involved variation in their definitions. In summary, addition of taxanes improves pCR, disease free survival and loco-regional recurrence free survival but has no major impact on BCT rates.

Table 2 Subgroup as well as overall meta-analysis for all considered outcomes

Outcome	Sub-Group	Number of studies	Events taxane	Events anthracycline	Egger's test P-value	P Statistic	Risk Ratio(95%CI)	Grade
pCR (BA)	Taxane v/s Anthracycline	2	21/125	24/128	-	38.3	0.74 (0.23-2.39)	High
	Taxane + Anthracycline v/s Anthracycline	6	106/562	103/627	0.110	41.9	1.23 (0.86-1.76)	High <sup>1</sup>
	Overall	8	127/687	127/755	0.573	34.4	1.14 (0.84-1.55)	High <sup>1</sup>
pCR (B)	Taxane v/s Anthracycline	1	16/47	8/50	-	-	2.13 (1.01-4.50)	Moderate <sup>2</sup>
	Taxane + Anthracycline v/s Anthracycline	6	443/1951	329/1959	0.475	72.6	1.48 (1.04-2.12)	Moderate <sup>1,3</sup>
	Overall	7	459/1998	337/2009	0.331	69.6	1.54 (1.11-2.15)	Moderate <sup>1,3</sup>
pCR (DCIS)	Taxane v/s Anthracycline	2	29/189	24/186	-	85.4	1.06 (0.25-4.47)	Moderate <sup>1,3</sup>
	Taxane + Anthracycline v/s Anthracycline	4	761/1679	183/683	0.339	71.4	1.23 (0.86-1.75)	Moderate <sup>1,3</sup>
	Overall	6	790/1868	207/869	0.277	71.7	1.20 (0.84 -1.70)	Moderate <sup>1,3</sup>
Overall response	Taxane v/s Anthracycline	4	249/356	221/349	0.956	66.2	1.12 (0.94-1.33)	Low <sup>3,4</sup>
	Taxane + Anthracycline v/s Anthracycline	7	1098/1348	1024/1345	0.045	71.4	1.14 (1.02-1.27)	Low <sup>3,4</sup>
	Overall	11	1347/1704	1245/1694	0.031	69.1	1.13 (1.04-1.24)	Low <sup>3,4</sup>
Complete clinical response	Taxane v/s Anthracycline	4	62/356	45/349	0.908	00.0	1.40 (1.01-1.93)	Moderate <sup>4</sup>
	Taxane + Anthracycline v/s Anthracycline	7	548/2231	511/2176	0.273	60.1	1.13 (0.88-1.43)	Low <sup>3,4</sup>
	Overall	11	610/2587	556/2525	0.106	49.5	1.18 (0.97-1.44)	Moderate <sup>4</sup>
Breast conserving surgery	Taxane v/s Anthracycline	1	40/86	30/85	-	-	1.32 (0.91-1.90)	Moderate <sup>2</sup>
	Taxane + Anthracycline v/s Anthracycline	8	1040/2206	1007/2199	0.633	00.0	1.03 (0.97-1.09)	High <sup>1</sup>
	Overall	9	1080/2292	1037/2284	0.406	01.1	1.04 (0.98-1.10)	High <sup>1</sup>
Overall survival	Taxane v/s Anthracycline	3	18/255	31/251	0.002	74.9	0.41 (0.13-1.31)	Low <sup>1,35</sup>
	Taxane + Anthracycline v/s Anthracycline	4	424/2026	456/1960	0.899	0.0	0.91 (0.79-1.05)	High <sup>1</sup>
	Overall	7	442/2281	487/2211	0.059	37.4	0.86 (0.70-1.05)	High <sup>1</sup>
Disease free survival	Taxane v/s Anthracycline	3	71/285	84/289	0.144	0.00	0.92 (0.63-1.36)	High <sup>1</sup>
	Taxane + Anthracycline v/s Anthracycline	4	722/2026	772/1958	0.685	0.00	0.89 (0.80-0.99)	High <sup>1</sup>
	Overall	7	793/2311	856/2247	0.791	0.00	0.89 (0.80-0.99)	High <sup>1</sup>
Loco-regional recurrence	Overall	4	120/2026	161/1960	0.808	0.00	0.74 (0.59-0.94)	High
Distant metastasis	Overall	4	426/2026	441/1960	0.264	0.00	0.94 (0.82-1.07)	High

<sup>1</sup>Involves non-blinded RCT(s) but objective measurement will not change the drawn evidences.



<sup>2</sup>Evidence is based on few sample (Imprecise).<sup>3</sup>Imprecision because of higher heterogeneity ( $I^2$ ).<sup>4</sup>Involves non-blinded RCTs which may change the drawn evidence.<sup>5</sup>Publication Bias.pCR: Pathological complete response; DCIS: Ductal carcinoma *in situ*.**Table 3 Pooled effect estimates for various toxicity in taxanes in comparison to anthracyclines**

Toxicity	Number of studies	RR (95%CI)
Hematological toxicity		
Neutropenia	7	1.00 (0.78-1.29)
Febrile neutropenia	4	2.67 (2.33-3.07)
Leucopenia	4	0.72 (0.36-1.45)
Anemia	3	0.75 (0.12-4.53)
Thrombocytopenia	4	0.07 (0.03-0.19)
Thrombosis	2	1.07 (0.59-1.96)
Cardiac and nervous system toxicity		
Neuropathy	2	1.01 (0.35-2.93)
Sensory neuropathy	3	18.26 (5.87-56.80)
Cardiac left ventricular function	1	0.33 (0.01-8.14)
Cardiovascular toxicity	1	2.74 (0.88-8.57)
Dermatological toxicities		
Hand foot syndrome	2	27.43 (3.75-200.84)
Rash	1	8.96 (0.48-166.20)
Dermatological toxicity	2	3.71 (1.18-11.67)
Alopecia	1	0.78 (0.73-0.83)
Diarrhea	5	1.90 (0.97-3.73)
Gastro	1	4.23 (1.43-12.53)
constipation	1	3.49 (0.73-16.73)
Oral toxicities		
Stomatitis	6	1.89 (1.23-2.91)
Musculoskeletal pain	1	1.01 (0.06-15.95)
General toxicity		
Nausea	7	0.33 (0.24-0.44)
Fatigue	5	1.29 (0.96-1.73)
Infection	4	2.53 (2.00-3.19)
Other	6	1.12 (0.61-2.06)
Vomiting	4	0.23 (0.16-0.33)
Allergic reaction	3	21.25 (2.74-164.67)
Myalgia	3	1.99 (0.36-11.01)
Serious adverse event	1	0.65 (0.29-1.45)
edema	1	6.97 (0.36-134.74)
Fever	1	15.85 (0.96-260.89)
Hypotension	1	6.97 (0.36-134.74)
Pulmonary	1	3.54 (0.92-13.65)
Arthelgia	3	0.02 (0.01-0.04)
Bone pain	1	0.07 (0.00-1.17)

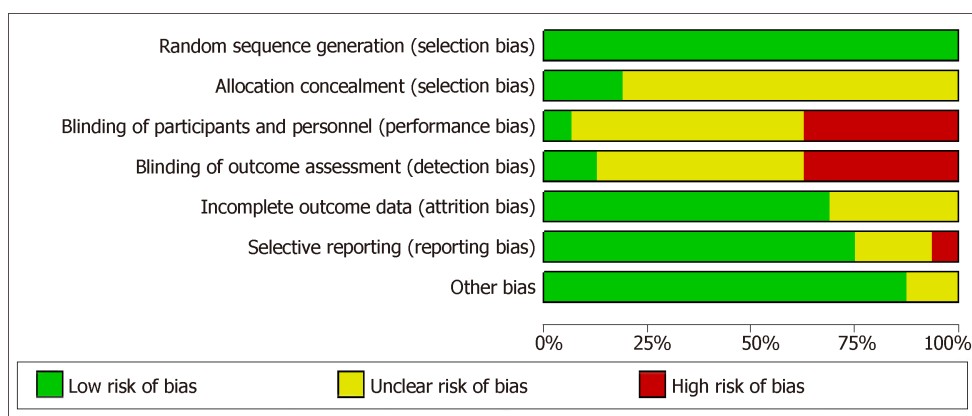


Figure 3 Summary risk of bias using Cochrane bias assessment tool.

## ARTICLE HIGHLIGHTS

### Research background

There are mainly two active group of chemotherapy regimen namely Anthracyclines and taxanes. Randomized controlled trials (RCTs) have reported variable evidences regarding efficacy of taxanes over anthracyclines especially for tumor response and survival outcomes. Hence, as required, the present study synthesizes the relative efficacy of taxanes over anthracyclines using pathological complete response, clinical responses, breast-conserving surgeries and survival outcomes in female breast cancer patients by systematic review and meta-analysis of available RCTs.

### Research motivation

There is contradictory reporting regarding relative efficacy of taxanes over anthracycline. To resolve this, for the first time, present meta-analysis is to assess the relative efficacy of taxanes (Docetaxel and Paclitaxel) alone or their addition to anthracyclines over anthracyclines alone in terms of pCR, clinical response, breast conserving surgery, survival outcomes and toxicity in neo-adjuvant setting. As and when there is further addition in regimes, such appraisals from time to time are unavoidable.

### Research objectives

Keeping in view of contradictory reporting, this study aimed to assess the relative effectiveness of taxanes over anthracyclines in neo-adjuvant setting in terms of tumor response and survival outcomes through systematic review and Meta analysis. This was expected to provide important clues regarding appropriate clinical practice.

### Research methods

The RCTs have reported contradictory findings on relative effectiveness of taxanes over anthracyclines in neo-adjuvant setting regarding treatment of breast cancer patients. In spite of this, no earlier attempt is made to synthesize relative effectiveness of taxanes over anthracyclines. For the first time, using a focused systematic review and Meta analysis of existing RCTs, this study attempted to synthesize relative effectiveness of taxanes over anthracyclines in terms of pCR, clinical response, BCS, survival outcomes and toxicity in neo-adjuvant setting. For this, all related RCTs were searched through PubMed and Cochrane register of controlled trials on 28 April 2017 and published in English language. Using PRISMA guidelines, retrieved records were screened and data were extracted by two independent reviewers. Depending on heterogeneity assessed through  $I^2$  statistic, Meta-analysis was performed using either fixed effect or random effect method. Subgroup meta-analyses were also performed for each considered outcomes on the basis of taxanes alone or taxanes along with anthracyclines in comparison to anthracyclines alone.

### Research results

Through a search through PubMed and Cochrane register of controlled trials on 28 April 2017, for this study, a total of 16 RCTs were found eligible in view of reporting at least one of the considered outcomes. The analytical results revealed that taxanes based chemotherapy significantly improved pCR, disease free survival and loco-regional recurrence free survival. Further, subgroup analysis showed that addition of taxanes to anthracyclines has better effectiveness regarding these survivals over anthracyclines alone than taxanes alone over anthracyclines alone.

### Research conclusions

This study hypothesized that effectiveness of neo-adjuvant chemotherapy may rely on used regimens. Keeping in view of varying reporting under related RCTs, as an appraisal, to assess

relative effectiveness of taxanes in comparison to anthracyclines was planned. For this, it was carried out as a first systematic review and Meta analysis of the related RCTS. As obvious, as a first-time observation, the synthesized results suggest that taxanes based chemotherapy may significantly improve pCR, disease free survival and loco-regional recurrence free survival. Further, as additional clues, subgroup analysis showed that addition of Taxanes to anthracyclines emerged to be more effective regarding these survivals over anthracyclines alone than taxanes alone over anthracyclines alone.

### Research perspectives

In presence of contradictory findings under RCTs, a systematic review and Meta analysis of available RCTs may provide important clues towards clinical practice. Completeness of data is crucial for such studies. To achieve this, as true in case of other study designs, an appropriate protocol needs to be written for carrying out such studies. Although such studies are being carried out on other study designs as well, to ensure high level of evidence, such studies on RCTs need to be preferred.

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## Current state and future direction of screening tool for colorectal cancer

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### Abstract

As the second-most-common cause of cancer death, colorectal cancer (CRC) has been recognized as one of the biggest health concerns in advanced countries. The 5-year survival rate for patients with early-stage CRC is significantly better than that for patients with CRC detected at a late stage. The primary target for CRC screening and prevention is advanced neoplasia, which includes both CRC itself, as well as benign but histologically advanced adenomas that are at increased risk for progression to malignancy. Prevention of CRC through detection of advanced adenomas is important. It is, therefore, necessary to develop more efficient detection methods to enable earlier detection and therefore better prognosis. Although a number of CRC diagnostic methods are currently used for early detection, including stool-based tests, traditional colonoscopy, etc., they have not shown optimal results due to several limitations. Hence, development of more reliable screening methods is required in order to detect the disease at an early stage. New screening tools also need to be able to accurately diagnose CRC and advanced adenoma, help guide treatment, and predict the prognosis along with being relatively simple and non-invasive. As part of such efforts, many proposals for the early detection of colorectal neoplasms have been introduced. For example, metabolomics, referring to the scientific study of the metabolism of living organisms, has been shown to be a possible approach for discovering CRC-related biomarkers. In addition, a growing number of high-performance screening methodologies could facilitate biomarker identification. In the present, evidence-based review, the authors summarize the current state as recognized by the recent guideline recommendation from the American Cancer Society, US Preventive Services Task Force and the United States Multi-Society Task Force and discuss future direction of screening tools for colorectal cancer. Further, we highlight the most interesting publications on new screening tools, like molecular biomarkers and metabolomics, and discuss these in detail.

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**Key words:** Colorectal cancer; Screening tool; Early detection; Biomarkers; Metabolomics

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**Core tip:** A large proportion of colorectal cancer (CRC) cases and deaths could be prevented by screening with early detection and removal of colorectal adenomas or early stage CRC. Reliable and non-invasive screening tools for early stage CRC and precancerous lesions, such as adenoma is indispensable. However, current screening methods have limitations. Therefore, it is important to review the current literature on new screening tools such as molecular biomarkers and metabolomics for the development of new diagnostic tools.

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## INTRODUCTION

Colorectal cancer (CRC) remains a global health problem and currently is considered one of the leading causes of death in the world<sup>[1]</sup>. The patient's survival is predicted by the tumor stage at the time of diagnosis. Early CRC diagnosis maximizes the benefit of treatment. Typically, it takes 7-10 years for an adenoma to become a carcinoma, which provides a timeframe allowing for early detection of CRC<sup>[2]</sup>. At present, the best available option for early detection and elimination of premalignant lesions is colonoscopy. However, it is invasive, expensive, and inconvenient for patients. Therefore, non-invasive and reliable methods for diagnosing CRC are valuable due to colonoscopy risks: Puncture of the colon, intraperitoneal bleeding, post-polypectomy, and infection. In particular, with regards to the detection of CRC precursor lesions, such as adenoma, the lack of sensitivity and specificity or an unacceptably wide range of the FOBT has hampered the clinical application in CRC screening<sup>[3]</sup>. Therefore, newer, non-invasive screening methods and biomarkers to permit identification of CRC and its precursors in easily accessible biospecimens are needed. Consequently, current screening methods have limitations, and it is necessary to find new screening methods that can detect CRC in the early phase to improve survival and quality of life for patients with CRC.

The following qualities are what an ideal screening method would possess: It should show high sensitivity and specificity, it must be safe and cost-effective to be widely used, it must be simple to measure, and readings must be consistent among patients of all genders and races. Acceptability of screening method is also important in target population<sup>[4]</sup>. In this review, we summarize the current status of screening tools for colorectal cancer and discuss the future direction of colon cancer screening, including metabolism and proteomics.

## CURRENT STATE OF SCREENING TOOLS FOR CRC

The American Cancer Society (ACS), US Preventive Services Task Force (USPSTF) and the United States Multi-Society Task Force (USMSTF) Guideline recommends stool-based tests and structural examinations as options for colorectal cancer screening<sup>[5-7]</sup>. Stool-based tests consist of guaiac-based tests, immunochemical tests, and mt-sDNA tests. Structural examinations consist of colonoscopy, computed tomography colonography, and flexible sigmoidoscopy (FS). These screening tests are currently in use, and we will first discuss the screening value and limitations of currently-used tests. The characteristics, advantages and disadvantages of colorectal cancer screening tests currently in use are shown in Table 1.

### Stool-based CRC screening tests

Stool-based tests are conventionally known as fecal occult blood tests (FOBTs) because they aim to discover the presence of occult blood in stool, which may derive from colorectal cancer or larger polyps of at least 2 cm in size. FOBT are divided into



Table 1 Characteristics of colorectal cancer screening tests currently in use in the United States

Screening test	Interval	Evidence	Advantages	Disadvantages	Other considerations
<b>Stool-based screening tests</b>					
FIT with high sensitivity <sup>123</sup>	Every year	Improved performance compared with high-sensitivity gFOBT Mortality reduction: indirect evidence from RCTs of guaiac-based stool tests	Can be performed at home Requires only a single specimen No diet or medication restrictions Does not require bowel preparation or anesthesia Inexpensive compared with structural examinations and mt-sDNA	High nonadherence to yearly testing (especially without reminder systems) Less effective for advanced adenoma detection Few accessible tests have published peer-reviewed performance data	Varies in test performance due to brand and version Follow-up colonoscopy for positive test may charge extra costs
gFOBT with high sensitivity <sup>12</sup> (HSGFOBT)	Every year	Good RCT evidence for incidence and mortality reduction <sup>[112-116]</sup> Varies in test performance characteristics by version of the test	Inexpensive compared with structural examinations and mt-sDNA Can be done at home Does not require bowel preparation or anesthesia	High nonadherence to yearly testing (especially without reminder system) Less effective for advanced adenoma detection Difficulty in determining test performance among the many FDA-cleared tests Requires multiple samples Requires dietary and medication restriction Higher false-positive rate than FIT leads to more colonoscopies	Follow-up colonoscopy for positive test may charge extra costs
mt-sDNA <sup>1</sup>	Every 3 yr	Mortality reduction: indirect evidence from RCTs of guaiac-based stool tests Improved sensitivity for cancer and AA and poorer specificity compared with FIT	Can be done at home Does not require bowel preparation or anesthesia	More expensive than other stool-based tests Higher false-positive rate than FIT	Follow-up colonoscopy for positive test may charge extra costs A new test with limited data on screening outcomes. Uncertainty in management of positive results followed by a negative colonoscopy
FIT-DNA <sup>23</sup>	Every 1 or 3 yr	Test characteristic studies	Improved sensitivity compared with FIT per single screening test Does not require bowel preparation or anesthesia Can be done at home	Higher false-positive rate than FIT	Uncertainty in management of positive results followed by a negative colonoscopy
<b>Direct visualization screening tests</b>					

Colonoscopy <sup>123</sup>	Every 10 yr	Non-RCT evidence of incidence and mortality reduction Prospective cohort study with mortality end point	Requires less frequent screening Screening, diagnosis, treatment and prevention through polypectomy can be done at the same-session. Gross visualization of the entire colon	Pain and discomfort Lower tolerability and compliance than FS <sup>[117]</sup> Possibility of bowel perforation / bleeding and cardiopulmonary complications from anesthesia Requires full bowel cleansing Performance varies upon adequacy of bowel preparation, the cecal intubation rate, withdrawal time, and adenoma detection rate Lower sensitivity for neoplasia in the proximal than the distal colon	Polypectomy and anesthesia may charge extra costs Most expensive test, but currently reimbursable with insurance Requires day-off (if sedation is used)
CTC <sup>123</sup>	Every 5 yr	Test characteristic studies Extrapolation from RCTs of sigmoidoscopy demonstrating mortality reduction	Rapid, non-invasive imaging method Well-tolerated by patients Does not require anesthesia Better tolerability and acceptance than colonoscopy and FS <sup>[118]</sup>	Exposure to low-dose radiation Requires full bowel cleansing A second bowel cleansing will be required before follow-up colonoscopy for positive test	Follow-up colonoscopy for positive test may charge extra costs Insufficient evidence about the benefit-burden balance of additional tests on incidental extracolonic findings Relatively expensive and may not be covered by insurance
FS <sup>123</sup>	Every 5 yr	RCTs with mortality end points:	Does not require anesthesia Requires more limited bowel cleansing Better acceptance than colonoscopy <sup>[117]</sup>	Pain and discomfort Does not examine the proximal Colon Requires enema prior to procedure Abnormal findings require second colonoscopy	Follow-up colonoscopy for positive test may charge extra costs Concerns about lack of quality standards, limited availability, failure to achieve a complete examination
FS with FIT <sup>2</sup>	FS every 10 yr plus FIT every year	RCT with mortality end point (subgroup analysis)	More benefits than when combined with FIT or compared with other strategies It may be an potentially option for patients who want endoscopy screening but do not want colonoscopy		Test declined in the US

<sup>1</sup>The American Cancer Society Guidelines recommend.<sup>2</sup>US Preventive Services Task Force Guidelines recommend.<sup>3</sup>The U.S. Multi-Society Task Force Guidelines recommend [Tier 1: Colonoscopy every 10 yr, annual fecal immunochemical test; Tier 2: CT colonography every 5 yr, FIT-fecal DNA every 3 yr, flexible sigmoidoscopy every 10 years (or every 5 yr); Tier 3: Capsule colonoscopy every 5 yr]. CRC: Colorectal cancer; CTC: Computed tomographic colonography; FDA: US Food and Drug Administration; FIT: Fecal immunochemical test; FS: Flexible sigmoidoscopy; gFOBT: Guaiac-based fecal occult blood test; mtsDNA: Multitarget stool DNA; RCT: Randomized controlled trial.

two primary categories according to the detected analyte: Guaiac-based fecal occult blood tests (gFOBT) and fecal immunochemical tests (FITs). gFOBT, which detects the peroxidase activity of hemoglobin, was first recognized as an effective screening for CRC. The use of this stool testing for CRC screening has been supported by multiple consistent randomized clinical trials<sup>[8]</sup>. However, the use of gFOBT is complicated by its poor sensitivity and specificity as the test shows false negatives when a patient uses antioxidants, like vitamin C, whereas false positives occur when a patient has upper GI bleeding from NSAIDs intake, or consumes red meat or dietary peroxidase from certain vegetables and fruits<sup>[9]</sup>. On the other hand, FITs specifically detect antibodies against human globin. Thus, it is not influenced by upper GI bleeding since globin is degraded in the upper GI tract, and the test result is well protected from the

influence of medications, red meat, or peroxidases from foods, eliminating the need for pre-testing food restrictions<sup>[9]</sup>.

Individual gFOBT and FIT versions show various performance characteristics. Although non-rehydrated, low-sensitivity gFOBT variants are still commercially available, it is not recommended or used for CRC screening test due to the poor performance of these gFOBT. Though there may be other tests having higher sensitivity, at the time of publication, only Hemoccult II Sensa (Beckman Coulter Inc., Brea, CA) performed as a high-sensitivity gFOBT (HSgFOBT) among the many guaiac-based tests evaluated in population-based studies. The ranges of sensitivity and specificity of HSgFOBT are from 62% to 79% and 87% to 96%, respectively<sup>[8,10,11]</sup>. The fact that gFOBT testing is often carried out in the physician's office in the form of a single-panel test after a digital rectal exam is a potential limitation of this testing. According to Collins *et al*<sup>[12]</sup>, for advanced neoplasia, the gFOBT testing sensitivity was merely 4.9%, and for cancer, it was just 9%. The accuracy of this method is so low that it cannot, under any circumstances or rationale of convenience, be endorsed as a method for CRC screening. The sensitivity of many individual tests requires optimum situations, and this is another limitation of gFOBT, which can be even more compromised by insufficient and imperfect specimen collection together with absent or inappropriate processing and interpretation. Meanwhile, FITs present higher sensitivity and slightly lower specificity for cancer and advanced neoplasia when compared with low-sensitivity gFOBTs, while demonstrating similar or higher sensitivity and specificity than HSgFOBTs. The ranges of sensitivity and specificity of single-sample FIT are 73% to 92% and 91% to 97%, respectively<sup>[9,13-16]</sup>. FIT is a non-invasive test. Moreover, in one meta-analysis it showed a one-time sensitivity for cancer of 79% and also had a reasonable sensitivity for advanced adenomas (about 30%), and it is very inexpensive (about \$20)<sup>[17]</sup>. Despite these numbers and advantages, the majority of the brands of FIT do not have sufficient data to show the accuracy of the test in identifying the presence of CRC, as Daly *et al*<sup>[18]</sup> were only able to review validation data for less than half of the versions of FIT available in the United States. The FITs were not tested in randomized studies and most of the evidence for the effectiveness of FIT test was based on indirect evidence of reduced mortality due to randomized controlled trials (RCTs) of the guaiac-based stool tests. Studies also used different versions of FITs tests to analyse their outcomes. As the FIT is intended to be repeated, the results for single-sample sensitivity and specificity alone are not sufficient. Although some evidence has started being published recently, the data for the long-term performance of FIT is still lacking<sup>[19]</sup>; thus, these studies should not be the basis for determining the performance qualities of FITs because they do not yet have adequate data.

Based on the general-population MISCAN modeling analysis that was conducted in 2018, annual FIT from 45–75 year-old adults became a recommendable strategy by providing 94% of the life-year gains (LYGs) compared with that of the standard screening test, a colonoscopy every 10 years for 45–75 year-old adults<sup>[20]</sup>. Compared to annual FIT, annual HSgFOBT from adults 45 to 75 years of age presented a higher rate of false positives, requiring more colonoscopies; thus, it was not considered to be a model-recommendable strategy, though LYGs of HSgFOBT were same as that of FIT (403 LYGs)<sup>[20]</sup>. Despite such limitations, HSgFOBT (*i.e.*, Hemoccult II Sensa) is considered to be an option for CRC screening in the updated ACS guidelines because of its high sensitivity and low cost. These benefits can be advantageous when FIT is not available.

No direct injury is caused by HSgFOBT or FIT screening. However, special care must be taken to avoid physical injury when a practitioner performs a colonoscopy to confirm a positive HSgFOBT<sup>[8]</sup>. Lately, in screening programs for CRC, the original, low-sensitivity guaiac test has been used over HSgFOBT or FIT, with the United States similarly changing their screening programs in accordance with this trend.

A third stool test is the multi-targeted stool DNA (mt-sDNA) test that uses an immunochemical assay for human hemoglobin and assays for aberrantly methylated BMP3 and NDRG4, mutated K-ras, and  $\beta$ -Actin from exfoliated cells from colonic neoplasms<sup>[21]</sup>. Based on a manufacturer-supported, multi-center comparative study of mt-sDNA and FIT testing in average-risk individuals, the sensitivity of mt-sDNA and FIT were 92.3% and 73.8%, respectively<sup>[21]</sup>. Although the sensitivity of FIT could improve to 77% when the specificity of FIT was as high as that of mt-sDNA (86.6%), the sensitivity of FIT is significantly lower than that of mt-sDNA and did not show sufficient specificity for screening program. Compared with FIT, mt-sDNA was superior in detecting advanced adenomas, especially sessile serrated polyps that were larger than 1 cm. The sensitivity for serrated sessile polyps was 42.4% for mt-sDNA but 5.1% for FIT. However, mt-sDNA had a higher false positive rate, indicating significantly lower specificity (89.8%) compared with that of FIT (96.4%).

In the case of mt-sDNA testing for detecting large adenomas and CRC, the fact that

the sensitivity of the test is based on a panel of markers, which seem to identify only a subset of CRC, presents an obvious drawback of mt-sDNA testing. Another possible drawback is the high expense per unit of the currently used test compared to other stool tests. It is also unclear how often the test should be performed. A benefit, though, is the lack of direct harm associated with mt-sDNA, but practitioners should, again, be careful in performing the necessary colonoscopy once a patient's stool test is positive. Unlike other stool-based tests, mt-sDNA has issues with interpretation of false positive results because the reported results from the mt-sDNA test currently available in the United States cannot distinguish between a positive result originating from FIT versus mt-sDNA testing. A false positive from the mt-sDNA test could result from a failure to detect a visible lesion, invisible neoplastic changes, or from the presence of a non-colonic digestive tract neoplasm. Patients may proceed to more aggressive short-term surveillance to confirm a false positive result from mt-sDNA. Some follow-up studies that observed patients with false positive results from mt-sDNA for approximately 4 years showed that no patient developed CRC or aerodigestive malignancies<sup>[22,23]</sup>. According to the follow-up study of Cooper *et al*<sup>[24]</sup>, only three out of 12 patients with previous false positive results on mt-sDNA had positive colonoscopy results upon follow-up. The study also emphasizes the importance of long-term follow-up, and high-quality colonoscopy, especially in the proximal colon, for patients presenting with positive mt-sDNA test results.

In the 2018 MISCAN modeling analysis, mt-sDNA was not a recommended test because it requires higher numbers of colonoscopies per LYGs<sup>[20]</sup>. Mt-sDNA done every 3 years resulted in 93% of the LYGs compared with annual FIT testing, but when compared with the LYGs of colonoscopy every 10 years, the percentage was 2% less than the *a priori* criterion of 90%<sup>[20]</sup>.

The ACS's 2018 guidelines decided to include mt-sDNA as one of the testing options for CRC screening at 3-year intervals, as mt-sDNA is superior in detecting advanced adenomas and serrated sessile polyps, and some adults prefer mt-sDNA over other screening tests. The USMSTF 2017 guidelines recommend that the combined FIT-fecal mt-sDNA test be performed at three-year intervals as a second-tier test (Table 1)<sup>[6]</sup>.

### Options for CRC structural (visual) examinations

Structural (visual) examinations, such as endoscopic and radiologic examinations [CT colonography (CTC)] are preferred options for CRC screening when bowel visualization is necessary. Since it directly visualizes the bowel, the screening interval is longer than for stool tests. Structural CRC screening tests require bowel preparation prior to implementing the test, which requires the patient's active participation for self-administering an enema for FS or ingesting polyethylene glycol oral laxative for CTC. For CTC, patients are also restricted to a liquid diet for one day prior to CTC for bowel cleansing. Unlike FS and CTC, colonoscopy is often performed with anesthesia; hence, the patient must be accompanied by a caretaker<sup>[25]</sup>.

**Colonoscopy:** Colonoscopy is a widely performed screening for CRC. Patients who are found to be positive for other CRC screening tests undergo colonoscopy for additional assessment. Colonoscopy has a high sensitivity to detect cancer and all classes of precancerous lesions, it allows single-session diagnosis and treatment, and the intervals between examinations are usually long (10 years) in subjects with normal findings. According to a large, prospective, observational cohort study by Nishihara *et al*<sup>[26]</sup>, the CRC mortality hazard ratio was 0.32 [95% confidence interval (95%CI): 0.24–0.45], when the comparison was made between colonoscopy done at least once and none done at all over 24 years. In addition, distal cancers had a lower hazard ratio of 0.18 (95%CI: 0.10–0.31) compared to proximal cancers, which had a hazard ratio of 0.47 (95%CI: 0.29–0.76). Furthermore, a decrease in incidence was shown in participants who were found to have negative results for colonoscopy (hazard ratio: 0.53, 95%CI: 0.40–0.71)<sup>[26]</sup>. According to the study by Lin *et al*<sup>[8]</sup>, the sensitivity and specificity of colonoscopy for identification of adenomas of at least 6 mm in size were 75%–93% and 94%, respectively, whereas those for identification of adenomas at least 1 cm were 89%–98% and 89%, respectively.

According to the three Cancer Intervention and Surveillance Modeling Network models that informed the USPSTF's 2016 CRC screening guide, CRC incidence and mortality would decrease by 62%–88% and 79%–90%, respectively, if colonoscopy was done every ten years from 50 through 75 years of age<sup>[27]</sup>. According to the study by Knudsen *et al*<sup>[28]</sup>, the median LYG for colonoscopy every 10 years was 270, which was higher than those of other exams. Based on the 2018 general-population MISCAN modeling, a large decrease in the incidence of CRC and the number of deaths from CRC along with an increase in LYG compared to other recommendable strategies were observed, although the frequency of lifetime colonoscopy is more than twice that

of stool-based testing<sup>[20]</sup>.

However, there are some drawbacks of colonoscopy. Although colonoscopy proved to be more effective as a screening tool than other models, its disadvantages include excessive detection and removal of minute low cancer risk polyps, increasing the risks associated with polypectomy as well as the possibility which could result in unnecessary follow-up evaluations. Thorough bowel cleansing is unavoidable, risk of bowel perforation is higher than for other screening methods, there is a greater risk of pneumonitis due to aspiration (especially when deep sedation is involved in the procedure), a slight risk of splenic injury necessitating splenectomy, and greater occurrence of bleeding after the procedure compared to other screening tests. Major complications of screening through colonoscopy are perforation and bleeding, amounting to approximately four cases per 10,000 screenings and eight cases per 10000 screenings, respectively, according to USPSTF<sup>[29]</sup>. The frequency of these dangers is increased when polypectomy is performed. There was a significantly greater rate of complications from performing colonoscopy after other positive non-colonoscopy screening tests than when performing an initial colonoscopy<sup>[8,30]</sup>. Injuries that result from undergoing colonoscopy increase significantly and nonlinearly with the comorbidity burden and age of the patients<sup>[31]</sup>. Colonoscopy has a greater probability of not being able to detect serrated polyps compared to typical adenomas<sup>[32]</sup>. Colonoscopy's performance is also operator-dependent. The skill of the operator affects the detection of cancer, adenomas, and serrated lesions, as well as the selection of appropriate screening and surveillance intervals after colonoscopy<sup>[33-42]</sup>. Despite these risks, colonoscopy is still the preferred approach to allow gross visualization of the entire colon and same-session detection, biopsy, and removal of polyps.

**CTC:** CTC, or virtual colonoscopy, produces multiple thin-slice CT images that can be printed on 2D film or compiled into 3D images, enabling examiners to observe internal organs without the use of colonoscopy. CRC detection rates with CTC were similar to that with colonoscopy based on two extensive studies<sup>[43,44]</sup>. A systemic review and meta-analysis of CTC and colonoscopy based on 49 studies calculated the sensitivity and specificity of CTC. The sensitivity of CTC for detecting CRC was 96.1%, and the sensitivity for detecting adenomas larger than 6 mm was 73%–98% with a specificity of 89%–91%<sup>[45]</sup>. In CTC, the chances of perforation are less than colonoscopy, and the 82%–92% sensitivity achieved for detecting adenomas larger than 1 cm is also an advantage of CTC<sup>[46-49]</sup>. Nevertheless, the sensitivity for detecting polyps less than 1 cm is inferior to colonoscopy, and a major deficiency of CTC is the difficulty in detecting flat and serrated lesions<sup>[50,51]</sup>. Patient who undergo CTC may experience some undesirable symptoms, such as abdominal pain resulting from bowel preparation, pain related to the examination, neuro-cardiogenic syncope and pre-syncope, and very rare worst adverse effects (*i.e.*, GI perforation and radiation exposure-induced cancer). The detection of incidental extracolonic findings is also an unresolved problem. There is the limited evidence about the cost-benefit balance for additional tests necessary for these incidental extracolonic findings<sup>[8]</sup>. There is insufficient proof that CTC reduces the mortality or incidence of CRC. Both ACS and USPSTF (2016) guideline agreed that CTC every 5 years beginning from 45 to 50 years of age and continuing through 75 years of age is a recommended strategy for CRC screening.

**FS:** FS, a procedure to evaluate the lower half of the colon, is the first visual examination used for CRC screening. Advantages of FS are that it is very cheap, has significantly lower risks than that of colonoscopy, does not require thorough bowel preparation, and sedation is unnecessary. A disadvantage of FS is that it provides less benefit in protecting against right-sided colon malignancy when compared with the amount of protection achieved by colonoscopy in case-control and cohort studies. In addition, since the procedure does not involve sedation, the patient might experience discomfort, could be dissatisfied by the procedure, and be more hesitant to repeat the examination compared to colonoscopy<sup>[52]</sup>.

By analyzing four RCTs of FS with one or two screening examinations at intervals of every 3–5 years, there was significant decrease in CRC incidence and mortality<sup>[53-56]</sup>. According to pooled analysis conducted for the USPSTF, patients who had regular follow-up over 11 or 12 years generally decreased their CRC mortality by 27% (relative risk: 0.73, 95%CI: 0.66–0.82), which is especially significant for distal CRC but not proximal CRC<sup>[8,27]</sup>. There was also a decrease in CRC incidence of 21%. According to the US-based Prostate, Lung, Colon, and Ovarian Cancer (PLCO) Screening Trial, proximal and distal CRC incidence were both significantly reduced with FS screening<sup>[27]</sup>. In a study by Atkin *et al*<sup>[57]</sup>, though, there was significant reduction in the incidence of CRC by 26% and mortality by 30%. The study concluded that the result derived from the detection of distal CRC because there was no significant reduction in



incidence or mortality of proximal CRC after FS screening. Overall, FS screening showed a 21% reduction in incidence and a 27% reduction in mortality for CRC when analyzing the pooled data from the three studies (PLCO, SCORE, and NORCCAP) that had an average follow-up of 10 to 12 years<sup>[58]</sup>. Since FS is not effective in screening proximal CRC, which disproportionately affects older women, the incidence and mortality of CRC was not reduced in women aged 60 years or older.

According to MISCAN modeling analysis adjusted for increased incidence, FS screening is recommended beginning at age 45 and repeated every 5 years until the age of 75 years; whereas, assuming stable incidence, USPSTF (2016) is not recommending FS alone for CRC screening<sup>[28]</sup>. Because the effectiveness of FS screening is mostly restricted to the rectum and distal colon, its use has been replaced by colonoscopy in the United States<sup>[20]</sup>. A recent report found that only 2.5% of adults who are recommended for CRC screening test underwent FS screening, while 60% of them received colonoscopy<sup>[59]</sup>. Even if there is solid evidence that FS is an effective CRC screening test, it is questionable whether community-based clinicians are receiving regular training or performing an adequate number of FSs to maintain their skill because it is less frequently used in the United States. This assumption is also supported by proposed FS screening standards that do not provide information about strict quality standards<sup>[60]</sup>. The reasons mentioned above are leading the ACS Guideline Development Group to remove FS from the list of recommended CRC screening tests. However, it is still considered to be one of the recommended tests for CRC screening in some countries where colonoscopy is not yet commonly performed due to its efficacy in reducing CRC mortality and its availability as a primary visual examination tool.

### **Emerging technologies not currently recommended for routine screening**

Blood screening for methylated SEPT9 DNA (mSEPT9) and capsule endoscopy are not recommended procedures but are FDA approved for certain situations.

**Blood screening test for methylated SEPT9 DNA (mSEPT9):** The FDA recently cleared a blood test that identifies a CRC biomarker, mSEPT9<sup>[61]</sup>. This blood screening test is performed on patients with average CRC risk who have declined other screening tests listed in the USPSTF CRC guidelines.

An advantage of the mSEPT9 test is that, as a serum assay, it is more convenient for patients. A disadvantage of the mSEPT9 test is that the performance characteristics are inferior to FIT, that is, sensitivity for cancer is lower than that of FIT, detection of advanced adenoma is impossible, and the cost is more than for other screening tests<sup>[62,63]</sup>. The test seems to be more sensitive for later stage compared to earlier stage cancer<sup>[64]</sup>. Patients who receive a positive result from this blood screening test should be ready to have follow-up tests, such as colonoscopy, which they have refused to undergo previously. Whether patients positive for mSEPT9 would be willing to undergo colonoscopy is questionable. There is also limited evidence in asymptomatic populations who are the targeted candidates for screening. Furthermore, no microsimulation modeling for the newer version of the test was done to evaluate the benefit and benefit-to-harm ratio or to determine the optimal timing for screening. Because of these limitations, most guidelines discourage the use of mSEPT9 for screening.

**Capsule endoscopy:** Initially, capsule endoscopy was predominantly used for gross assessment of the small bowel, but later, there were attempts to use it as a tool to screen the large bowel for CRC. In a systematic review that studied patients with a high risk or who presented with signs or symptoms of CRC, the pooled sensitivity was 87% (95% CI: 77%–93%), and the pooled specificity was 76% (95% CI: 60%–87%) for capsule endoscopy in identification of colorectal polyps at least 6 mm in size<sup>[65]</sup>. Increased pooled sensitivity and specificity were observed (89%, 95% CI: 77%–95% and 91%, 95% CI: 86%–95%, respectively) in tests on larger colorectal polyps that were at least 10 mm in size<sup>[65]</sup>. However, the use of capsule endoscopy as a screening tool is limited due to its side effects. Adverse effects were found to include predominantly gastrointestinal problems such as nausea, vomiting, abdominal pain, and fatigue from the required bowel preparation, and these were found in less than 4% of patients<sup>[65]</sup>. The most severe problem was capsule retention (0.8% of patients with 95% CI: 0.2%–2.4%). Capsule endoscopy also necessitates sufficient colon preparation and further evaluation with colonoscopy if polyps are detected. Capsule endoscopy is not presently approved by the FDA for use in CRC screening.

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## **FUTURE DIRECTION OF SCREENING TOOLS FOR CRC**

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Currently, finding a CRC-specific tumor marker for the development of a new, non-invasive screening method is a primary focus among researchers. CRC is a disease of a highly heterogeneous nature. To interpret the heterogeneous mechanisms that bring about tumorigenesis, “-omics” data derived from genomic, transcriptomic, epigenetic, and proteomic analysis through multi-omics is required. With a single “-omics” approach, the degree of internal and individual variability related to tumor composition and oncogenic signals may be misinterpreted. Therefore, in order to understand the occurrence of tumor, various approaches are required and being studied. We will review the molecular biomarker studies that have been carried out so far and identify new approaches and studies, including metabolomics for the discovery of new CRC biomarkers.

### **Molecular biomarkers**

CRC is a multifactorial disease caused by genetic and epigenetic changes in oncogenes, mismatch repair genes, tumor suppressor genes, and cell cycle regulating genes of the colon mucosal cells. As these molecular changes provide indications for diagnosis, prognosis, and information on treatment response, they were considered possible CRC biomarkers. The three main molecular pathways contributing to the genetic alterations responsible for carcinogenesis are microsatellite instability (MSI), chromosomal instability (CIN), and the CpG island methylator phenotype. Recently, new methods of molecular detection are being evaluated. Nonetheless, the majority of these methods have not yet been validated in larger, preclinical research using randomized study designs. Studies characteristics, patients characteristics, major markers and diagnostic performance of various molecular biomarkers studies are shown in [Table 2](#).

**Adenomatous polyposis coli mutation:** Multifunctional proteins that control Wnt signaling, cell cycle regulation, cytoskeleton stabilization, intracellular adhesion, and apoptosis are encoded by the adenomatous polyposis coli (*APC*) gene. The *APC* gene mutation qualifies as a molecular biomarker for CRC diagnosis because approximately 90% of patients with CRC show *APC* gene mutation<sup>[66]</sup>. Liang *et al*<sup>[67]</sup> have performed meta-analysis study between 1997 and 2010 to correlate *APC* polymorphisms and CRC risk. It was found that while E1317Q significantly increased the risk of adenoma, I1307K was linked to a high risk of CRC.

**MSI:** Commonly, MSI is diagnosed by estimating missing MMR gene products, amplification via polymerase chain reaction (PCR), or immunohistochemistry (IHC)<sup>[68]</sup>. Through meta-analysis and prospective studies, it was shown that MSI serves as an exclusive marker with significant prognostic value in early-stage CRC<sup>[69]</sup>. The prognosis for MSI CRC was found to be superior to that of microsatellite stable (MSS) CRC<sup>[70]</sup>. The Bethesda panel consists of five microsatellite loci (BAT25, BAT26, D17S250, D5S346, and D2S123)<sup>[71]</sup>. At present, most clinical laboratories use a panel of five mononucleotide markers (Bat-25, Bat-26, NR-21, NR-24, and mono-27) to detect MSI<sup>[72]</sup>. MSI is found to be highly prevalent in stage II CRC with an approximate 20% incidence and rare in stage IV CRC with about a 4% prevalence. Hence, MSI screening may aid in early detection of CRC<sup>[72,73]</sup>.

**Detection of CRC-specific RNA Markers in stool:** While the fecal occult blood test (FOBTs) is commonly used as a screening tool, it still has poor sensitivity and specificity. Many tools using protein, DNA and RNA to detect various markers in stool were recently developed<sup>[74]</sup>. The idea is such that miRNA not only regulates specific mRNAs and serves a fundamental role in oncogenesis, but also plays critical role in normal development or in tumor cell multiplication, division, and death<sup>[75,76]</sup>. To diagnose CRC early, several miRNAs were recently assessed. Wu *et al*<sup>[77]</sup> obtained 424 stool specimens from adenoma, CRC, and control patients to investigate miRNA. They found out that when compared with the control, expression of miRNA-135b was significantly increased in advanced adenoma and CRC stool specimens. According to a study performed by Kalimutho *et al*<sup>[78]</sup>, investigating hypermethylated miR-148a in stool specimens may be capable of early CRC detection. Also, following examination of 648 miRNAs from stool specimens of CRC, Kalimutho *et al*<sup>[78]</sup> have determined that fecal miR-144 may be used as a tool for CRC diagnosis. With 74% sensitivity and 87% specificity, miR-144 expression was found to be highly significant in CRC stool specimens. Additionally, PTGS2, a transcript of a specific colorectal tumor gene, expression is extremely specific for early diagnosis of CRC<sup>[79]</sup>. Koga *et al*<sup>[80]</sup> acquired stool specimens from 206 patients with CRC and 134 normal individuals and performed a study on miRNA expression in desquamated colonocytes from stool specimens. The result showed a sensitivity of 74.1% and specificity of 74.1%. Although it did not show sufficient specificity to be used as a screening test, they proposed that the profile of miRNA expression may be useful as a CRC screening test.

**Table 2 Summary of the current and potential biomarkers for early diagnosis of colorectal cancer**

Characteristics of the studies			Training set [test set] (if applicable)				Diagnostic performance (if applicable)			
Ref.	Study type, country	Study group	Population (n)	Male (%)	Age (mean/SD)	Stage (0) / I/ II/ III/ IV/ (?)	Sample	Marker	Sn / Sp	AUC / P-value
Microsatellites loci										
Piñol <i>et al</i> <sup>[119]</sup> , 2005	Prospective, multicenter, nation-wide study/Spain	CRC	1222	59.8	70/11	161/510/337 /214	Blood	Bethesda panel	81.8/98	N/A
Umar <i>et al</i> <sup>[71]</sup> , 2004	Guidelines	N/A	N/A	N/A	N/A	N/A	Blood	Bethesda panel	81.8/98	N/A
Berg <i>et al</i> <sup>[120]</sup> , 2009	Recommendations	N/A	N/A	N/A	N/A	N/A	Blood	Microsatellites instability (MSI)	55-90/90	N/A
Liang <i>et al</i> <sup>[67]</sup> , 2013	Meta analysis/China	N/A	N/A	N/A	N/A	N/A	Blood	APC Polymorphisms	N/A	N/A
CRC-specific RNA markers										
Wu <i>et al</i> <sup>[77]</sup> , 2014	Case-control, China	Normal	109	45.9	60.4/7.0	I + II/III + IV/(?) 24/76/4	Stool	MiRNA-135b	78 (CRC) 73(Advanced adenoma) 65(any adenoma) /68	0.79 (CRC) 0.71 (adenoma) / <0.0001
			Adenoma < 1cm	110	53.6					
			Advanced adenoma	59	50.7					
			CRC	104	57.7					
			IBD	42	61.9					
Kalimutho <i>et al</i> <sup>[78]</sup> , 2011	Case-control, Italy	CRC	28	46	66	(5)/2/6/3/0 / (NA:12)	Stool	miRNA-148	74/87	N/A
		HGD	12	67	62					
		Cn	39	28	58					
Koga <i>et al</i> <sup>[74]</sup> , 2010	Case-control, Japan	CRC	206	67	63	23/46/133/4	Stool	PTGS2	74.1/74.1	N/A, <0.0001
		Cn	134	44	60					
Methylation biomarkers										
Luo <i>et al</i> <sup>[86]</sup> , 2011	Meta Analysis/China	N/A	N/A	N/A	N/A	N/A	Stool	VIM	80/80	N/A
Guo <i>et al</i> <sup>[88]</sup> , 2013	Case-control, China	CRC	75	61	58.5 (12.5)	12/30/30/3	Stool	FBNI	72/93.3	N/A, <0.001
		Cn	30	67	58.4 (12.9)					
Glockner <i>et al</i> <sup>[89]</sup> , 2009	Case-control, United States	CRC	26 [47]	52 [45]	69.33 [71.1]	Stage I to III	Stool	TFP12	89/93	N/A
		Adenoma	[19]		[61.4]					
		Cn	45 [30]	46 [54]	55 [52.3]					
Oh <i>et al</i> <sup>[90]</sup> , 2013	Case-control, South Korea	CRC	131	69	58.4	26/57/36/12	Blood	SDC2	87/95	0.927, <0.0001
		Cn	125	64	51					
Grützmann <i>et al</i> <sup>[121]</sup> , 2008	Case-control, Germany	CRC	252[126]	57 [60]	61 [67]	63/83/59/29/ (NA:19)	Blood	Septin 9	48/93	N/A
		Cn	102[183]	35 [41]	59 [56]					
Warren <i>et al</i> <sup>[91]</sup> , 2011	Case-control, United States	CRC	50	54	62	I + II/III + IV 38/12	Blood/Stool	Septin 9	90/88	N/A
		Cn	94	45	58					
Tóth <i>et al</i> <sup>[92]</sup> , 2012	Case-control, Hungary	CRC	93	52	67.8 (9.8)	25/14/36/18	Stool	Septin9 (gFOBT)	100/100	N/A
		Cn	94	38	62.6 (9.9)					

SD: Standard deviation; Sn: Sensitivity; Sp: Specificity; AUC: Area under the curve; CRC: Colorectal cancer; N/A: Not available; Cn: control; IBD: Inflammatory bowel disease; HGD: High grade dysplasia; VIM: Vimentin; TFP12: Tissue factor pathway inhibitor 2.

from stool specimens.

**Methylation biomarkers:** A number of factors, including one's lifestyle, diet, aging,

reduction of folate levels, exposure to arsenic, and health problems (such as colitis) can lead to colorectal mucosa's abnormal DNA methylation<sup>[81-84]</sup>. One can detect patterns of aberrant DNA methylation from CRC cells in the DNA derived from blood or stool specimens from patients with colorectal cancer<sup>[85]</sup>. Along with the various levels of specificity and sensitivity, several abnormally methylated genes that have been identified in either blood or stool can be used as diagnostic biomarkers in CRC patients. In the United States, for example, vimentin (VIM) gene methylation analysis in a stool-based test is readily available, with about 80% specificity and sensitivity<sup>[86]</sup>. These abnormally methylated genes are also AIX4, SEPT9, FBNI, WiF-1, P53, PGR, MGMT, TIMP3, and GATA4<sup>[81,87]</sup>. Guo *et al*<sup>[88]</sup> used PCR to study hypermethylation of FBNI in patients with CRC. The study involved tissues and stool specimens from 75 patients with CRC and 30 normal individuals. FBNI hypermethylation was found in 78.7% of CRC tissue specimens and 72% in stool specimens compared to 6.7% of controls, showing a specificity of 93.3% and a sensitivity of 72%. According to Guo *et al.*, estimating hypermethylated FBNI in stool specimen can be a useful non-invasive biomarker for identification of CRC. One of the genes, tissue factor pathway inhibitor 2 (TFPI2), was methylated in almost all patients with CRC of all stages with 97% in adenoma and 99% in CRC<sup>[89]</sup>. TFPI2 gene methylation in CRC patient's stool specimens yielded up to a 93% specificity and a 89% sensitivity. Oh *et al*<sup>[90]</sup> conducted a study to measure methylation of the SDC2 gene in blood specimens. This study included 131 patients with CRC representing all stages and 125 normal individuals. The results showed a high level of specificity, 95.2%, and an 87.0% level of sensitivity. Also, the sensitivity for early-stage was 92.3%. Therefore, SDC2 methylation in blood was suggested to be a non-invasive, highly sensitive, and specific biomarker for CRC screening<sup>[90]</sup>. There are a number of CRC screening tests available on the market detecting aberrant gene methylation from either blood or stool. As described previously, the mSEPT9 assay is an example of these available tests. Warren *et al*<sup>[91]</sup> conducted a study on the efficacy of the blood-based mSEPT9 assay for CRC detection using blood specimens from 50 CRC patients and 94 healthy individuals. The results showed 90% sensitivity and 88% specificity for all stages. Accordingly, Tóth *et al*<sup>[92]</sup> studied the efficiency of detection of mSEPT9, gFOBT, and CEA from CRC and normal plasma. As mSEPT9 achieved high sensitivity and specificity levels of 100%, it is considered to be a superior screening test for CRC detection over CEA and gFOBT.

Despite the wide variety of molecular techniques, More research is needed to produce a new molecular biomarker or biomarker panel that could be used for a broad range of screening. In the future, studies should provide solutions to resolve the predictive and prognostic problems of the proposed and presently used molecular biomarkers. Developing effective molecular screening for CRC capable of detecting early-stage colorectal malignancies would be an innovation. In considering the molecular background of the tumor, molecular markers ensure that the field develops a more personalized approach. Identifying clinically-related, cost-effective and easily tested biomarkers to facilitate patient management decisions and provide direct benefits to the patient is, after all, the goal.

### Metabolomics

One option for non-invasive screening is metabolomics, which is a potential tumor marker for CRC. It is important to have a comprehensive understanding of all small-molecule marker metabolites of CRC to accurately understand the tumor metabolic pathway that will assist diagnosis and become the basis for novel preventive and therapeutic methods.

Published studies that attracted large amounts of publicity have recently peaked interest in the possibilities of metabolomic analysis to identify biomarkers for advanced identification of disease progression from easily obtainable biofluids. Therefore, metabolomics analysis had only just started to join the conventional practices of cancer diagnosis and treatment.

One of newly rising "omics" studies, metabolomics investigates global, or system-wide, metabolic profiles, offering a dynamic portrait of the metabolic status of living systems. Being highly potent for diagnosing various cancers using advanced analytic techniques and biometric tools, this approach has been used for therapeutic monitoring and drug development. There are some metabolic markers always found in CRC; however, metabolic profiles of patients with early-stage CRC, including precancerous lesions, are not clearly understood. Due to the non-invasive nature of the approach, it warrants further investigation.

**Characteristics of Colorectal Cancer Screening By Biofluid Sample Type (Blood, Urine, Stool):** Novel diagnostics can be subdivided based on the type of biofluid sample to be analyzed, primarily blood, urine, or stool specimens. The pros and cons of each specimen are shown in [Table 3](#).

**Table 3** Characteristics of colorectal cancer screening of bio fluidic sample types (blood, urine, stool)

Sample types	Evidence of efficacy	Advantage	Disadvantage
Blood-based biomarkers (serum, plasma, and dried blood spot)	A combination of 8 metabolites (99.3% sensitivity, 93.8% specificity, and AUC 0.996) <sup>[94]</sup> Gastrointestinal tract acid 446 (83.3% sensitivity, 84.8% specificity, 85.7%, and 52.1% , respectively) <sup>[96,97]</sup> Decanoic acid (87.87% sensitivity, 80.0% specificity, 71.0%, and 75.0%, respectively) <sup>[98,99]</sup>	Easily accessible Less affected by diet than urine Less diurnal variation and Less inter- and intra-subject variability than urine Stable over a 4-mo period frozen at -80 °C except at room temperature	Affected by smoking status More invasive than urine and stool Analysis can be more complex than urine
Urine	Cross-validated panel of seven metabolites (97.5% sensitivity, 100% specificity, and AUC 0.998) <sup>[104]</sup> 10 different metabolites (100% sensitivity, 80% specificity but small sample size) <sup>[103]</sup> N1, N12-Diacetylspermine <sup>[105,106]</sup>	Easily accessible Less invasive than blood	More affected by diet than serum samples More diurnal variation and More inter- and intra-subject variability than serum A full day storing at room temperature or on cool packs altered metabolite concentration More than 2 freeze and thaw cycles affected the metabolic profile significantly
Stool	A three metabolite panel (AUC 1.0 but very small sample size) <sup>[107]</sup> A metabolomics panel (AUC 0.94) <sup>[108]</sup>	Easily accessible Less invasive than blood	Inconvenient to collect of stool samples Low compliance

(1) Blood-based biomarkers: Blood-based markers can be found in either plasma or serum samples, as well as in dried blood spots, which only requires minimal amounts of blood. Moreover, blood-based markers from dried blood spots have particular advantages, such as easy transportation, convenient storage, and ability to delay processing<sup>[93]</sup>.

In a study of blood-based biomarkers, a dried blood spot biomarker that was composed of four amino acids and four acylcarnitines resulted a quite reasonable sensitivity (81.2%) and specificity (84.0%)<sup>[93]</sup>. One issue of this study, however, was that the 62% of participants were already in a later stage (III or IV) of CRC. Among the available blood-based panels, the most effective biomarker was introduced by Nishiumi *et al*<sup>[94]</sup>, who combined eight metabolites to detect early-stage CRC. The panel showed 99.3% sensitivity, 93.8% specificity, and an area under the curve (AUC) of 0.996. The highest sensitivity and specificity were reported for a single marker, but the study involved limitations, such as a small study population and relatively young age (18–22 years) of healthy controls<sup>[95]</sup>. Most of all, the study was not validated. Gastrointestinal tract acid 446 (GTA-446) is a rising biomarker that has been newly introduced by Hata *et al*<sup>[96]</sup> (83.3% sensitivity, 84.8% specificity) and Ritchie *et al*<sup>[97]</sup> (85.7% sensitivity, 52.1% specificity). In addition, two independent studies found that decanoic acid could be a promising biomarker candidate (87.87% and 71.0% sensitivity, 80.0% and 75.0% specificity)<sup>[98,99]</sup>.

(2) Urine: Most studies of biomarkers found in urine have discovered that a panel is more suitable than solitary metabolites. The outcomes of three Canadian studies were based on identical study settings<sup>[100–102]</sup>. Among the studies, the assay with the highest sensitivity used ten distinct metabolites. However, no additional categorization was done for the latter<sup>[103]</sup>. The study showed 100% sensitivity and a specificity of 80%. However, it had a small sample size. A cross-validated panel that included seven metabolites had a sensitivity of 97.5% (AUC: 0.998) and a specificity of 100%, the highest percentage<sup>[104]</sup>. Two studies, one by Deng, Deng *et al*<sup>[101]</sup> and another by H. Wang *et al*<sup>[102]</sup> reported similarly high sensitivities. In addition, two separate studies detected N1, N12-diacetylspermine as a distinct biomarker that could be used for a future screening test<sup>[105,106]</sup>.

(3) Stool: In a systematic review of studies on early identification of abnormal colorectal growths using biomarker detection, one study reported an AUC of 1.0 based on a three-metabolite panel<sup>[107]</sup>. However, the research only had a small population size. Participants from true screening study showed another metabolomics panel to identify advanced colorectal neoplasms. The panel demonstrated good performance (AUC: 0.94)<sup>[108]</sup>.

Sample type, analytical techniques, major metabolites, outcomes, sensitivity, specificity and significant findings of various metabolomic studies are shown in Table 4. It seems that a panel of metabolites is superior to a single marker for advanced colorectal neoplasms. As for amino acids in blood specimens and nucleosides in urine samples, the findings were consistent.



Table 4 High-throughput metabolomic studies of potential biomarkers in CRC screening

Sample type	Ref.	Analytical technique(s)	Major metabolites	Out-comes	Sn / Sp	Significant finding(s)
Dried blood	Jing <i>et al</i> <sup>[93]</sup> , 2017	Direct infusion MS	AA (4) FA (4)	CRC	81.2/84	Establishing a reasonable diagnostic regression model with eight blood parameters
SERUM BP	Zhang <i>et al</i> <sup>[122]</sup> , 2018	UPLC-MS/MS	FA(2): Eicosanoids	CRC	N/A	Identification of eicosanoids as potential biomarkers for identifying among health, enteritis and CRC
	Guo <i>et al</i> <sup>[123]</sup> , 2017	FTICR MS	FA(5): Male FA(2): Female	CRC	77.3/92.4 80.8/85.9	Presenting the relationship between the change trends of six phospholipids and cancer stages
	Farshidfar <i>et al</i> <sup>[124]</sup> , 2016	GC-MS	AA (9) FA(7) CH (12) Others (13)	CRC	85.0/86.0	Discovery of a suite of CRC biomarkers that provide early detection, prognostication and preliminary staging information
	Zhang <i>et al</i> <sup>[125]</sup> , 2016	FTICR MS	FA (6)	CRC	93.8/92.2	Identification of Free Fatty Acids as diagnostic indicators of early-stage CRC patients
	Gu <i>et al</i> <sup>[126]</sup> , 2015	LC-MS/MS	AA (8)	CRC	65.0/95.0	Performing a combined analysis of amino acids in three different domains: FAAs, FSPAAAs, and IPAAAs
	Zhu <i>et al</i> <sup>[127]</sup> , 2014	LC-MS	AA (7) FA (3) CH (3)	CRC	96.0/80.0	Establishing Partial least-squares-discriminant analysis (PLS-DA) models for distinguishing CRC patients
	Li <i>et al</i> <sup>[128]</sup> , 2013	DI-ESI (±)-FTICR MS	FA (9)	CRC	86.5/96.2	Emphasize that the facile loss of methyl chloride from the [M + Cl] (-) form of LPC (16:0) in its tandem mass spectrum
	Tan <i>et al</i> <sup>[129]</sup> , 2013	UPLC-QTOFMS	AA (6) FA (1) CH (3)	CRC	83.7/91.7	Identification of serum metabolite markers as diagnostic indicators for the detection of CRC
	Ma <i>et al</i> <sup>[130]</sup> , 2012	GC-MS	AA (3) CH (3)	CRC	93.3 <sup>1</sup> /96.7 <sup>1</sup>	Emphasize integrated network connectivity analysis for the diagnosis

S	Nishiumi <i>et al</i> <sup>[131]</sup> , 2012	GC-MS	AA (3) CH (1)	CRC	83.1/81.0	Establishing potential predictive model for early detection of colorectal cancer
	Ritchie <i>et al</i> <sup>[132]</sup> , 2010	FTICR MS	FA (3)	CRC	75.0/90.0	Identification of a systemic metabolic dysregulation comprising previously unknown hydroxylated polyunsaturated ultra-long chain fatty acid metabolites in CRC patients
	Ludwig <i>et al</i> <sup>[133]</sup> , 2009	Hadamard-encoded TOCSY spectra	FA (1) CH (4)	CRC	70.0/95.0	Showing the potential of fast Hadamard-encoded TOCSY spectra for improved classification of serum samples from colorectal cancer patients using a metabolomics approach
	Hata <i>et al</i> <sup>[96]</sup> , 2017	FIA-MS/MS	FA (1: GTA-446)	CRC	83.3/84.8	Identification of GTA-446 as promising tool for primary colorectal cancer screening
	Uchiyama <i>et al</i> <sup>[96]</sup> , 2017	CE-TOFMS	FA (1): Benzoic FA (1): Octanoic FA (1): Decanoic AA (1): Histidine	CRC	89.0/82.0 76.0/71.0 71.0/75.0 63.0/82.0	The first report to determine the correlation between serum metabolites and CRC stage using CE-TOFMS Identification of benzoic acid as diagnostic indicators
	Ritchie <i>et al</i> <sup>[97]</sup> , 2013	TQ-MS	FA (1)	CRC	85.7/~52.1 <sup>2</sup>	Identification of low-serum GTA-446 as significant risk factor for CRC and sensitive predictor of early-stage disease
	Ikeda <i>et al</i> <sup>[134]</sup> , 2012	GC-MS	AA (1): Alanine CH (1): GluL AA(1): Glutamine	CRC	54.5/91.6 75.0/75.0 81.8/66.7	Showing the potential of metabolomics as an early diagnostic tool for cancer
	Leichtle <i>et al</i> <sup>[135]</sup> , 2012	TIS-MS	AA (1)	CRC	N/A	Showing serum glycine and tyrosine in combination with CEA are superior to CEA for the discrimination

PLASMA	BP	Nishiumi <i>et al</i> <sup>[94]</sup> , 2017	GC/QqQMS	AA (3) FA (3) CH (2)	Stage 0/I/II	99.3/93.8	Establishing potential predictive model of colorectal cancer that do not involve lymph node or distant metastasis
		Li <i>et al</i> <sup>[136]</sup> , 2013	Lipid extraction MS	FA (3)	CRC	88.3/80.0	Identification of the plasma choline-containing phospholipid levels as potential biomarkers to distinguish between healthy controls, AP and CRC cases, implying their clinical usage in CRC and/or AP-CRC progression detection
		Miyagi <i>et al</i> <sup>[137]</sup> , 2011	HLPC-ESI-MS	AA (10)	CRC	N/A	Showing the potential of plasma free amino acids profiling for improving cancer screening and diagnosis and understanding disease pathogenesis
		Okamoto <i>et al</i> <sup>[138]</sup> , 2009	HLPC-ESI-MS	AA (6)	CRC	N/A	Presenting the possibility of plasma free amino acids profiling
		Zhao <i>et al</i> <sup>[139]</sup> , 2007	LC- MS	FA (4)	CRC	82.0/93.0	Identification of percentage of 18:1-LPC or 18:2-LPC plasma levels compared with total saturated LPC levels, either individually or in combination as potential biomarkers for CRC
	S	Liu <i>et al</i> <sup>[140]</sup> , 2018	N/A	AA(1):Homocysteine	CRC/A	43.5/98.8	Presenting the possibility of using homocysteine with CEA in screening of early rectal cancer
		Shen <i>et al</i> <sup>[95]</sup> , 2017	2D LC-QToF/MS	FA (1): PG FA (1): SM	CRC	1.00/1.00 1.00/1.00	Presenting the possibility of 2D LC-QToF/MS-based lipidomics profiling
		Crotti <i>et al</i> <sup>[99]</sup> , 2016	GC-MS	FA (1)	CRC	87.8/80.0	Identification of the C10 fatty acid as valuable early diagnostic biomarker of CRC
		Cavia-Saiz <i>et al</i> <sup>[141]</sup> , 2014	high pressure-LC	AA (1)	CRC	85.2/100	Identification of the plasma levels of l-kynurenine as a potential biomarkers of CRC

URINE	BP	Nakajima <i>et al</i> <sup>[105]</sup> , 2018	LC- MS	AA (2)	CRC	N/A	Presenting the potential of polyamines and a machine-learning method as a screening tool of CRC
		Deng,Fang <i>et al</i> <sup>[142]</sup> , 2017	1-dimensional NMR	AA (7) FA (2) CH (8)	A	82.6/42.4	Presenting novel urine-based metabolomic diagnostic test for the detection of adenomatous polyps
		Deng <i>et al</i> <sup>[101]</sup> , 2017	LC- MS	FA (1) CH (2)	A	82.4 <sup>3</sup> /36.0 <sup>3</sup>	Presenting a clinically scalable MS-based urine metabolomic test for the detection of adenomatous polyps
		Wang <i>et al</i> <sup>[143]</sup> ,2017	H-NMR	AA (3) CH (1)	Stage I/II	87.5/91.3	Supporting the utility of NMR-based urinary metabolomics fingerprinting in early diagnosis of CRC
		Rozalski <i>et al</i> <sup>[144]</sup> , 2015	GC-MS	CH (3)	CRC	78.6/75.0	Identification of Urinary 5-hydroxymethyluracil and 8-oxo-7,8-dihydroguanine as potential biomarkers
		Wang <i>et al</i> <sup>[102]</sup> , 2014	1-dimensional NMR	AA (7) FA (2) CH (8)	A	82.7/51.2	Presenting a proof-of-concept spot urine-based metabolomic diagnostic test
		Hsu <i>et al</i> <sup>[145]</sup> , 2013	HPLC-MS/MS	CH (6)	CRC	69.0/98.0	Identification of a set of six targeted nucleosides as marker
		Eisner <i>et al</i> <sup>[100]</sup> , 2013	H-NMR	AA (2) CH (2)	Polyps	64.0/65.0	Presenting a machine-learned predictor of colonic polyps based on urinary metabolomics
		Yue <i>et al</i> <sup>[103]</sup> , 2013	RRLC-QTOF/MS	FA (9) Others (1)	CRC	100/80.0	Identification of CRC urinary metabolites as marker
		Cheng <i>et al</i> <sup>[104]</sup> , 2012	GC/TOF-MS UPLC-QTOFMS	AA (4) FA (1) CH (2)	CRC	97.5/100	Reporting a second urinary metabonomic study on a larger cohort of CRC ( <i>n</i> = 101) and healthy subjects ( <i>n</i> = 103)
		Chen <sup>[146]</sup> , 2012	CE-MS	AA (8) CH (4)	CRC	N/A	Presenting the usefulness of the technique of CE-MS based on moving reaction boundary
		Wang <i>et al</i> <sup>[147]</sup> , 2010	UPLC-MS SPE-HPLC	AA(4) FA(5) / CH (7)	CRC	N/A	Identification of urinary metabolic biomarker based on UPLC-MS and SPE-HPLC

		Feng <sup>[148]</sup> , 2005	RP-HPLC	CH (2)	CRC	71.2/93.3	Identification of Pseu and m1G as novel biomarkers for colorectal cancer diagnosis and surgery monitoring
		Zheng <i>et al</i> <sup>[149]</sup> , 2005	Column switching HPLC	CH (14)	CRC	71.0/96.0	Identification of urinary nucleosides determined by column switching high performance liquid chromatography method
	S	Johnson <i>et al</i> <sup>[150]</sup> , 2006	LC- MS	FA (1)	ACN	90.0/45.0	Identification of urinary PGE-M as a potential biomarker of ACN
		Hiramatsu <i>et al</i> <sup>[106]</sup> , 2005	ELISA	AA (1)	CRC	75.8/96.0	Indicating that urinary N(1), N(12)- Diacetylspermine is a more sensitive marker than CEA, CA19-9, and CA15-3
FECES	BP	Amiot <i>et al</i> <sup>[108]</sup> , 2015	H-NMR	AA (2) FA (4) CH (1)	ACN	N/A	Identification of (1)H NMR Spectroscopy of Fecal Extracts as biomarker
		Phua <i>et al</i> <sup>[107]</sup> , 2014	GC/TOF-MS	FA (1) CH (2)	CRC	N/A	Establishing proof-of-principle for GC/TOFMS-based fecal metabonomic detection of CRC
		Bezabeh <i>et al</i> <sup>[151]</sup> , 2009	(1)H-MRS	AA (6) FA (1) CH (3)	CRC	85.2/86.9	Detecting colorectal cancer by 1H magnetic resonance spectroscopy of fecal extracts
	S	Lin <i>et al</i> <sup>[152]</sup> , 2016	H-NMR	FA (1): Acetate FA (1): Succinate	Early stage	94.7/92.3 91.2/93.5	Identification of the potential utility of NMR-based fecal metabolomics fingerprinting as predictors

<sup>1</sup>Sensitivity and specificity calculated from available data.

<sup>2</sup>Specificity was calculated for the intention to screening population (40–74 year-olds in the colonoscopy population).

<sup>3</sup>Additional results for different cut-off values can be read from the original article. BP: Biomarker panels; S: Single markers; MS: mass spectrometer; FTICR: Fourier transform ion cyclotron resonance; SM: sphingomyelins; PC: phosphatidylcholine; FIA-MS/MS: flow injection analysis-mass spectrometry; Arg: arginine; Val: valine; Phe: phenylalanine; Tyr: tyrosine; Ala: alanine; TQ-MS: triple-quadrupole tandem mass spectrometry; GluL: glucuronic lactone; TIS-MS: Turbo Ion Spray Source mass spectrometer; AP: adenomatous polyps; HPLC-ESI-MS: high-performance liquid chromatography-electrospray ionization-mass spectrometry; A: adenomas; GC/QqQMS: gas chromatography/triple-quadrupole mass spectrometry; 2D LC-QToF/MS: two dimensional liquid chromatography-quadrupole time-of-flight mass spectrometry; PG: phosphatidylglycerol(34:0); SM: sphingomyelin (38:8); CE-TOFMS: capillary electrophoresis-time-of-flight mass spectrometry; GC-MS: gas chromatography-mass spectrometry; LC-MS/MS: liquid chromatography tandem MS; FAAs: free amino acids; FSPAAs: free and soluble-proteome amino acids; IPAAs: insoluble-proteome amino acids; DI-ESI(±): Direct-infusion positive and negative ion electrospray ionization; ACN: advanced colorectal neoplasms; NMR: nuclear magnetic resonance spectra; H-NMR: proton nuclear magnetic resonance spectroscopy; RRLC: rapid resolution liquid chromatography; UPLC-QTOFMS: Ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry; SPE, solid phase extraction; RP, reverse-phase; ELISA: Enzyme-linked immunosorbent assay; (1)H-MRS: (1)H magnetic resonance spectroscopy.

### Limitations of current studies on metabolic biomarkers and influences on metabolomics profiles

Due to some drawbacks, interpreting and implementing metabolomics studies becomes complicated, in particular, poor standardization is a major concern. The sample to be analyzed also has advantages and disadvantages depending on the type,



and the results can be influenced by various situations (Table 3). For future, practical use, the Standard Metabolomics Reporting Structure Group attempted to standardize protocols for metabolomics studies beginning with the design of the study, collection and preparation of specimens<sup>[109]</sup>. Poor standardization could reduce the comparability of studies.

Another limitation is that there is insufficient individual validation of the biomarkers in controlled clinical settings or in a true screening setting for early detection of malignancy in a cohort of asymptomatic individuals<sup>[110]</sup>. The majority of studies report biomarker panels used in their studies that have not been validated. Insufficient validation could lead to overestimation of the performance of biomarker panels because of overfitting. There are concerns of generalization in the case of studies that only used internal validation. Also, the ability to detect valid biomarkers is limited because most of the studies were performed with comparatively small sample sizes<sup>[111]</sup>. In clinical practice, before using metabolomics for early detection, significant effort should be devoted to screening large cohorts under standardized circumstances. Also, since the majority of subjects in these studies were Asian, there may be limited generalization and transferability to other races.

## CONCLUSION

Herein, we provide a review of the literature on the current state and future direction of screening tools for colorectal cancer. Generally, detecting cancer and its precursors at an early stage and initiating treating can prevent unnecessary deaths from colorectal cancer. However, because of the limitations of the screening tools currently in use, the development of new screening tools is required, and studies on metabolomics and proteomics are currently underway. It may be possible to develop a new non-invasive diagnostic test based on biomarkers, which is simple, cost-effective, and highly specific and sensitive. Yet, due to heterogeneity of the biomarkers, more research on this topic needs to be conducted before implementing these potential screening biomarkers in clinical settings. Especially important for achieving better efficacy in colorectal cancer screening are establishing standardized protocols in research for metabolomics and proteomics, carrying out larger studies in true screening settings, and external validation of the outcomes. For better diagnostic performance of non-invasive tests in detecting CRC or its precursors, combining various approaches, such as metabolomics and proteomics, should also be considered.

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## Hepatitis B reactivation in patients with hepatitis B core antibody positive and surface antigen negative on immunosuppressants

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### Abstract

Hepatitis B viral (HBV) reactivation in the immunosuppressed is a significant problem even in patients who have achieved serological clearance due to the persistence of HBV as cccDNA. HBV reactivation will continue to pose a significant healthcare burden given the high prevalence of HBV and increasing use of immunosuppressants. Screening of hepatitis B surface antigen, antibody to Hepatitis B core antigen antibody and HBV DNA levels should be done routinely in all patients planned for significant immunosuppressant use. We aimed to examine the factors affecting reactivation risk. This depended on HBV disease status, the underlying disease requiring immunosuppression, and the specific immunosuppressive regime. While antiviral prophylaxis can prevent reactivation, it increases cost and still has risk of delayed reactivation after stopping antivirals and close follow-up and on-demand treatment is a good alternative for patients at risk of reactivation.

**Key words:** Previous hepatitis B exposure; Immunosuppression; Cost-effectiveness; Hepatitis B reactivation

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**Core tip:** Hepatitis B reactivation remains a common clinical problem, in countries with high endemicity of Hepatitis B, prevalence of Hepatitis B exposure can be very high where hepatitis B virus (HBV) DNA and HbsAg is negative. This group of patients when undergoing chemotherapy or immunosuppression can have reactivation and HBV DNA can be positive, this review summarizes the key studies and guide in rationale approach.

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## INTRODUCTION

Hepatitis B virus (HBV) is a common disease, with approximately 250 million people being HBsAg positive<sup>[1]</sup> and 1.6 billion people being antibody to hepatitis B core antigen (anti-HBc) positive<sup>[2]</sup>. Even in patients who have achieved serological clearance, HBV persists in the host's hepatocytes as covalently closed circular DNA (cccDNA)<sup>[3]</sup>. The natural history of HBV infection involves an interaction between HBV viral replication and the host's immune response<sup>[4]</sup>. In HBV reactivation, initiation of immunosuppression removes immune surveillance pressure against HBV, enhancing viral replication<sup>[5]</sup>. This phase is defined by an increase in serum HBV DNA levels without any hepatitis. Upon cessation of immunosuppression, host immunity recovers and there is a rapid T-cell-mediated damage of infected hepatocytes, resulting in hepatitis<sup>[6]</sup>. Both processes can occur even in patients with serological clearance of HBV due to its persistence as cccDNA<sup>[3,6]</sup>. Reactivation can lead to hepatitis, liver failure, disruption or delay of immunosuppressive treatment, and can be fatal<sup>[7]</sup>. From an economic point of view, resultant morbidity from HBV reactivation is associated with significant healthcare costs<sup>[8]</sup>.

The management of HBV reactivation in immunosuppressed patients with chronic Hepatitis B is well established<sup>[9-11]</sup>. However, the management of patients with past or resolved HBV infection remains controversial<sup>[12]</sup>. Although patients with hepatitis B surface antigen (HBsAg)-negative, anti-HBc positive patients have a lower risk of HBV reactivation compared to HBsAg-positive patients, the prevalence of anti-HBc is higher than that of HBsAg, ranging from 5% in countries in the western hemisphere, to > 50% in east Asian countries<sup>[7,11]</sup>. Coupled with the increased development and use of immunosuppressants for various conditions<sup>[13]</sup>, HBV reactivation will continue to be an important clinical challenge, with HBsAg-negative, anti-HBc positive patients forming a significant portion of patients at risk of HBV reactivation. In this article, we will review literature pertaining to screening, treatment and follow-up strategies in patients with previous Hepatitis B exposure who are planned for immunosuppression.

In this narrative review, we carried out a search of the PubMed database for studies published until January 2019 using the keywords "Hepatitis B reactivation, HBsAg negative, anti-HBc positive, antiviral prophylaxis, immunosuppression, monitoring, cost-effectiveness" and identified relevant literature. The references cited in these papers were also checked to complement the search.

## IMPORTANT DEFINITIONS

The definition of key terms in the study of HBV reactivation has been varied. This has resulted in a wide range of HBV reactivation rates reported in literature<sup>[14,15]</sup>. Compounded with the lack of systematically collected data, this made it difficult to discern the risk of reactivation in specific populations of immunocompromised individuals and specific classes of immunosuppressants<sup>[10]</sup>. We defined these key terms in accordance with the American Association for the Study of Liver Diseases (AASLD) 2018 Hepatitis B Practice Guidance<sup>[10]</sup> for ease of reference (Table 1). Risk of HBV reactivation was classified as low when the incidence rate of HBV reactivation was < 1%, moderate when the incidence rate was between 1%-10% and high when incidence rate exceeded 10%.

## RISK FACTORS FOR HBV REACTIVATION

The main risk factors for HBV reactivation can be subdivided into: (1) Virologic factors; (2) Patient factors; and (3) Type of immunosuppression (Table 2). The virologic factors associated with an increased risk of reactivation in HBsAg-negative and anti-HBc-positive patients include high baseline HBV DNA levels, absence of anti-HBs antibody and presence of Hepatitis B core-related antigen (HBcrAg). High



**Table 1** Definition of key terms used in the study of hepatitis B virus reactivation

Key term	Definition
Resolved Hepatitis B infection	Negative HBsAg, positive anti-HBc, positive anti-HBs
Past Hepatitis B infection	Negative HBsAg, positive anti-HBc, negative or unknown anti-HBs
HBV reactivation	In HBsAg-negative, anti-HBc-positive patients: Newly detectable HBV DNA level OR; Occurrence of HBsAg sero-reversion (reappearance of HBsAg)
Hepatitis B flare	ALT increase to $\geq 3$ times the baseline level and $> 100$ U/L
HBV-associated liver failure	Impaired synthetic function (Total bilirubin $> 3$ mg/dL or international normalized ratio $> 1.5$ ) OR Ascites OR Encephalopathy OR Death following HBV-associated liver failure attributed to HBV reactivation
Antiviral prophylaxis	Antiviral treatment in a patient where there are no other indications for commencing treatment other than an anticipated HBV reactivation
On-demand treatment	Commencement of antiviral treatment upon diagnosis of HBV reactivation

HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; Anti-HBc: Antibody to Hepatitis B core antigen; Anti-HBs: Antibody to Hepatitis B surface antigen.

HBV DNA at baseline was found to be the most important risk factor for HBV reactivation in this population of patients<sup>[16]</sup>, and major guidelines have recommended that HBsAg-negative, anti-HBc positive patients with detectable serum HBV DNA be managed similarly to HBsAg-positive patients, in view of increased HBV reactivation risk<sup>[9]</sup>. A meta-analysis which investigated the effect of anti-HBs status in patients with past Hepatitis B infection found that the presence of anti-HBs reduced HBV reactivation risk overall. The rate of HBV reactivation was 5% in patients who had both positive anti-HBc and anti-HBs serology, compared to 14% in patients who only had a positive anti-HBc<sup>[17]</sup>. This effect was true for higher risk patients who had lymphoma or underwent rituximab-containing chemotherapy<sup>[17]</sup>. Baseline HBcrAg positivity was also associated with increased HBV reactivation risk. In a prospective study involving 124 HBsAg-negative, anti-HBc positive patients undergoing high-risk immuno-suppression (either rituximab-containing chemotherapy or allogeneic hematopoietic stem cell transplant, cumulative HBV reactivation rates were significantly higher in HBcrAg patients compared to those who were HBcrAg negative (71.8% *vs* 31%,  $P = 0.002$ )<sup>[18]</sup>.

Patient factors increasing HBV reactivation risk include increased age, male gender, presence of liver cirrhosis and underlying disease that required immuno-suppression<sup>[19,20]</sup>. A meta-analysis performed by Cholongitas *et al*<sup>[21]</sup> showed that rates of HBV reactivation were higher in patients with hematological disease (10.9%) compared to patients with non-hematological disease (3.6%). Patients with liver cirrhosis and hepatocellular carcinoma (HCC) are at a higher risk of chemotherapy-related reactivation of HBV<sup>[22]</sup>.

The type of immunosuppression plays a key role in a patient's HBV reactivation risk. Patients on B-cell depleting-therapies including rituximab have a high risk of HBV reactivation. In a meta-analysis done by Mozessohn *et al*<sup>[23]</sup>, HBsAg-negative and anti-HBc-positive patients on Rituximab had a pooled reactivation rate of 16.4%. B cell-depleting agents also been well described to have a significantly increased risk of HBV-associated liver failure<sup>[24]</sup>. Notably, the increased risk of HBV reactivation appears more pronounced in patients who are on rituximab for hematological malignancies<sup>[17]</sup>, compared to rheumatological conditions such as rheumatoid arthritis<sup>[25,26]</sup>.

Tumor necrosis factor- $\alpha$  inhibitors such as infliximab and tyrosine kinase inhibitors such as imatinib have moderate risk of reactivation in HBsAg-negative, anti-HBc-positive patients<sup>[27,28]</sup>. Anthracycline derivatives such as doxorubicin are associated with a moderate risk of HBV reactivation in patients with previous HBV exposure, and is particularly concerning for patients with HBV-related HCC undergoing doxorubicin-containing transarterial chemoembolization<sup>[9]</sup>. Patients who receive high dose corticosteroids as monotherapy or in conjunction with other immunosuppressive agents have an increased risk of HBV reactivation<sup>[29]</sup>.

Immune checkpoint inhibitors such as anti-CTLA4 (ipilimumab) and anti-PD-L1 (pembrolizumab) have been increasingly used to treat numerous cancers<sup>[30]</sup>. A retrospective study by Wen *et al*<sup>[31]</sup> found that the use of these drugs in HBsAg-negative, anti-HBc-positive patients did not result in any cases of HBV reactivation, even in the absence of antiviral therapy. Thus far, there has only been one reported case of HBV reactivation in a patient with HIV co-infection who received pembrolizumab for treatment of non-small cell lung cancer<sup>[32]</sup>. Data on the risk of HBV



**Table 2 Risk factors determining risk of hepatitis B reactivation in HBsAg negative, anti-HBc positive patients. Hepatitis B chemoprophylaxis can be considered in patients with 2 or more high risk factors for hepatitis B reactivation**

Category	Risk factor
Virological factors	(1) High baseline HBV DNA; (2) Absence of anti-HBs antibody; (3) Presence of Hepatitis B core-related antigen
Patient factors	(1) Male gender; (2) Liver cirrhosis; (3) Immunosuppression required because of hematological malignancy
Type of immunosuppressive regime	High risk: B-cell depleting therapies  Medium risk: Anthracycline derivatives, high dose corticosteroids, systemic cancer chemotherapy, cytokine-based therapies, immunophilin inhibitors, tyrosine-kinase inhibitors, protease inhibitors  Low risk: Immune checkpoint inhibitors, low-moderate dose corticosteroids, antimetabolite therapies

reactivation in such patients remains limited, and more studies are needed to verify these findings.

## HBV SCREENING

Most patients with previous or chronic HBV infection are asymptomatic and may not be diagnosed prior to commencement of immunosuppression. There is wide variability in the approach to HBV screening before immunosuppression<sup>[33]</sup>. The decision for hepatitis B screening prior in patients who will be receiving immunosuppression is based on a balance between the cost savings of early HBV detection and subsequent administration of antiviral prophylaxis *vs* the cost of screening large numbers of patients.

Major clinical practice guidelines differ in their recommendations for HBV screening in individuals undergoing immunosuppression. The American Society of Clinical Oncology presented a more selective screening strategy<sup>[33]</sup>, and recommended HBV screening only in patients with HBV risk factors<sup>[34,35]</sup> or those who will be receiving cancer therapy associated with a high risk of reactivation, such as B-cell depleting agents or stem cell transplantation<sup>[22]</sup>. A cost effectiveness modelling study<sup>[8]</sup> in a population of patients with hematologic neoplasms at high risk of HBV reactivation showed screening for HBV infection to be both clinically efficacious and cost effective, with initial cost of the screening strategy being offset by monetary savings from prevention of clinical events.

Given the increased use of immunosuppressants, with an increased risk of severe HBV reactivation, universal HBV screening has been found to be cost effective in populations with high prevalence of HBV<sup>[10]</sup>. In populations with lower prevalence of HBV, screening has been found to be cost effective only in patients with higher HBV reactivation risk and those with comorbidities predisposing to higher risk of mortality following reactivation<sup>[36]</sup>. While cost effectiveness arguments are compelling, it is difficult to quantify the negative impact of inability or delay in administration of chemotherapy in patients with HBV reactivation. The AASLD, Asia Pacific Association for the Study of the Liver, European Association for the Study of the Liver are in favor of universal screening prior to commencement of immunosuppressants<sup>[9-11]</sup>. HBV screening should occur at least 1 wk prior to initiation of immunosuppression, with the view of starting antiviral prophylaxis at least 1 wk or as soon as possible upon commencement of immunosuppression.

In the setting of long-term or indefinite immunosuppressive therapy, HBV screening is cost effective even when prevalence of Hepatitis B infection is as low as 0.3%<sup>[22]</sup>. However, HBV screening should not be routine in patients undergoing a low risk immunosuppression regime given that prophylaxis or pre-emptive treatment would not be routinely offered.

Both HBsAg and anti-HBc should be used for HBV screening<sup>[10]</sup> prior to commencement of immunosuppression. Further testing for HBV DNA should be done only if HBsAg is reactive, or in HBsAg-negative patients with positive anti-HBc antibodies. This is to detect occult infection which needs to be managed in the same manner as HBsAg-positive serology. Presence of detectable HBV DNA levels in HBsAg-negative patients with positive anti-HBc antibodies is associated with higher reactivation rates compared to patients who have an undetectable serum HBV DNA<sup>[9]</sup>.

For patients with resolved HBV infection receiving chemotherapy for hematological malignancies, further testing for anti-HBs may be useful to stratify

HBV reactivation risk status. A meta-analysis performed by Paul *et al*<sup>[17]</sup> showed that the absence of anti-HBs increased HBV reactivation risk<sup>[37]</sup>, and such patients should be considered for antiviral prophylaxis. Another study found that patients with resolved HBV infection who underwent allogeneic bone marrow transplant for hematological malignancies and received post-transplant HBV vaccination for augmentation of did not have any cases of HBV reactivation compared to 12 cases of reactivation in patients who did not undergo vaccination. This was postulated to be due to an augmentation of the anti-HBs response<sup>[38]</sup>. The utility of anti-HBs screening in HBsAg-negative, anti-HBc positive patients on other immunosuppressive regimens has not been investigated.

In recent years, HBcrAg has been advocated as a novel biomarker for screening and monitoring of HBsAg-negative, anti-HBc-positive patients who have undetectable HBV DNA titres<sup>[39]</sup>. These patients could either have prior self-limiting HBV exposure or have chronic HBV infection with HBsAg seroclearance, where HBV remains in very low replicative and transcriptional levels despite HBsAg negativity<sup>[40]</sup>. Serum HBcrAg correlates well with intrahepatic cccDNA<sup>[41,42]</sup>, itself being a surrogate measure of the proliferative potential of HBV<sup>[3]</sup>. Seto *et al*<sup>[18]</sup> showed that in HBsAg-negative, anti-HBc-positive patients with lymphoma undergoing rituximab-containing chemotherapy, HBcrAg positivity was associated with HBV reactivation ( $P = 0.011$ , HR: 3.65). This association was found in the same study to be stronger than anti-HBs negativity in predicting HBV reactivation risk<sup>[18]</sup>. The use of HBcrAg for further risk stratification holds much promise in areas of high anti-HBc seroprevalence, where cost effectiveness of antiviral prophylaxis remains a major concern<sup>[43]</sup>. The use of HBcrAg remains nascent and has not entered widespread clinical use<sup>[44]</sup>. Further studies of HBcrAg in patients with moderate and low HBV reactivation risk would enhance the generalizability of this test for screening.

## TREATMENT STRATEGIES

Two main strategies - Antiviral prophylaxis and On-demand treatment (Table 1), are used to attenuate the risk of HBV reactivation in HbsAg-negative, anti-HBc positive immunocompromised individuals. Antiviral prophylaxis involves the initiation of HBV antiviral therapy in patients where there are no other indications for commencing treatment other than an anticipated HBV reactivation. On-demand treatment refers to commencement of antiviral treatment only upon diagnosis of HBV reactivation, but usually involves more intensive surveillance both during and after an immunosuppressive regimen.

## DIFFERENCE IN CLINICAL OUTCOMES BETWEEN TREATMENT STRATEGIES

Choosing between the two strategies involves weighing the difference in clinical outcomes, and well as cost effectiveness. Major guidelines<sup>[9-11]</sup> acknowledge that HBsAg-negative, anti-HBc-positive patients are generally of a lower risk profile than patients who are HBsAg-positive, and permit close surveillance with early on-demand treatment in patients with a moderate (1%-10%) and low risk (< 1%) risk of HBV reactivation. In patients with high risk of reactivation (> 10%), such as those with hematological malignancies receiving B-cell depleting chemotherapy, antiviral prophylaxis was found to be superior to on-demand treatment<sup>[45]</sup>. Recent studies have shown that on-demand treatment strategy may be comparable to antiviral prophylaxis even in this high-risk group, albeit with some caveats. Liu *et al*<sup>[46]</sup> described a randomized control trial where lymphoma patients with past Hepatitis B infection were randomized to start antiviral prophylaxis with entecavir *vs* close follow-up with on-demand treatment prior to commencement of chemotherapy. The study contained both patients who were receiving B cell-depleting therapy, and those who were not. Despite the incidence of reactivation being higher in the on-demand treatment arm, there were no reported HBV-related liver decompensation events or deaths. It should be noted that a large proportion of patients in this study (73.2%) were anti-HBs-positive, which could have protected against HBV reactivation.

Retrospective analyses<sup>[25,26]</sup> found that patients with past (HBsAg-negative, anti-HBc positive) HBV infection who received rituximab for rheumatological diseases without antiviral prophylaxis had low (3.3%-5%) rates of HBV reactivation. The HBV reactivation episodes were also mild, with no HBV-related hepatitis, hepatic decompensation or death. However, these retrospective studies were done in patients with rheumatological disease, with a lower risk profile than patients undergoing a

similar immunosuppressive regime for hematological malignancy.

## COST EFFECTIVENESS OF TREATMENT STRATEGIES

While cost effectiveness of antiviral prophylaxis in HBsAg-positive patients is known<sup>[10]</sup>, the cost effectiveness of strategies to attenuate the risk of HBV reactivation in patients with past HBV infection is not well established<sup>[9]</sup>. The cost effectiveness of antiviral prophylaxis is broadly dependent on disease prevalence, and the savings arising from the clinical events that are prevented as a result of prophylaxis. In turn, the savings accrued from prevented clinical events is correlated with the severity of the events prevented<sup>[43]</sup>. HBV reactivation in HBsAg-negative, anti-HBc-positive patients are usually milder than patients who are HBsAg positive, and rarely result in hepatitis, hepatic decompensation or death<sup>[25,26,46]</sup>. In a cost effectiveness modelling study<sup>[8]</sup> in a population of patients with hematologic neoplasms at high risk of HBV reactivation, antiviral prophylaxis was found to be cost effective in HBsAg-positive patients, but not in patients who were HBsAg-negative and anti-HBc-positive. A prospective study by Seto *et al.*<sup>[47]</sup> found that in HBsAg-negative, anti-HBc-positive patients with lymphoid malignancies undergoing systemic therapy with B-cell depleting agents (Rituximab or obinutuzumab), close biweekly surveillance with early on-demand therapy in event of HBV reactivation was a viable option compared to antiviral prophylaxis, and may be cost effective. Liu *et al.*<sup>[46]</sup> also found that patients with past HBV infection, particularly those with positive anti-HBs, had a low risk of HBV reactivation and monitoring HBV DNA and ALT closely was more cost effective than antiviral prophylaxis.

It must be emphasized, however, that a strategy of close HBV serology monitoring with early on-demand treatment is logistically demanding. Patients would be required to come for frequent follow-up appointments and existing cost-effectiveness studies do not take into account the productivity costs borne by the patient as a result of this strategy. Similarly, it is also difficult to quantify the negative impact of inability or delay in administration of chemotherapy in patients with HBV reactivation. Decisions regarding risk attenuation strategies of HBV reactivation in immunocompromised patients with previous HBV exposure must be individualized. We suggest a treatment algorithm (Figure 1) which took into account various factors determining the clinical risk and outcomes of both treatment strategies in HBsAg-negative, anti-HBc-positive patients undergoing immunosuppression.

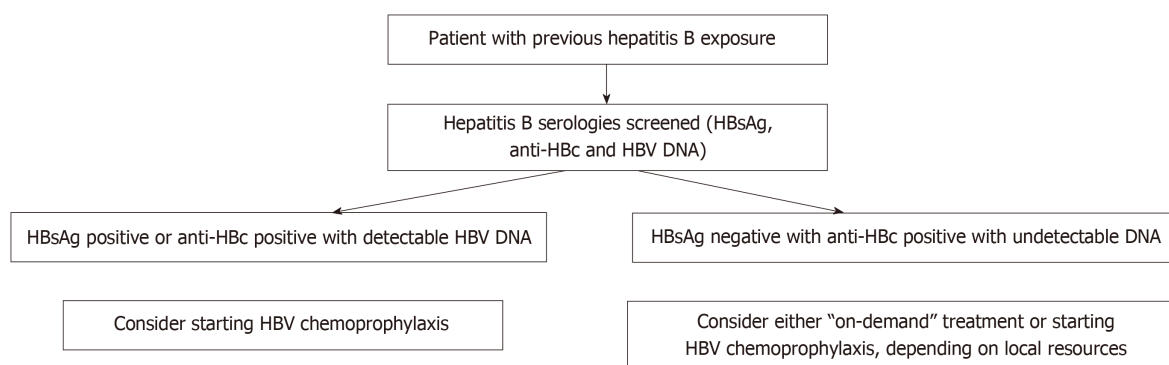
## CHOICE OF TREATMENT AGENT

For patients who are commenced on antiviral treatment either as prophylaxis or as on-demand treatment, antiviral agents with a high genetic barrier to resistance was recommended<sup>[23]</sup>. However, the cost of these medications is high. The major clinical societies' guidelines have noted that in the event of resource constraints, less expensive earlier-generation antivirals with a lower genetic barrier to resistance may be used in patients with a lower risk of resistance (*i.e.*, Undetectable HBV DNA at baseline, and an anticipated prophylaxis duration of less than 6 mo). Patients who are started on antiviral prophylaxis should be continued on antiviral treatment for at least 12 mo after cessation of rituximab-containing immunosuppressants, due to risk of delayed reactivation with rituximab<sup>[48]</sup> and 6 mo for all other immunosuppressive regimes. Patients should undergo routine testing for HBV DNA and serum ALT and AST for 3-6 mo after discontinuation of antiviral therapy to monitor for HBV reactivation post withdrawal.

## CONCLUSION

HBV reactivation remains a concern even after serological clearance due to virus persistence in the host genome as cccDNA. HBV reactivation will continue to remain a significant problem due to high global prevalence of HBV and the increasing use of immunosuppressants. HBV screening with HBsAg, anti-HBc, anti-HBs and HBV DNA should be done in all patients prior to commencement of immunosuppression. HBcrAg shows promise in further characterization of patient's reactivation risk profile. More studies should be done to assess applicability in low and moderate risk immunosuppressants and determining if HBcrAg can be used to identify optimal timepoint of stopping antiviral therapy.

The decision for prophylaxis or close monitoring with early on-demand treatment



**Figure 1 Treatment algorithm for HBsAg negative, anti-HBc positive patients undergoing immunosuppression.** Future research may help determine whether HBcrAg has a role in determining whether patients with HBsAg negative with anti-HBc positive with undetectable DNA undergo HBV chemoprophylaxis or "on-demand" treatment. HBV: Hepatitis B virus.

needs to be individualized, and depends on the risk of reactivation, cost effectiveness and difference in clinical outcome between the two scenarios. More research is needed to better identify patients who are at elevated risk for HBV reactivation, but concurrently low risk for HBV reactivation associated complications, in order to ascertain the population in whom close monitoring with early on-demand treatment would be a viable option. The risk profile of emerging medications including immune checkpoint mediators needs to be further studied to expand our knowledge on the optimal method of attenuating HBV reactivation risk in immunosuppressed patients with previous HBV exposure.

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## Present state of endoscopic ultrasonography-guided fine needle aspiration for the diagnosis of autoimmune pancreatitis type 1

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### Abstract

Autoimmune pancreatitis (AIP) is defined as pancreatitis caused by irregular narrowing of the pancreatic duct accompanied by pancreatic swelling, fibrosis and lymphocyte infiltration, events that are related to autoimmune mechanisms. The 2010 International Consensus Diagnostic Criteria for AIP defined pancreatitis as "type 1" when increased levels of serum IgG4 were present and other organs were involved; lymphoplasmacytic sclerosing pancreatitis was the main histological characteristic. Apart from surgery, endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) is the only method for the histological diagnosis of AIP; however, this method is difficult. The use of larger-diameter FNA needles and trucut biopsy did not improve the diagnostic performance of EUS-FNA, but it has improved gradually. In this review, we look back at past efforts to improve the diagnostic performance of EUS-FNA and reveal the present state of EUS-FNA for the histological diagnosis of AIP type 1.

**Key words:** Autoimmune pancreatitis type 1; Endoscopic ultrasonography-guided fine needle aspiration; IgG4-related disease; Lymphoplasmacytic sclerosing pancreatitis

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**Core tip:** Apart from surgery, endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) is the only method for the histological diagnosis of autoimmune pancreatitis (AIP). However, this method is difficult. Several attempts to improve the diagnostic

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performance of EUS-FNA have been undertaken, with gradual success. In this review, we examine past efforts and discuss the present state of EUS-FNA for the histological diagnosis of AIP type 1.

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## INTRODUCTION

In 1993, a case accompanied by lymphadenopathy and IgG4 hypergammaglobulinemia was reported by Suzuki *et al*<sup>[1]</sup>. Thereafter, the concept of IgG4-related disease (IgG4-RD) was established as follows: IgG4-RD is characterized by elevated serum IgG4 accompanied by the swelling of multiple organs or tumoral lesions infiltrated by lymphocytes, with IgG4-positive plasma cells and fibrosis observed throughout the body<sup>[2,3]</sup>. In gastroenterology, autoimmune pancreatitis (AIP) type 1<sup>[4,5]</sup> and IgG4-related sclerosing cholangitis (IgG4-SC)<sup>[6-8]</sup> are the primary presentations.

AIP was defined by Yoshida *et al*<sup>[9]</sup> as pancreatitis caused by irregular narrowing of the pancreatic duct accompanied by pancreatic swelling, fibrosis and lymphocyte infiltration, events that are related to autoimmune mechanisms. Moreover, Hamano *et al*<sup>[4]</sup> reported elevated levels of serum IgG4 in AIP patients. The 2010 International Consensus Diagnostic Criteria (ICDC) for AIP defined pancreatitis as “type 1” when the levels of serum IgG4 are elevated and other organs are involved; lymphoplasmacytic sclerosing pancreatitis (LPSP) is considered the main histological characteristic<sup>[10]</sup>. Four characteristics have been identified as important for diagnosing LPSP, namely, periductal lymphoplasmacytic infiltrate without granulocytic infiltration, obliterative phlebitis, storiform fibrosis, and abundant (> 10 cells/HPF) IgG4-positive cells (Figure 1). The presence of three of these four characteristics is defined as level 1 histological findings. The presence of only two of these characteristics is defined as level 2 histological findings.

AIP can be diagnosed by imaging and increased serum IgG4 levels or by other methods<sup>[11]</sup>. However, a histological diagnosis of AIP requires level 1 pancreatic histological findings. Apart from surgery, endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) is the only method for the histological diagnosis of AIP. Furthermore, AIP cases associated with pancreatic cancer have been reported<sup>[12-16]</sup>. Therefore, EUS-FNA is important for the safe and noninvasive distinction of AIP from pancreatic cancer. In this report, we discuss the efficacy and present diagnostic performance of EUS-FNA for AIP type 1.

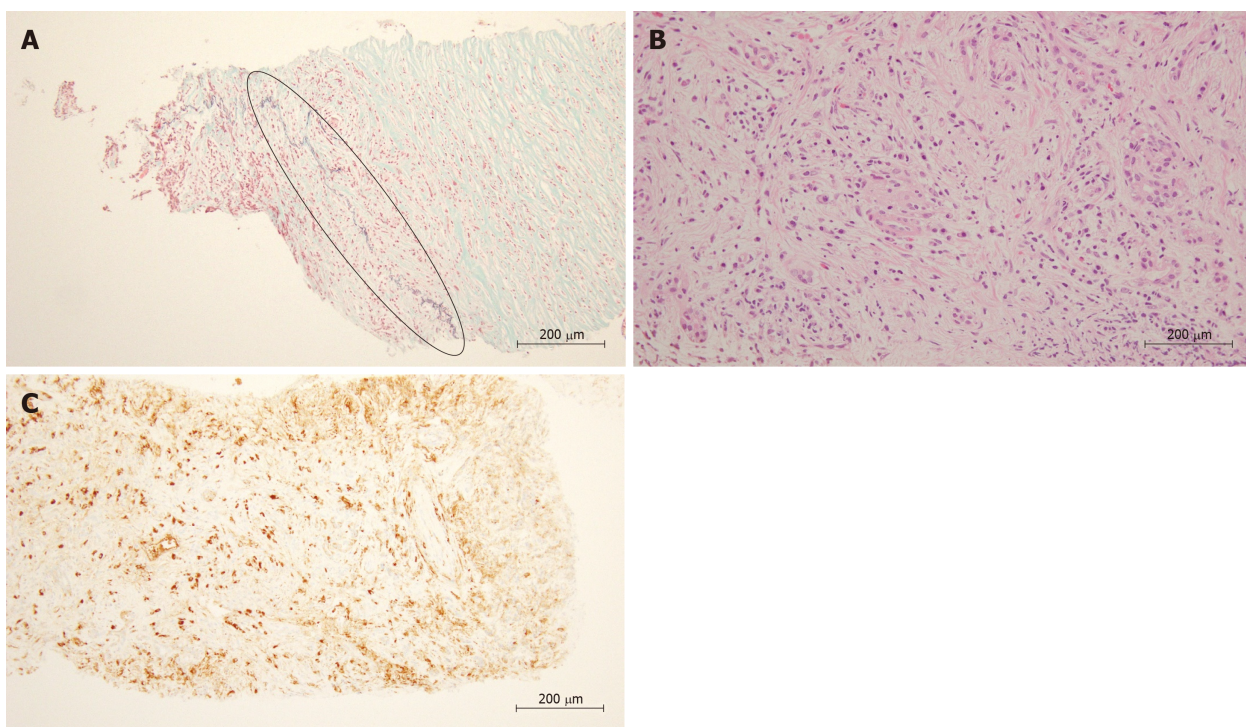
## SEARCH METHODS

Reports were searched in PubMed and the Cochrane Library by using the following keywords: “autoimmune pancreatitis” and “endoscopic ultrasonography-guided fine needle aspiration”. Among the searched reports, only original articles were included in this review. Furthermore, we performed a manual search and added such articles to this review as necessary.

We introduce the achievements in the included reports in the following order: Before and after the ICDC, conventional EUS-FNA (22-gauge, 19-gauge), EUS-TCB, multicenter studies, and special needles.

## REPORTS BEFORE THE ICDC

Before the ICDC, few reports described the use of EUS-FNA for the histological diagnosis of AIP type 1. In 2005, Deshpande *et al*<sup>[17]</sup> performed EUS-FNA in 16 AIP patients. Among these patients, three were found to have false-positive cytological diagnoses (one adenocarcinoma, one solid pseudopapillary neoplasm, and one



**Figure 1** Histological findings in lymphoplasmacytic sclerosing pancreatitis by endoscopic ultrasonography-guided fine needle aspiration. A: Obliterative phlebitis was observed, highlighted in an ellipse (EM  $\times$  200); B: Plasma cells and storiform fibrosis were observed (HE  $\times$  400); C: IgG4-positive plasma cells were observed (IgG4 immunostaining  $\times$  200).

mucinous neoplasm). The cellularity of the stromal fragments was significantly higher in the AIP samples than in the control samples (adenocarcinoma, chronic pancreatitis, *etc.*). In the same year, researchers performed EUS-guided trucut biopsy (EUS-TCB) in three AIP patients. In two of the three patients, fibrosis and lymphoplasmacytic infiltration were observed. In 2009, Mizuno *et al*<sup>[18]</sup> performed EUS-TCB, and among nine AIP patients, four were diagnosed with probable LPSP. In 2011, Khalid *et al*<sup>[19]</sup> reported the diagnosis of two of 14 AIP patients with LPSP. In this period, the performance of EUS-FNA for the histological diagnosis of AIP was very poor.

## REPORTS AFTER THE ICDC

After the ICDC were established, several reports described the use of EUS-FNA for the diagnosis of AIP. The results of these studies (excluding case reports) are shown in [Table 1](#). In the following sections, we describe the details of these studies.

### CONVENTIONAL EUS-FNA BY 22-GAUGE NEEDLE

First, the results of EUS-FNA using a 22-gauge needle were described. In 2011, Imai *et al*<sup>[20]</sup> reported the results of 21 AIP patients who underwent EUS-FNA. The AIP patients could not be histologically diagnosed with AIP. In 2012, Ishikawa *et al*<sup>[21]</sup> reported that level 1 histological findings as defined by the ICDC were observed in 9 of 39 EUS-FNA specimens from AIP type 1 patients, and level 1 or level 2 histological findings were observed in 14 of 39 patients. In 2018, Cao *et al*<sup>[22]</sup> reported the results of EUS-FNA in 27 AIP patients: 18.5% (5/27) of the AIP patients showed level 1 histological findings, and 62.96% (17/27) showed level 1 or level 2 histological findings. Although these results were somewhat insufficient, they represented a gradual improvement.

### CONVENTIONAL EUS-FNA BY 19-GAUGE NEEDLE

In 2012, Iwashita *et al*<sup>[23]</sup> reported the results of 44 AIP patients who underwent EUS-FNA using a 19-gauge needle. In this report, 39% (17/44) showed level 1 histological



**Table 1 Studies on the use of endoscopic ultrasonography-guided fine needle aspiration for the diagnosis of autoimmune pancreatitis after the International Consensus Diagnostic Criteria (excluding case reports)**

Ref.	Yr	Needle (G)	No. of needle passes	n	LPI	OP	SF	IgG4 (+) PC	Level 1 HF	Level 1 or 2 HF
Conventional EUS-FNA of single-center study										
Imai <i>et al</i> <sup>[20]</sup>	2011	22	3.5 ± 0.9	21	11	0	0	0	0	0
Ishikawa <i>et al</i> <sup>[21]</sup>	2012	22	2.0 ± 0.48	39	16	0	34	10	9	14
Cao <i>et al</i> <sup>[22]</sup>	2018	22	3.59 ± 1.28	27	18	0	18	8	5	17
Iwashita <i>et al</i> <sup>[23]</sup>	2012	19	3.0	44	23	21	38	5	17	19
Multicenter study										
Morishima <i>et al</i> <sup>[24]</sup>	2016	22	2.02 ± 0.48	41	36	0	0	27	0	27
Kanno <i>et al</i> <sup>[25]</sup>	2016	22	3.4 ± 1.3	78	43	38	49	19	32	45
EUS-FNA by automated spring-loaded Powershot needle										
Kanno <i>et al</i> <sup>[26]</sup>	2012	22	3-7	25	23	4	20	9	14	20

EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration; LPI: Lymphoplasmacytic infiltrate without granulocytic infiltration; OP: Obliterative phlebitis; SF: Storiform fibrosis; IgG4 (+) PC: Abundant (> 10 cells/HPF) IgG4-positive cells; Level 1 HF: Level 1 histological findings; Level 2 HF: Level 1 or level 2 histological findings.

findings, and 43% (19/44) showed level 1 or level 2 histological findings. Although the diameter of the needle was larger, more specimens could not be sampled.

## EUS-TCB

As mentioned above, EUS-TCB achieved somewhat modest results before the ICDC were announced. Before the ICDC, almost all of the reports on using EUS-FNA to identify AIP type 1 asserted that AIP could not be histologically diagnosed by EUS-FNA. However, Mizuno *et al*<sup>[18]</sup> reported the efficacy of EUS-TCB (referred to in the section REPORTS BEFORE THE ICDC).

## MULTICENTER STUDIES

Because AIP is a relatively rare disease, studies of EUS-FNA in AIP patients have had limited sample sizes. However, in 2016, two multicenter studies were performed, both of which used a 22-gauge needle for EUS-FNA.

Morishima *et al*<sup>[24]</sup> performed a multicenter study in which 18 hospitals took part, and 41 AIP type 1 patients were entered in the study. The number of needle passes was  $2.01 \pm 0.48$  (1-4). In this report, 65.8% (27/41) of the patients showed level 2 histological findings. However, none of the patients showed level 1 histological findings. In addition, storiform fibrosis and obliterative phlebitis were not observed.

Kanno *et al*<sup>[25]</sup> performed a multicenter study in which twelve institutions participated. The report included 78 AIP type 1 patients. The number of needle passes was  $3.4 \pm 1.3$ . In this report, 41.0% (32/78) of patients showed level 1 histological findings, and 57.7% (45/78) showed level 1 or level 2 histological findings. Twenty-four (19/78) patients showed abundant (> 10 cells/HPF) IgG4-positive cells. A total of 62.8% (49/78) of patients showed storiform fibrosis, and 48.7% (38/78) of patients showed obliterative phlebitis. These two reports provided sufficient evidence that EUS-FNA could be used for the diagnosis of level 2 histological findings.

## EUS-FNA BY SPECIAL NEEDLES

Due to the difficulty of the histological diagnosis of AIP, EUS-FNA was performed using special needles. Kanno *et al*<sup>[26]</sup> used an automated spring-loaded Powershot needle (NA11J-KB; Olympus, Tokyo, Japan). In this report, 56% (14/25) of patients showed level 1 histological findings, and 80% (20/25) showed level 1 or level 2 histological findings. Obliterative phlebitis was observed in 40% (10/25) of patients. Although it is very difficult to prove obliterative phlebitis by EUS-FNA, this report showed promising results.

Recently, the use of SharkCore (Medtronic, Sunnydale, Calif) needles for EUS-guided fine needle biopsy (EUS-FNB) in AIP patients was reported. According to a



report written by Detlefsen *et al*<sup>[27]</sup> in 2017, one AIP type 1 patient showed level 2 histological findings. However, obliterative phlebitis was observed in this patient. In the same year, Runge *et al*<sup>[28]</sup> reported EUS-FNB in two patients. Both patients showed a marked increase in IgG4-positive plasma cells. Although storiform fibrosis and obliterative phlebitis were not reported, these two patients likely showed level 2 or higher histological findings. In 2018, Bhattacharya *et al*<sup>[29]</sup> reported two patients who showed level 1 histological findings by EUS-FNB. Although the three reports on EUS-FNB using the SharkCore needle were case reports, all cases showed some histological findings as defined by the ICDC. The diagnostic performance of EUS-FNA for AIP could be improved by the further development of such needles.

## CONCLUSION

Diagnosing AIP type 1 by EUS-FNA is currently difficult. However, the diagnostic performance of EUS-FNA for AIP is gradually improving over time and with the further development of special needles. In the future, further advancement of FNA needles and puncture methods will be warranted to improve the histological diagnosis of AIP.

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# Hepatic gastrointestinal stromal tumor: Systematic review of an exceptional location

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## Abstract

### BACKGROUND

A minor subset of primary gastrointestinal stromal tumors (GIST) can also arise outside the gastrointestinal tract, which is known as an extra-GIST (E-GIST). Primary GIST of the liver is an exceptional location.

### AIM

To characterize epidemiological, clinical and pathological features and options of treatments.

### METHODS

We performed a systematic review to search for articles on primary hepatic GIST.

### RESULTS

This review shows that right hepatic lobe was the most frequent location. Regarding pathological and immunohistochemical features, mitotic count was  $\geq 5/50$  High Power Fields in more than 50%; and CD117 was negative in only 1 patient. More than 70% of patients had a lesion with high risk of malignancy.

### CONCLUSION

The diagnosis of E-GIST must be considered in a liver mass. Rendering an accurate diagnosis is a challenge, as well as the confirmation of their primary or metastatic nature.

**Key words:** Gastrointestinal stromal tumors; Extra-gastrointestinal stromal tumor; Primary hepatic tumor; CD117; Primary hepatic gastrointestinal stromal tumor; Primary gastrointestinal stromal tumor of the liver

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**Core tip:** A great majority of primary gastrointestinal stromal tumors (GISTs) outside the gastrointestinal tract (GI) are metastases; however, a minor subset of primary GISTs can

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also arise outside the GI tract which is known as an extra-GIST (E-GIST). Among E-GIST, liver is an exceptional location. We systematically review the literature on primary GIST of the liver. Primary hepatic EGISTs have a male predominance and usually are incidental findings. The surgical approach is commonly performed, and the final diagnosis is made by pathological, immunohistochemical and molecular analysis. Primary hepatic EGISTs are often high-risk lesions. Literature is scarce and it is very difficult to establish guidelines for clinicians.

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## INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are a group of mesenchymal tumors characterized by the expression of KIT protein (CD117), with an overall incidence between 10-20 per million, which harbor different clinical behavior<sup>[1,2]</sup>. These are the most common mesenchymal neoplasm of the gastrointestinal tract, which represent 0.1%-3% of all gastrointestinal neoplasms<sup>[3,4]</sup>.

Regarding the pathogenesis, GISTs are believed to originate from interstitial cells of Cajal (ICC), the pacemaker of gastrointestinal tract<sup>[5-7]</sup>, or to a common precursor cell of ICCs and smooth muscle cells<sup>[8]</sup>. So, GISTs may arise anywhere along the gastrointestinal tract. Approximately 60%-70% of GIST occurs in the stomach, followed by 20%-30% in small intestine; colon and rectum (5%), esophagus (< 2%) and appendix are less frequent<sup>[9,10]</sup>.

A great majority of GISTs outside the GI tract are metastases from GI GISTs; however, a minor subset of primary GIST can also arise outside the GI tract which is known as an extra-GIST (E-GIST). According to Miettinen *et al*<sup>[11]</sup>, the frequency of EGISTs is no higher than 1% of all GISTs of defined origin, and they are characterized by the same morphological, immunohistochemical and molecular characteristic than conventional GIST<sup>[12]</sup>.

Regarding embryology, the origin of EGISTs remains controversial, with certain hypotheses. Since some researchers have observed "ICC-like" cells with a similar structure and function to ICCs in organs outside of the GI tract<sup>[13,14]</sup>, it is reasonable to presume that EGISTs originate from this common precursor cells of ICC. Other authors suggest that EGISTs may originate from a pool of undifferentiated pluripotent mesenchymal stem cells located outside GI tract, and then differentiate into ICCs<sup>[15-17]</sup>.

Among E-GIST, mesentery, retroperitoneum and pancreas are the most frequent and this type of tumor has been reported in the omentum, bladder, gallbladder and ureter also; while, primary GIST of the liver is an exceptional location and its definitive diagnosis requires ruling out other types of liver mesenchymal tumors and other tumor types, such as sarcoma<sup>[6,12,15]</sup>.

Most studies of E-GISTs are case reports, lacking enough information to clarify the disease; therefore, it is very difficult to establish guidelines for clinicians. In addition, if we refer to primary hepatic E-GIST, the literature is much scarcer due to this exceptional location.

This study is a systematic review of the literature on primary GIST of the liver. Our aim is to identify clinical and diagnostic features and treatment in this exceptional location of this type of mesenchymal tumor.

## MATERIALS AND METHODS

We performed a search for articles on primary hepatic GIST in MEDLINE (PubMed), Tripdatabase, and Cochrane Library databases, with no restrictions on publication dates or author up until January 31, 2019.

The search items comprise the following MESH terms: "Extra-gastrointestinal stromal tumors" OR "extra-gastrointestinal stromal tumor" OR "extra-gastrointestinal stromal neoplasm" OR "extra-gastrointestinal stromal neoplasms" OR "E-GIST" OR

“E-GISTs” and the following no MESH terms: “Primary malignant gastrointestinal stromal tumor of the liver” OR “Primary hepatic gastrointestinal stromal tumor” OR “Primary gastrointestinal stromal tumor of the liver”.

The articles were included or rejected based on the information obtained from the title and summary, and in case of doubt, after reading the complete article.

To evaluate the quality of the studies selected, we used the scale designed by Manterola *et al*<sup>[18]</sup> which evaluates each publication individually depending on the type of study, the sample size, and the methodology used. It has a range of 6 to 36 points, with a quality cutoff point of 18. We carried out a qualitative analysis of the studies included and their conclusions, based on the levels of evidence and degrees of recommendation proposed by Cook *et al*<sup>[19]</sup>.

## RESULTS

After the both initial searches, 420 articles were obtained. Only 23 (5.48%) met the search criteria, one of which were excluded because language (one in Romanian). The flowchart diagram is shown in [Figure 1](#). We included 22 articles, including 23 patients<sup>[1,3,6,20-38]</sup>.

The mean age was 56.18 years ( $\pm 15.4$  SD), with a slightly male predominance (12/23). According country, 18 patients were from Southeast Asian, with 11 cases from China; while only 5 came from Western (France, Spain, Italy, United States). Right lobe was the most frequent location (12/23; 52.17%), and bilobar extension was present in 4 patients. Liu *et al*<sup>[28]</sup> report the coexistence of a hepatic and a pancreatic primary lesion. The median size was 15 cm (range: 2.2-27 cm). Among the symptoms, nearly 50% of patients (10/23) have no symptoms, being incidental finding during follow-up or extension study for gastric cancer in 2 of them ([Table 1](#)).

Regarding the diagnosis referred in [Table 2](#), upper and lower endoscopy studies were performed in 9 patients, with no findings in 8 cases and an early gastric cancer in the other (biopsy: signet ring cell carcinoma). Biopsy of hepatic lesion was performed in 8 patients; one of them was surgical one.

Regarding the treatment of the selected patients, the management was surgical in 16 cases, ranging from limited surgical excision or anatomic resection to liver transplantation or extracorporeal resection and auto transplantation.

Two patients were received local treatment, with radiofrequency ablation (RFA) and microwave ablation. Luo *et al*<sup>[37]</sup> report a central hepatic lesion in a young patient. Liu *et al*<sup>[28]</sup> report a pancreatic primary E-GIST coexisting with another hepatic primary E-GIST, where both lesions were treated by RFA.

There were two refusals for treatment; in one case the patient refused surgery and received imatinib mesylate, and the other refused any action. In only one patient, the finding was a non-resectable hepatic lesion and the patient was treated with imatinib mesylate ([Table 3](#)).

Regarding pathological, molecular and immunohistochemical findings, the most frequent cell type was spindle cells (17/23, 73.91%); molecular analysis was performed in 6 patients, with mutation in 5/6; mitotic count was  $\geq 5/50$  High Power Fields in 12 cases (52.17%); and CD117 was negative in only 1 patient. The risk of malignancy was classified according to Fletcher *et al*<sup>[2]</sup> and 17/23 (73.91%) patients had a lesion with high risk of malignancy ([Table 4](#)).

Mean follow-up was 14 mo (range: 3-252). During the follow-up, 10 patients were disease-free (follow-up: 3-30 mo) ([Table 5](#)).

## DISCUSSION

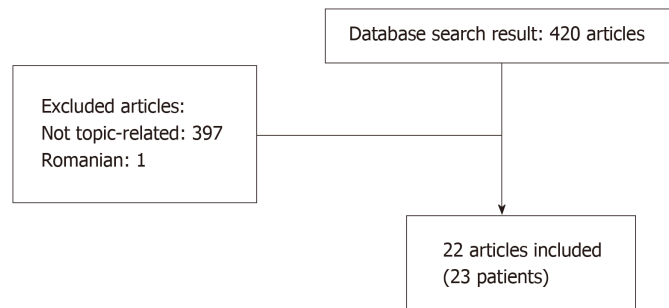
Since Hu *et al*<sup>[6]</sup> reported the first primary hepatic GIST in 2003, we should consider that not all tumors of the liver with GIST features are metastasis and the liver could itself be the primary GISTs location.

Primary hepatic E-GISTs are extremely uncommon compared with their alimentary counterparts; thus, E-GISTs presenting in the liver raise a difficult diagnosis, management and prognosis<sup>[22]</sup>.

In this review, primary E-GISTs of the liver have a slightly male predominance and the reported cases have a Southeast Asian predominance. Regarding symptoms, almost 50% have no clinical manifestations; among symptomatic patients, the symptoms are vague compared to GISTs, which commonly present with GI bleeding, abdominal pain, a palpable mass, weight loss, nausea and vomiting<sup>[12]</sup>. Also, the size of the hepatic lesion may become larger, mean 15 cm in our review.

Once we find a tumor with GIST characteristics presenting in the liver, the main





**Figure 1** Flowchart.

challenge is determining whether this lesion is primary or metastatic, considering the liver is the most common site of distant metastasis for malignant GIST<sup>[21,28]</sup>.

All of the studies included in this systematic review are case report, lacking enough information to clarify the disease and making difficult to establish protocols. The differential diagnosis is the main challenge for primary hepatic E-GISTs, but there are no consensus guidelines. According to Joyon *et al*<sup>[21]</sup>, the diagnosis of primary E-GIST of the liver could be considered if these conditions are present: (1) Absence of GIST in the GI tract, with endoscopic and imaging studies; and absence of connection with the muscularis propria of the GI tract; (2) Absence of any past medical history which might suggest the resection of an overlooked or misdiagnosed GIST; and (3) Absence of GI tumor diagnosed during follow-up.

Thus, every hepatic GIST should be considered metastatic until no grossly nor histologically evidence of association with the muscularis propria have been shown and the remote medical history has been carefully explored, and no primary tumor could be found on long-term follow up<sup>[39]</sup>. Not all case included in this systematic review have been described enough information to be sure that all these requirements are fulfilled. In one hand, Kim *et al*<sup>[32]</sup> reported a patient with two synchronous lesions, an early gastric cancer and a primary hepatic GIST. Their preoperative diagnosis was a malignant hepatic lesion with an early gastric cancer, due to presence of signet ring cells in gastric carcinoma and radiological features of liver tumor. After surgery, the pathological study showed an early gastric cancer in the lesser gastric curvature, with signet ring cell carcinoma features, and spindle cells with a positive reactivity CD117 in hepatic tumor. Nagai *et al*<sup>[25]</sup> reported a hepatic primary E-GIST in a patient with a previous gastric cancer 7 years earlier. Histologically, gastric cancer was a poorly differentiated adenocarcinoma, with lymph node involvement. When the hepatic mass was founded, upper and lower gastrointestinal endoscopic studies were performed, with no findings. Microscopically, the hepatic tumor was composed of spindle cells, with positive results for KIT and CD34<sup>[25]</sup>. Ochiai *et al*<sup>[38]</sup> reported a patient with surgical resection of a primary hepatic GIST, based on the positive immunostaining for CD34 and c-kit, and recurrent hepatic lesion and submucosal gastric tumor 2 years after first operation. After surgical removal of both hepatic and gastric lesions, both specimens showed GIST features with expression of c-kit and CD34, but the different morphological and molecular findings (gastric lesion: spindle cells and mutation in exon of c-kit; hepatic tumor: round cells and no mutation at exon 11) were enough for the authors to conclude that the hepatic GIST and gastric tumor were independent. In these patients, the different histological and immunohistochemical findings were the reason to define the hepatic lesion as a primary E-GIST. On the other hand, Liu *et al*<sup>[28]</sup> reported two synchronous E-GISTs. The patient presented a 5 cm-pancreatic mass and a 2 cm-hepatic lesion, with the same radiological features, and no other lesions in upper/lower gastrointestinal endoscopic examinations. With the diagnosis of malignant pancreatic cancer with hepatic metastases, the authors performed a surgical fine-needle aspiration and pathological findings in the hepatic and pancreatic biopsy tissues indicated that the tumors were mitotic spindle cell with CD117+<sup>[28]</sup>. The authors conclude that they are two independent lesions due to the pancreas and liver are exceptional location for primary GIST.

EGISTs have the same morphological, immunohistochemical and molecular features than conventional GISTs, including metastatic ones<sup>[12]</sup>. The criteria for the histopathological diagnosis are now firmly established; tumor cells might present a spindle or an epithelioid appearance and show a distinctive immunophenotype characterized by the expression of KIT (CD 117)<sup>[2,28,40]</sup>. Thus, the definitive diagnosis relies on the histopathological examination<sup>[41]</sup>; however, none of the pathological

**Table 1 Clinical characteristics and location in selected patients with extra-gastrointestinal stromal tumor of the liver**

Ref.	Yr	Age / Sex	Country	Presentation	Location	Size (cm)	Multifocal
Hu <i>et al</i> <sup>[20]</sup>	2018	79/F	China	Epigastric discomfort	RL	3.2	No
Joyon <i>et al</i> <sup>[21]</sup>	2018	56/M	France	Abdominal pain	Bilobar (Segments VII/VIII and LL)	10	Yes
	2018	59/F	France	Abdominal pain, weight loss	RL	23	No
Carrillo <i>et al</i> <sup>[22]</sup>	2017	41/M	Spain	Abdominal pain, weight loss	RL (S. V-VI)	20	No
Lok <i>et al</i> <sup>[23]</sup>	2017	50/F	China	Abdominal pain	RL	15	Yes
Cheng <i>et al</i> <sup>[24]</sup>	2016	63/M	China	No symptoms	RL	15	No
Nagai <i>et al</i> <sup>[25]</sup>	2016	70/F	Japan	Follow-up gastric cancer, No symptoms	LL	6	No
Liu <i>et al</i> <sup>[26]</sup>	2016	NA	China	NA	NA	NA	NA
Wang <i>et al</i> <sup>[27]</sup>	2016	61/M	China	No symptoms	Caudate lobe	7.3	No
Liu <i>et al</i> <sup>[28]</sup>	2016	56/F	China	No symptoms	LL + Pancreas	2.2	No
Su <i>et al</i> <sup>[29]</sup>	2015	65/M	Taiwan	Malaise, loss of appetite, abdominal pain	LL	12	No
Bhoy <i>et al</i> <sup>[3]</sup>	2014	41/F	India	Abdominal pain, weight loss	RL (S.VI-VI) I	15	Yes
Lin <i>et al</i> <sup>[30]</sup>	2015	67/F	China	No symptoms	RL	7.4	No
Mao <i>et al</i> <sup>[31]</sup>	2015	60/F	China	No symptoms	Bilobar (S I, IV, V, VIII)	12.8	No
Kim <i>et al</i> <sup>[32]</sup>	2014	71/M	Korea	Study for early gastric cancer, No symptoms	LL	7	No
Louis <i>et al</i> <sup>[33]</sup>	2014	55/F	India	Abdominal pain, loss of appetite	Bilobar (SII, III, VI, VIII)	14.5	Yes
Zhou <i>et al</i> <sup>[34]</sup>	2014	56/M	China	No symptoms	RL	10	No
Li <i>et al</i> <sup>[35]</sup>	2012	53/M	China	Abdominal discomfort	RL	20	No
Yamamoto <i>et al</i> <sup>[36]</sup>	2010	70/M	Japan	Loss of appetite (12 years after gastric cancer)	LL	20	No
Luo <i>et al</i> <sup>[37]</sup>	2009	17/M	China	No symptoms	RL	5	No
Ochiai <i>et al</i> <sup>[38]</sup>	2009	30/M	Japan	Abdominal fullness	Bilobar	27	No
De Chiara <i>et al</i> <sup>[1]</sup>	2006	37/M	Italy	No symptoms	RL (SV)	18	No
Hu <i>et al</i> <sup>[6]</sup>	2003	79/F	USA	Shortness of breath, pleuritic chest pain	RL	15	No

M: Male; F: Female; RL: Right lobe; LL: Left lobe; NA: Not available.

features is constant and required for a definitive diagnosis, including KIT mutation, which is not detectable in almost 5% of cases<sup>[29,34]</sup>. The concerning of KIT-negative GISTs could be solved after the discovery of novel mutations of the platelet derived growth factor receptor alpha oncogene as alternative pathogenetic mechanism<sup>[39]</sup>.

In the majority of cases of E-GISTs, preoperative diagnosis is not possible; therefore, patients may be easily misdiagnosed with different types of cancer and surgery is performed to make a confirm diagnosis after histological examination.

The overall management of hepatic E-GISTs is generally based on the recommendations for GI GIST. A large spectrum of therapeutic options has been proposed depending on the initial presentation and clinical context. As with GISTs, complete surgical resection is the mainstay of treatment for E-GISTs, as long as the lesion is resectable<sup>[24,28]</sup>.

A guided tumor biopsy must be considered for non-resectable tumors in order to assess the diagnosis and to offer another option for treatment such as radiofrequency, arterial embolization or chemoembolization may be considered<sup>[24,28,37]</sup>. The challenge is the risk of tumor rupture by the biopsy, well-known adverse prognostic factor in conventional GIST<sup>[42]</sup>.

For E-GIST and as with GI GISTs, imatinib mesylate may be administered preoperatively in locally advanced tumor in order to minimizing the size, for adjuvant treatment for patients with a high recurrence risk or for palliative treatment in non-resectable lesions, which is similar to the guidelines for their alimentary counterparts<sup>[1,28,29,30,43]</sup>. Rediti *et al*<sup>[44]</sup> in 2014 and Wada *et al*<sup>[45]</sup> in 2012 reported the complete remission in a patient with greater omentum-mesentery E-GIST and a peritoneal E-GIST, respectively, who received only imatinib mesylate as a treatment.

On 2001, National Institutes of Health proposed a consensus classification system for defining the risk of malignant behavior, based on mitotic count and tumor size and now is widely in use, also for E-GIST<sup>[2]</sup>. Joensuu *et al*<sup>[46]</sup> proposed a new modified classification including primary tumor site and tumor rupture in the item for classifying the risk of GIST and the indication for adjuvant treatment.

**Table 2** Diagnostic characteristics in selected patients with extra-gastrointestinal stromal tumor of the liver

Ref.	Yr	Age / Sex	Endoscopy	Imaging	Biopsy
Hu <i>et al</i> <sup>[20]</sup>	2018	79/F	Yes (no findings)	CT	No
Joyon <i>et al</i> <sup>[21]</sup>	2018	56/M	NA	CT	Percutaneous
	2018	59/F	Yes (no findings)	CT	Guided
Carrillo <i>et al</i> <sup>[22]</sup>	2017	41/M	Yes (no findings)	CT / MRI	No
Lok <i>et al</i> <sup>[23]</sup>	2017	50/F	Yes (no findings)	CT	No
Cheng <i>et al</i> <sup>[24]</sup>	2016	63/M	NA	CT	No
Nagai <i>et al</i> <sup>[25]</sup>	2016	70/F	Yes (no findings)	CT / MRI	No
Liu <i>et al</i> <sup>[26]</sup>	2016	NA	NA	NA	NA
Wang <i>et al</i> <sup>[27]</sup>	2016	61/M	NA	CT	No
Liu <i>et al</i> <sup>[28]</sup>	2016	56/F	NA	CT	Surgical
Su <i>et al</i> <sup>[29]</sup>	2015	65/M	NA	CT	CT-guided
Bhoy <i>et al</i> <sup>[3]</sup>	2014	41/F	NA	US/ CT	FNA
Lin <i>et al</i> <sup>[30]</sup>	2015	67/F	Yes (no findings)	CT	No
Mao <i>et al</i> <sup>[31]</sup>	2015	60/F	Yes (no findings)	CT / MRI	No
Kim <i>et al</i> <sup>[32]</sup>	2014	71/M	Early gastric cancer	CT / MRI	No
Louis <i>et al</i> <sup>[33]</sup>	2014	55/F	NA	CT	US-FNA/CT-FNA
Zhou <i>et al</i> <sup>[34]</sup>	2014	56/M	Yes (no findings)	NA	No
Li <i>et al</i> <sup>[35]</sup>	2012	53/M	NA	CT	US-FNAB
Yamamoto <i>et al</i> <sup>[36]</sup>	2010	70/M	NA	CT	No
Luo <i>et al</i> <sup>[37]</sup>	2009	17/M	NA	CT/US	US-FNA
Ochiai <i>et al</i> <sup>[38]</sup>	2009	30/M	NA	CT/ MRI	No
De Chiara <i>et al</i> <sup>[1]</sup>	2006	37/M	NA	CT	No
Hu <i>et al</i> <sup>[6]</sup>	2003	79/F	NA	CT	No

M: Male; F: Female; RL: Right lobe; NA: Not available; US: Ultrasound; CT: Computerized tomography; MRI: Magnetic resonance image; FNA: Fine needle aspiration.

Compared with GIST, E-GISTs have been reported to be accompanied by adverse prognostic factors, including a high proliferative index, large size and distant metastasis<sup>[15]</sup>; so E-GIST is considered to exhibit a worse prognosis, with a higher malignant potential and risk of recurrence following surgery compared with GISTs in the GI tract<sup>[2,11,15,24,26,42]</sup>. Disease free-survival and disease specific-survival of hepatic GISTs are significantly worse than those of gastric and small intestine GISTs and the location is an independent prognostic factor<sup>[26]</sup>. There is a trend that E-GIST is an aggressive group with worse outcome. In this review, which includes only case reports, 10/23 patients are DF with a follow-up between 3 and 30 mo, and 8/23 patients had progression of E-GIST or metastasis, with not available data in 5 patients.

In conclusion, the diagnosis of E-GIST must be considered in a liver mass. Rendering an accurate diagnosis is a challenge, as well as the confirmation of their primary or metastatic nature. The optimal treatment is surgery and imatinib mesylate has a role as neoadjuvant treatment for locally advanced tumors, adjuvant treatment if the lesion has a high risk for recurrence, and palliative treatment if there is distant metastasis. Literature on hepatic E-GIST is scarce and further studies such as multicentric databases are needed to clarify diagnosis and treatment.

**Table 3 Treatment and adjuvant treatment in selected patients with extra-gastrointestinal stromal tumor of the liver**

Ref.	Yr	Age / Sex	Treatment	Adjuvant imatinib mesylate
Hu <i>et al</i> <sup>[20]</sup>	2018	79/F	Curative surgical resection	Yes
Joyon <i>et al</i> <sup>[21]</sup>	2018	56/M	OLT	No
	2018	59/F	Refused surgery: imatinib mesylate	No
Carrillo <i>et al</i> <sup>[22]</sup>	2017	41/M	Segmentectomy V-VI	Yes
Lok <i>et al</i> <sup>[23]</sup>	2017	50/F	Right hepatectomy	Yes
Cheng <i>et al</i> <sup>[24]</sup>	2016	63/M	Right hepatectomy	Yes
Nagai <i>et al</i> <sup>[25]</sup>	2016	70/F	Left lateral segmentectomy	No
Liu <i>et al</i> <sup>[26]</sup>	2016	NA	NA	NA
Wang <i>et al</i> <sup>[27]</sup>	2016	61/M	Caudate lobe resection	No
Liu <i>et al</i> <sup>[28]</sup>	2016	56/F	Microwave ablation	Yes
Su <i>et al</i> <sup>[29]</sup>	2015	65/M	Irresectable: imatinib mesylate	No
Bhoy <i>et al</i> <sup>[3]</sup>	2014	41/F	Right hepatectomy	Yes
Lin <i>et al</i> <sup>[30]</sup>	2015	67/F	Surgical excision	Yes
Mao <i>et al</i> <sup>[31]</sup>	2015	60/F	ECHRA	Yes
Kim <i>et al</i> <sup>[32]</sup>	2014	71/M	Left lateral segmentectomy+excision of 1 intrabdominal nodule+total gastrectomy	NA
Louis <i>et al</i> <sup>[33]</sup>	2014	55/F	Segmentectomy III and atypical resection (segments II, VI and VIII)	Yes
Zhou <i>et al</i> <sup>[34]</sup>	2014	56/M	Anterior and median segmentectomy	No
Li <i>et al</i> <sup>[35]</sup>	2012	53/M	Refused treatment	No
Yamamoto <i>et al</i> <sup>[36]</sup>	2010	70/M	Left hepatectomy	NA
Luo <i>et al</i> <sup>[37]</sup>	2009	17/M	RFA	NA
Ochiai <i>et al</i> <sup>[38]</sup>	2009	30/M	L-Trisegmentectomy	Yes
De Chiara <i>et al</i> <sup>[1]</sup>	2006	37/M	NA	No
Hu <i>et al</i> <sup>[6]</sup>	2003	79/F	Right lobectomy	NA

M: Male; F: Female; OLT Ortotopic liver transplantation; ECHRA Extracorporeal hepatic resection and autotransplantation; NA: Not available; RFA: Radiofrequency ablation.

**Table 4 Pathological, immunohistochemical and molecular findings, and risk of malignancy according to Fletcher *et al*<sup>[2]</sup>**

Ref.	Yr	Age / Sex	Cell type	Molecular analysis	Mitotic count (n° / 50 HPF)	IH	Risk of malignancy
Hu <i>et al</i> <sup>[20]</sup>	2018	79/F	Spindle cells	NA	NA	CD117+, CD34+	NA
Joyon <i>et al</i> <sup>[21]</sup>	2018	56/M	Spindle cells	NA	8	CD117+, CD 34+	High risk
	2018	59/F	Mixed (spindle and epithelioid)	6 bp deletion in KIT exon 11	42	CD117+	High risk
Carrillo <i>et al</i> <sup>[22]</sup>	2017	41/M	Spindle cells	9 deletion in KIT	5	CD 117+, CD 34-	High risk
Lok <i>et al</i> <sup>[23]</sup>	2017	50/F	Spindle cells	NA	70	CD117+, CD 34+	High risk
Cheng <i>et al</i> <sup>[24]</sup>	2016	63/M	Spindle cells	NA	>5	CD 117 +, CD 34-	High risk
Nagai <i>et al</i> <sup>[25]</sup>	2016	70/F	Spindle cells	NA	40	CD117+, CD 34+	High risk
Liu <i>et al</i> <sup>[26]</sup>	2016	NA	NA	NA	NA	NA	NA
Wang <i>et al</i> <sup>[27]</sup>	2016	61/M	Spindle cells	NA	NA	CD117+/CD34+	High risk
Liu <i>et al</i> <sup>[28]</sup>	2016	56/F	Spindle cells	NA	2	CD117+	Low risk
Su <i>et al</i> <sup>[29]</sup>	2015	65/M	Spindle cells	NA	5	CD117+, CD 34-	High risk
Bhoy <i>et al</i> <sup>[3]</sup>	2014	41/F	NA	NA	NA	CD 117+	High risk
Lin <i>et al</i> <sup>[30]</sup>	2015	67/F	Mixed (spindle and epithelioid)	Mutation in exon 11	8	CD117+, CD 34+	High risk
Mao <i>et al</i> <sup>[31]</sup>	2015	60/F	Spindle cells	Mutation in exon 11	>10	CD 117+, CD 34 -	High risk
Kim <i>et al</i> <sup>[32]</sup>	2014	71/M	Spindle cells	NA	30-32	CD117+	High risk
Louis <i>et al</i> <sup>[33]</sup>	2014	55/F	Spindle cells	NA	10	CD117+	High risk
Zhou <i>et al</i> <sup>[34]</sup>	2014	56/M	Spindle cells	NA	<5	CD117+/CD34+	Intermediate
Li <i>et al</i> <sup>[35]</sup>	2012	53/M	Spindle cells	NA	NA	CD117+, CD34+	High risk

Yamamoto <i>et al</i> <sup>[36]</sup>	2010	70/M	Epithelioid cells	mutation PDGFRA exon 12	1	CD 117-/CD34+	High risk
Luo <i>et al</i> <sup>[37]</sup>	2009	17/M	Spindle cells	NA	0	CD117+, CD 34+	Low risk
Ochiai <i>et al</i> <sup>[38]</sup>	2009	30/M	Mixed (spindle and epithelioid)	No mutation at exon 11	75	CD117+, CD 34+	High risk
De Chiara <i>et al</i> <sup>[1]</sup>	2006	37/M	Spindle cells	NA	20	CD117+, CD 34+	High risk
Hu <i>et al</i> <sup>[6]</sup>	2003	79/F	Spindle cells	NA	20	CD117+, CD 34+	Low risk

M: male; F: female; NA: not available; PDGFRA: Platelet derived Growth Factor Receptor Alpha; HPF: High Power Fields.

**Table 5 Outcome and follow-up in selected patients with EGIST of the liver**

Ref.	Yr	Outcome	Follow-up (mo)
Hu <i>et al</i> <sup>[20]</sup>	2018	NA	NA
Joyon <i>et al</i> <sup>[21]</sup>	2018	Local recurrence (12 yr)	252
	2018	DF	18
Carrillo <i>et al</i> <sup>[22]</sup>	2017	DF	18
Lok <i>et al</i> <sup>[23]</sup>	2017	Brain metastasis (6 mo)	6
Cheng <i>et al</i> <sup>[24]</sup>	2016	DF	30
Nagai <i>et al</i> <sup>[25]</sup>	2016	DF	10
Liu <i>et al</i> <sup>[26]</sup>	2016	NA	NA
Wang <i>et al</i> <sup>[27]</sup>	2016	DF	12
Liu <i>et al</i> <sup>[28]</sup>	2016	Abdominal metastasis	17
Su <i>et al</i> <sup>[29]</sup>	2015	Progression of disease (died 6 mo)	6
Bhoy <i>et al</i> <sup>[3]</sup>	2014	DF	5
Lin <i>et al</i> <sup>[30]</sup>	2015	Hepatic recurrence (24 mo)	72
Mao <i>et al</i> <sup>[31]</sup>	2015	DF	12
Kim <i>et al</i> <sup>[32]</sup>	2014	NA	NA
Louis <i>et al</i> <sup>[33]</sup>	2014	DF	6
Zhou <i>et al</i> <sup>[34]</sup>	2014	DF	12
Li <i>et al</i> <sup>[35]</sup>	2012	NA	NA
Yamamoto <i>et al</i> <sup>[36]</sup>	2010	NA	NA
Luo <i>et al</i> <sup>[37]</sup>	2009	DF	3
Ochiai <i>et al</i> <sup>[38]</sup>	2009	Hepatic recurrence, submucosal gastric tumor (24 mo)	25
De Chiara <i>et al</i> <sup>[1]</sup>	2006	Lung metastasis (14 mo)	39
Hu <i>et al</i> <sup>[6]</sup>	2003	Portal lymph node metastasis (16 mo)	16

NA: Not available; DF: Disease free.

## ARTICLE HIGHLIGHTS

### Research background

A minor subset of primary gastrointestinal stromal tumors (GIST) can also arise outside the gastrointestinal tract, which is known as an extra-GIST (E-GIST).

### Research motivation

Primary GIST of the liver is an exceptional location and this study aimed to characterize epidemiological, clinical and pathological features and options of treatments.

### Research objectives

Our aim is to characterize epidemiological, clinical and pathological features and options of treatments.

### Research methods

We perform a system review including all patients with hepatic GISTs.

### Research results

This review shows that right hepatic lobe was the most frequent location, the median size was higher, there was a Southeast Asian predominance, and nearly 50% of patients have no



symptoms. The most frequent treatment was surgery and more than 70% of patients had a lesion with high risk of malignancy.

### Research conclusions

Literature on hepatic EGIST is scarce and further studies such as multicentric databases are needed to clarify diagnosis and treatment.

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## Scoring criteria for determining the safety of liver resection for malignant liver tumors

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### Abstract

#### BACKGROUND

Liver resection has become safer as it has become less invasive. However, the minimum residual liver volume (RLV) required to maintain homeostasis is unclear. Furthermore, the formulae used to calculate standard liver volume (SLV) are complex.

#### AIM

To review previously reported SLV formulae and the methods used to evaluate the minimum RLV, and explore the association between liver volume and mortality.

#### METHODS

A systematic review of Medline, PubMed, and grey literature was performed. References in the retrieved articles were cross-checked manually to obtain further studies. The last search was conducted on January 20, 2019. We developed an

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SLV formula using data for 86 consecutive patients who underwent hepatectomy at our institution between July 2009 and August 2011.

## RESULTS

Linear regression analysis revealed the following formula:  $SLV (mL) = 822.7 \times \text{body surface area (BSA)} - 183.2$  ( $R^2 = 0.419$  and  $R = 0.644$ ,  $P < 0.001$ ). We retrieved 25 studies relating to SLV formulae and 12 studies about the RLV required for safe liver resection. Although the previously reported formulae included various coefficient and constant values, a simplified version of the SLV, the common SLV (cSLV), can be calculated as follows:  $cSLV (mL) = 710$  or  $770 \times BSA$ . The minimum RLV for normal and damaged livers ranged from 20%-40% and 30%-50%, respectively. The Sapporo score indicated that the minimum RLV ranges from 35%-95% depending on liver function.

## CONCLUSION

We reviewed SLV formulae and the minimum RLV required for safe liver resection. The Sapporo score is the only liver function-based method for determining the minimum RLV.

**Key words:** Standard liver volume; Residual liver volume; Hepatectomy; Mortality; Liver failure; Liver function

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**Core tip:** We systematically reviewed standard liver volume (SLV) formulae, methods for assessing the minimum residual liver volume (RLV) required for safe liver resection, and the association between liver volume and mortality. Although the reported SLV formulae contained different coefficient/constant values, a simplified version of the SLV, the common SLV (cSLV), can be calculated as follows:  $cSLV (mL) = 710$  or  $770 \times \text{body surface area}$ . The Sapporo score is the only liver function-based method for determining the minimum RLV.

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## INTRODUCTION

Liver resection is a potentially curative treatment for malignant liver tumors, such as hepatocellular carcinoma (HCC) and metastatic liver cancer, in cases in which no metastasis is present in other organs<sup>[1,2]</sup>. Although the mortality rate associated with liver resection has decreased, surgical complications still occur<sup>[3-7]</sup>. To ensure that liver resection is performed safely, it is important to preoperatively evaluate patients' liver function so that it is possible to estimate the maximum liver volume that can be safely removed<sup>[8,9]</sup>. The Child-Pugh classification and the liver damage classification established by the Liver Cancer Study Group of Japan are used to evaluate liver function<sup>[10-13]</sup>. The indications for liver resection are grade A or B liver function, according to either classification system. However, liver function varies greatly between grade A and B in patients with HCC. Therefore, in HCC patients it is difficult to accurately predict the maximum safe extent of liver resection.

Recent advances in radiological assessments of the liver have made it possible to precisely calculate liver volume prior to liver resection<sup>[8,9,14-16]</sup>. Multi-detector-row computed tomography (MDCT) can be used to evaluate not only liver volume, but also patients' individual anatomies prior to liver resection<sup>[17-19]</sup>. The aim of this systematic review was to summarize the methods used to assess liver volume in order to aid the establishment of a standard formula for calculating standard liver volume (SLV). In addition, we attempted to summarize the relationship between liver volume and liver failure in order to facilitate safe liver resection.



## MATERIALS AND METHODS

This study was approved by the internal review board of Sapporo Medical University (approval ID: 302-195 and approval date: February 14, 2019). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines for conducting and reporting meta-analyses were followed<sup>[15]</sup>. To conduct this study, the study protocol was published on PROSPERO, which is the international prospective register of systematic reviews (reference: No. CRD42019123642).

### Estimation of SLV

Between July 2009 and August 2011, 86 consecutive patients who underwent liver resection for malignant tumors were enrolled in this study. Clinical laboratory tests, including of the serum levels of aspartate aminotransferase, alanine aminotransferase, albumin, hyaluronic acid, hepatocyte growth factor, and antithrombin III (ATIII); the prothrombin time (PT); the indocyanine green retention rate at 15 min (ICGR15); and the platelet count were evaluated prior to liver resection. The uptake ratio of the heart at 15 min to that seen at 3 min (HH15) and the uptake ratio of the liver to the liver plus heart at 15 min (LHL15) were obtained from time activity curves of 99 m Tc-galactosyl human serum albumin scintigraphy.

Liver volume was evaluated using 64-row MDCT (LightSpeed VCT VISION; GE Healthcare, Milwaukee, WI, United States). The images were obtained in four phases, the early arterial phase, portal vein phase, hepatic vein phase, and late phase. A ZIO STATION 2 (Ziosoft Inc., Tokyo, Japan) was used to calculate liver volume. The images of the hepatic vein phase were used for volumetry, and image analysis was restricted to the first and second branches of the portal and hepatic veins, as described previously<sup>[20]</sup>.

### Definition of liver dysfunction

Liver failure was defined as a serum bilirubin concentration of > 10 mg/dL for > 2 d. Liver dysfunction was defined as a total bilirubin level of  $\geq$  3 mg/dL and a PT value of < 50% within 7 d after liver resection<sup>[21]</sup>.

### Database searches

A systematic review of Medline, PubMed, and grey literature was performed. References from the retrieved articles were also cross-checked manually to obtain further studies. When more than one study from the same institution was found, only the publication with the most complete data was included. The last search was conducted on January 20, 2019. The search strategy for the PubMed database was as follows: {[“liver” (MeSH Terms) OR “liver” (All Fields)] AND volume (All Fields)} AND calculation (All Fields). The searches of other databases were conducted using the same medical subject headings (MeSH) and keywords in various combinations.

### Statistical analyses

Patient demographics and perioperative laboratory tests were extracted from the database, and differences between the groups were compared using the chi-square test followed by a post-hoc 2  $\times$  2 Fisher’s exact test. The unpaired t-test was used for comparisons between the no liver dysfunction group ( $n = 78$ ) and the liver dysfunction group ( $n = 8$ ). The relationships among the various clinical parameters were evaluated using Spearman’s rank correlation coefficient. The intraclass correlation coefficient (ICC) was used to assess inter-rater reliability. All calculations were performed using the SPSS 20.0 software program (SPSS Inc., Chicago, IL, United States). All results are expressed as the mean together with minimum and maximum levels.  $P$ -values of < 0.05 were considered to be statistically significant.

## RESULTS

### SLV at our institution

We investigated the cases of patients who underwent hepatectomy for various malignancies, including HCC, at our institution between July 2009 and August 2011. Table 1 shows the clinical demographics of the patients in three groups; *i.e.*, all patients ( $n = 86$ ), the no liver dysfunction group ( $n = 78$ ), and the liver dysfunction group ( $n = 8$ ).

The ICGR15, serum ATIII level, operation time, the background of the malignancy, and the reduction in liver volume differed significantly between the no liver dysfunction group and liver dysfunction group (Table 1). The results of the linear regression analysis of the relationship between resectable liver volume and body surface area (BSA) are shown in Figure 1. The latter analysis resulted in the deve-



**Table 1** Clinical demographics of the patients who underwent liver resection for malignant tumors in the no liver dysfunction group (*n* = 78) and liver dysfunction group (*n* = 8)

Clinical variables / characteristics	Total values ( <i>n</i> = 86)	No liver dysfunction ( <i>n</i> = 78)	Liver dysfunction ( <i>n</i> = 8)	<i>P</i> -values
Age (yr)	67.0 ± 10.3	66.8 ± 10.5	68.7 ± 9.3	NS
BSA (cm <sup>2</sup> )	1.61 ± 0.18	1.60 ± 0.18	1.65 ± 0.18	NS
Albumin (g/dL)	4.0 ± 0.4	4.0 ± 0.4	3.8 ± 0.4	NS
Bilirubin (mg/dL)	0.7 ± 0.3	0.6 ± 0.3	0.8 ± 0.4	NS
PT (%)	93.4 ± 11.9	93.9 ± 12.1	88.4 ± 9.7	NS
ICGR15 (%)	9.0 ± 4.6	8.4 ± 4.3	14.9 ± 2.5	< 0.001
HH15	0.578 ± 0.079	0.575 ± 0.078	0.617 ± 0.010	NS
LHL15	0.940 ± 0.027	0.940 ± 0.028	0.935 ± 0.024	NS
ATIII (%)	94.9 ± 17.2	96.0 ± 17.3	83.9 ± 13.4	0.036
Intraoperative bleeding (mL)	481.2 ± 468.5	455.8 ± 472.4	675.0 ± 430.0	NS
Operative time (min)	404.5 ± 135.2	390.8 ± 129.3	525.9 ± 140.9	0.014
Sex (male:female)	49:37	44:34	5:3	NS
Background (HCC: meta or CCC)	57:29	49:29	8:0	0.047
Hr (0/S:1:2/3)	53:17:16	49:16:13	4:1:3	NS
Sapporo score	13.2 ± 2.4	13.4 ± 2.5	11.0 ± 3.2	0.037
MELD score	7.6 ± 1.6	7.6 ± 1.7	7.8 ± 0.7	0.103
Child-Pugh score	3.2 ± 0.4	3.2 ± 0.4	3.4 ± 0.5	0.119
Total liver volume (cc)	1137.8 ± 222.9	1136.2 ± 228.5	1153.5 ± 221.4	NS
Reduction in liver volume (%)	19.0 ± 13.0	17.6 ± 12.1	32.8 ± 15.0	0.006
Residual liver volume (%)	85.1 ± 14.4	86.0 ± 13.7	75.4 ± 17.9	0.039

Continuous variables are expressed as the mean and standard deviation. BSA: Body surface area; PT-INR: Prothrombin time-international normalized ratio; ICG15: Indocyanine green retention rate at 15 min; ATIII: Serum anti-thrombin III level; HCC: Hepatocellular carcinoma; meta: Metastatic tumor; CCC: Cholangiocellular carcinoma; Hr: Hepatic resection; HH15: Uptake ratio of the heart at 15 min to that seen at 3 min on 99m Tc-galactosyl human serum albumin scintigraphy (GSA); LHL15: The uptake ratio of the liver to the liver plus heart at 15 min on GSA; MELD: Model For End-Stage Liver Disease; NS: Not significant.

lopment of the following formula for SLV:  $SLV (mL) = 822.7 \times BSA - 183.2$  ( $R^2 = 0.419$  and  $R = 0.644$ ,  $P < 0.001$ ). On the other hand, MELD score and Child-Pugh score did not correlate with resectable liver volume at all (Supplement Figure 1 and 2).

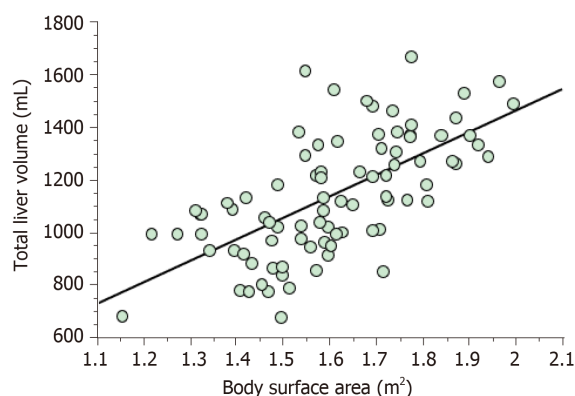
### Estimation of the minimum liver volume required for safe hepatectomy

The results of the linear regression analysis of the serum ATIII level and ICG15 are shown in Figure 2. The sum of the values for the serum ATIII level and ICG15 was almost 100%, which is consistent with the findings of previous studies<sup>[20]</sup>. We have also previously reported Sapporo scores for liver resection for malignant tumors (Table 2). The Sapporo score consists of four clinical variables, including the ICG15, serum ATIII level, HH15, and LHL15. Each factor is awarded 1 to 4 points. Thus, the maximum total score is 16 points, and the minimum total score is 4 points.

The results of the linear regression analysis of the reduction in liver volume and the Sapporo score are shown in Figure 3. The closed circles indicate the patients who exhibited liver dysfunction after hepatectomy, and the open circles indicate the patients who did not display liver dysfunction after hepatectomy. Linear regression analysis revealed a formula for predicting the risk of liver dysfunction after hepatectomy. The linear coefficient was almost 5, which meant that each extra Sapporo score point indicated that a further 5% of the total liver volume could be removed safely *via* liver resection. Therefore, the maximum Sapporo score (16 points) indicates that 65% of the total liver volume can be removed safely. On the other hand, the minimum score (4 points) only allows 5% of the total liver volume to be safely removed. The relationships between the Sapporo score, the resectable liver volume, and residual liver volume (RLV) are shown in Figure 3B.

### Systematic review of SLV and the minimum residual liver volume

A PRISMA flow diagram is shown in Figure 4. Among the 25 studies about SLV formulae, three types of calculations were reported. The first group used height and weight as independent factors (Table 3)<sup>[22-28]</sup>; the second group used BSA (Table 4)<sup>[24,25,29-36]</sup>; and the third group used other variables including age, gender, race, and radiological findings (Table 5)<sup>[37-43]</sup>. Although the SLV formulae in the first and second



**Figure 1** Linear regression analysis of total liver volume and body surface area. Standard liver volume (mL) =  $822.7 \times \text{BSA} - 183.2$ ;  $R^2 = 0.419$ ,  $R = 0.644$ ;  $P < 0.001$ .

groups included a variety of coefficient and constant values, they exhibited very similar ICC of between 0.70 and 0.78. On the other hand, in the third group the ICC of the SLV values obtained using age alone were very low (0.39 and -0.39, respectively). A combination of age and other variables gave ICC of between 0.66 and 0.79. If BSA were fixed at the mean value for the second group, the SLV formulae for the second group could be simplified as shown in Table 6. Although the previously reported SLV formulae included different coefficients and constant values, they could be grouped into two clusters (Figure 5). In cluster A, a simplified version of the SLV, the common SLV (cSLV), could be calculated as follows: cSLV (mL) =  $710 \times \text{BSA}$ , whereas in cluster B the cSLV could be calculated as follows: cSLV (mL) =  $770 \times \text{BSA}$ .

The minimum RLV required for safe liver resection has been debated for several decades. Most studies that examined this issue involved the use of normal livers containing metastatic liver tumors or transplanted livers for volume estimation<sup>[44-48]</sup>. All of the studies except ours calculated minimum cut-off values based on pathological findings (Table 7)<sup>[16,17,49-57]</sup>. According to previous reports, the minimum RLV for normal livers ranged from 20%-40%<sup>[54-56]</sup>, whereas that for damaged livers ranged from 30%-50%<sup>[16,52]</sup>. In contrast, the cut-off values obtained in the present study depended on liver function. According to the Sapporo score, the RLV cut-off values ranged from 35%-95%<sup>[20]</sup>. In addition, mortality rate ranged from 0.8% to 11% (Table 7).

## DISCUSSION

We reviewed the previously described formulae for calculating SLV and the minimum RLV required for safe liver resection. Although various SLV formulae have been reported, some of them were similar<sup>[24,30,32-35]</sup>. Therefore, we simplified the formula for estimating SLV to produce the cSLV. Furthermore, we found that the minimum RLV required for a safe hepatectomy ranged from 25%-50% depending on the pathological background. The Sapporo score is the only liver function-based method for determining the minimum RLV.

### Relationships between physical parameters and liver volume

Liver volume is obviously correlated with physical parameters<sup>[24,32-35]</sup>. However, the physiques of children and adults are markedly different<sup>[24]</sup>. In addition, the coefficients for the relationships among liver volume and physical parameters change during growth<sup>[24]</sup>. Yu *et al.*<sup>[24]</sup> attempted to develop a non-linear or stepwise model for estimating liver volume, whereas the other reported models were linear models. Unfortunately, this elaborate model did not become popular. One possible explanation for this is that it might be too elaborate for estimating SLV, and the use of other simpler models does not result in favorable outcomes.

BSA-based models for calculating SLV are very simple and are widely used in the clinical setting. However, 25 different formulae for calculating BSA have been proposed<sup>[58]</sup>. The first formula for calculating BSA was reported in 1879 by Meeh *et al.*<sup>[59]</sup>. Subsequently, the DuBios brothers developed a formula that included height and weight as variables<sup>[59,60]</sup>. This has remained the standard formula over the past century and we also used it for this study. However, these formulae do not produce precise estimates of BSA and provide no information regarding interindividual variability<sup>[58]</sup>.

**Table 2** Sapporo scores for liver resection of malignant tumors

Factors	Scores			
	4	3	2	1
ICGR15 (%)	≤ 10	10-19	20-29	≥ 30
ATIII (%)	≥ 90	80-89	70-79	≤ 69
HH15	≤ 0.55	0.56-0.59	0.60-0.64	≥ 0.65
LHL15	≥ 0.95	0.90-0.94	0.85-0.89	≤ 0.84

The total number of points was calculated as the sum of the scores for the ICGR15, serum ATIII level, HH15, and LHL15. Hr 2 or Hr 3: 16 points, Hr 1: ≥ 12 points, Hr S: ≥ 8 points, Hr 0: ≤ 7 points. ICG15: Indocyanine green retention rate at 15 min; ATIII: Serum anti-thrombin III level; HH15: Uptake ratio of the heart at 15 min to that seen at 3 min on 99m Tc-galactosyl human serum albumin scintigraphy (GSA); LHL15: The uptake ratio of the liver to the liver plus heart at 15 min on GSA.

Therefore, SLV varies markedly depending on which BSA formula is used.

Since the variation in SLV is not as large as that in BSA, similar coefficient and constant values were used to calculate SLV in previous studies. We identified two clusters of SLV formulae, as shown in Figure 5, and created a simplified cSLV formula for each cluster. The cluster analysis actually identified three clusters, but two of the clusters were very similar and not significantly different (data not shown). Therefore, we combined them together as cluster B. The differences among the clusters related to age or BSA. The age and BSA of cluster A tended to be younger and smaller, respectively, than those of cluster B. Therefore, differences in the patients' background data might have affected the coefficients used and the resultant ICC. Cluster B displayed ICC greater values than cluster A, although the exact reason for this was unclear. One possible explanation is that cSLV stabilized in elderly patients, and so the error range became smaller than that found in younger patients.

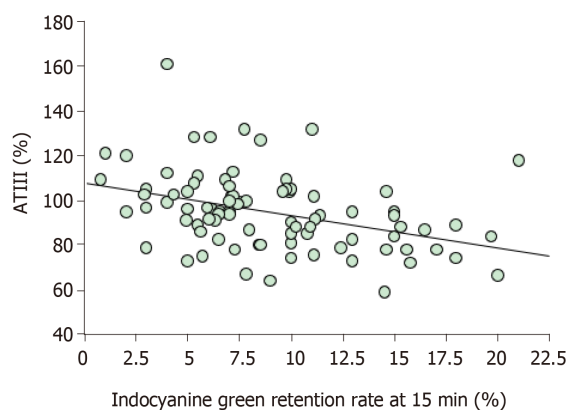
### Aging and SLV

SLV is affected by aging; *i.e.*, it was reported to be 4% of body weight at birth, but only 2%-2.7% of body weight in adults<sup>[30,61]</sup>. Therefore, age is an important factor when comparing the formulae used to calculate SLV. A study by Urata *et al.*<sup>[30]</sup> involved young patients, whereas other studies involved adults<sup>[24,30,32-35]</sup>. Our study population was older than those employed in previous studies. However, the formulae produced in each study were very similar. Although the SLV is affected by aging, it might remain relatively constant in all patients.

Takahashi *et al.*<sup>[37]</sup> and Kanamori *et al.*<sup>[38]</sup> proposed that SLV can be assessed using age alone. However, their approach would not have been appropriate for our patient population, in which most patients were elderly. On the other hand, a combination of age and other variables provided ICC of between 0.66 and 0.79. Thus, it is likely that SLV is partially affected by aging. Although elaborate formulae were created in the third group, this did not result in better ICC compared with those seen in the other groups. Therefore, simple SLV formulas could be applied to patients who are > 10 years old.

### Minimum RLV required for maintaining homeostasis after surgery

The issue of the minimum RLV does not only involve the reduction in liver volume, but also several other factors. For example, bile duct reconstruction could be one of the predictors of short-term clinical outcomes<sup>[62,63]</sup>. The frequency of bile leakage is higher in cases involving biliary reconstruction after hepatectomy than in cases in which biliary reconstruction is not performed<sup>[64]</sup>. In addition, biliary reconstruction can cause intra-abdominal leakage followed by intra-abdominal infection<sup>[64,65]</sup>. Therefore, the minimum RLV might differ between cases that do and do not involve biliary reconstruction. Second, the background of the liver also plays an important role in determining clinical outcomes. The general question is how we could evaluate liver damage before surgery. Several liver function evaluation methods have been proposed, including methods based on serum protein levels, serum enzyme levels, the ICGR15, and radiological assessments<sup>[66-68]</sup>. However, none of them represent liver function perfectly. For example, ICGR15 has been used for several decades; however, it does not reflect liver function in patients that exhibit ICG intolerance or possess an arteriovenous shunt. Radiological evaluations are also affected by the systemic circulation, *e.g.*, by dehydration and heart failure. Serum protein and enzyme levels are too stable to allow them to be used to evaluate liver function in the initial stages of liver damage, and they might not be valuable until the terminal stages of disease



**Figure 2** Linear regression analysis of serum antithrombin III levels and the indocyanine green retention rate at 15 min.  $ATIII + 1.461 \times ICGR15 = 108.068$ ;  $R^2 = 0.151$ ,  $R = 0.389$ ;  $P < 0.001$ . ATIII: Antithrombin III; ICGR15: Indocyanine green retention rate at 15 min.

progression. Therefore, the Sapporo score is still the only method for evaluating liver function, regardless of the degree of disease progression.

The other factors that might affect postoperative liver function include the concordance rate of the removed segments and the blood supply<sup>[69]</sup>. The patency of veins is also considered to affect back-flow control<sup>[70,71]</sup>. Therefore, evaluations of liver function should take both biochemical and anatomical findings into account. Although the Sapporo score is a useful method for evaluating liver function, some technical issues need to be solved before it is used to assess liver function in the clinical setting.

In conclusion, we reviewed SLV formulae and the minimum RLV required for safe liver resection. Although several SLV formulae have been presented, we created two simple SLV formulae that could be applied to the clinical setting. The Sapporo score is the only liver function-based method for estimating the minimum RLV.

**Table 3** Formulae for the calculation of standard liver volume based on height and weight

	Authors	Journals	Formulae	ICC
Height and weight	Ogiu <i>et al</i> <sup>[22]</sup>	<i>Health Phys</i> , 1997	LV = $576.9 \times H + 8.9 \times BW - 159.7$ (males); LV = $674.3 \times H + 6.5 \times BW - 214.5$ (females)	0.73
	Lin <i>et al</i> <sup>[23]</sup>	<i>Hepatogastroenterology</i> , 1998	LV = $133 \times H + 12 \times BW - 1530$	0.78
	Yu <i>et al</i> <sup>[24]</sup>	<i>Liver Transpl</i> , 2004	LV = $21.585 \times BW^{0.7322} \times H^{0.225}$	0.77
	Chandramohan <i>et al</i> <sup>[25]</sup>	<i>Indian J Gastroenterol</i> , 2007	LV = $18.51 \times BW + 191.80$	0.77
	Fu-Gui <i>et al</i> <sup>[26]</sup>	<i>Transplant Proc</i> , 2009	LV = $11.508 \times BW + 334.024$	0.71
	Poovathumkadavil <i>et al</i> <sup>[27]</sup>	<i>Transplant Proc</i> , 2010	LV = $12.26 \times BW + 555.65$	0.72
	Herden <i>et al</i> <sup>[28]</sup>	<i>Transpl Int</i> , 2013	LV = $-143.062973 + 4.274603051 \times H + 14.78817631 \times BW$ (Age: 0-1); LV = $-20.2472281 + 3.339056437 \times H + 13.11312561 \times BW$ (Age: 1-16)	0.76

ICC: Intraclass correlation coefficient; LV: Liver volume; BW: Body weight; H: Height.

**Table 4** Formulae for the calculation of standard liver volume based on body surface area

	Authors	Journals	Formulae	ICC
BSA	DeLand <i>et al</i> <sup>[29]</sup>	<i>Radiology</i> , 1968	LV = $1020 \times BSA - 220$	0.77
	Urata <i>et al</i> <sup>[30]</sup>	<i>Hepatology</i> , 1995	LV = $706.2 \times BSA + 2.4$	0.71
	Murry <i>et al</i> <sup>[31]</sup>	<i>Drug Metab Dispos</i> , 1995	LV = $710 \times BSA$	0.71
	Heinemann <i>et al</i> <sup>[32]</sup>	<i>Liver Transpl Surg</i> , 1999	LV = $1072.8 \times BSA - 345.7$	0.78
	Vauthey <i>et al</i> <sup>[33]</sup>	<i>Liver Transpl</i> , 2002	LV = $1267.28 \times BSA - 794.41$	0.78
	Yoshizumi <i>et al</i> <sup>[34]</sup>	<i>Transplant Proc</i> , 2003	LV = $772 \times BSA$	0.74
	Yu <i>et al</i> <sup>[24]</sup>	<i>Liver Transpl</i> , 2004	LV = $1145.4 \times BSA - 506.1$ (adults)	0.78
	Hashimoto <i>et al</i> <sup>[35]</sup>	<i>J Gastroenterol Hepatol</i> , 2006	LV = $961.3 \times BSA - 404.8$	0.77
	Chandramohan <i>et al</i> <sup>[25]</sup>	<i>Indian J Gastroenterol</i> , 2007	LV = $1267.28 \times BSA - 794.41$	0.78
	Saeki <i>et al</i> <sup>[36]</sup>	<i>Pediatr Transplant</i> , 2012	LV = $689.9 \times BSA - 24.7$	0.70
	Our study		LV = $822.7 \times BSA - 183.2$	0.74

ICC: Intraclass correlation coefficient; LV: Liver volume; BSA: Body surface area.



**Table 5** Formulae for the calculation of standard liver volume based on age, gender, or radiological findings

	Authors	Journals	Formulae	ICC
Others	Takahashi <i>et al</i> <sup>[37]</sup>	<i>Clin Pharmacol Ther</i> , 2000	LV = 15 × (4.6 × Age + 19.8), Age: 1-18; LV = 15 × (0.31 × Age + 97.8), Age: 30-40; LV = 15 × (-0.91 × Age + 149), Age: ≥ 41	0.39
	Kanamori <i>et al</i> <sup>[38]</sup>	<i>Int J Clin Pharmacol Ther</i> , 2002	LV = 67.3 × Age + 229.8	-0.39
	Choukèr <i>et al</i> <sup>[39]</sup>	<i>Liver Transpl</i> , 2004	LV = 452 + 16.34 × BW + 11.85 × Age - 166 × Gender (Age: 16-50, M: 0, F: 1); LV = 1390 + 15.94 × BW - 12.86 × Age (Age: 51-70)	0.79
	Chan <i>et al</i> <sup>[40]</sup>	<i>World J Gastroenterol</i> , 2006	LV = 218 + BW × 12.3 + Gender × 51 (F: 0, M: 1)	0.74
	Yuan <i>et al</i> <sup>[41]</sup>	<i>Transplant Proc</i> , 2008	LV = 949.7 × BSA - 48.3 × Age - 247.4 (Age: 1: < 40, 2: 41-60, 3: > 60)	0.77
	Kokudo <i>et al</i> <sup>[42]</sup>	<i>J Hepatol</i> , 2015	LV = 203.3 - 3.61 × Age + 58.7 × Thoracic width - 463.7 × Race (Asian: 1, Caucasian: 0)	0.66
	Ma <i>et al</i> <sup>[43]</sup>	<i>Liver Transpl</i> , 2017	LV = (2 × Depth) + (10 × BW) + 190	0.75

ICC: Intraclass correlation coefficient; LV: Liver volume; BSA: Body surface area; BW: Body weight; F: Female; M: Male.

**Table 6** Characteristics of the patients and simple standard liver volume formulae based on a mean body surface area of 1.61

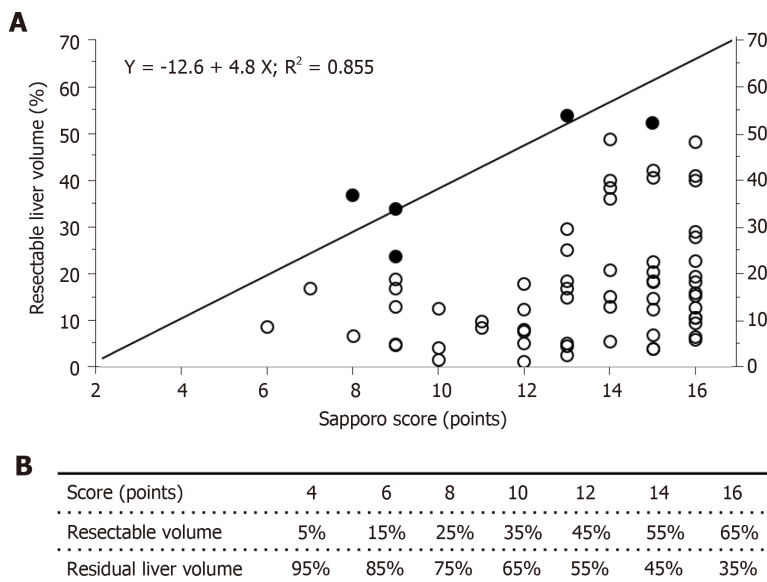
Authors	Mean age ± SD (range)	BSA	Simple formulae (tentative mean BSA = 1.61)	ICC	Clusters
DeLand <i>et al</i> <sup>[29]</sup>	ND	ND	LV = 883 × BSA	0.75	B
Urata <i>et al</i> <sup>[30]</sup>	11.1 ± 8.8	1.078 ± 0.528 (0.248-1.935)	LV = 707 × BSA	0.71	A
Murry <i>et al</i> <sup>[31]</sup>	9.7 (3.3-18.8)	Median: 1.37 (0.57-2.0)	LV = 710 × BSA	0.71	A
Heinemann <i>et al</i> <sup>[32]</sup>	50.6 ± 18.9	ND	LV = 858 × BSA	0.75	B
Vauthey <i>et al</i> <sup>[33]</sup>	Mean: 54, Median: 56 (14-90)	Median: 1.82 (1.32-2.90)	LV = 770 × BSA	0.74	B
Yoshizumi <i>et al</i> <sup>[34]</sup>	38.6 ± 20.6 (0-87) for males; 47.0 ± 19.7 (0-85) for females	1.86 ± 0.36 (0.24-2.88); 1.68 ± 0.28 (0.28-2.38)	LV = 772 × BSA	0.74	B
Yu <i>et al</i> <sup>[24]</sup>	42.4 ± 16.5	1.65 ± 0.26	LV = 831 × BSA	0.74	B
Hashimoto <i>et al</i> <sup>[35]</sup>	(17-66)	1.67 ± 0.18 (1.25-2.56)	LV = 710 × BSA	0.71	A
Chandramohan <i>et al</i> <sup>[25]</sup>	46.5 (10-70)	Median: 1.60 (0.88-2.25)	LV = 774 × BSA	0.73	B
Saeki <i>et al</i> <sup>[36]</sup>	5.8 (0 d-15)	ND	LV = 675 × BSA	0.70	A
Our study	67.0 ± 10.3	1.61 ± 0.18	LV = 709 × BSA	0.71	A

ICC: Intraclass correlation coefficient; BSA: Body surface area; ND: Not describe; LV: Liver volume.

**Table 7** Minimum residual liver volume based on various functional assessments

Authors	Publications	Functional assessments	Minimum residual LV			Mortality
			NL	CH, liver injury	LC	
Shirabe <i>et al</i> <sup>[49]</sup>	<i>J Am Coll Surg</i> , 1999	Pathology (HCC, HB, or HC)	250 mL/m <sup>2</sup> (40%)			8.8% (180 d)
Shoup <i>et al</i> <sup>[50]</sup>	<i>J Gastrointest Surg</i> , 2003	Pathology (NL, CRC metastasis alone)	25%	-	-	ND
Schindl <i>et al</i> <sup>[51]</sup>	<i>Gut</i> , 2005	Pathology (NL, 99% metastasis)	26.6%	-	-	ND
Ferrero <i>et al</i> <sup>[17]</sup>	<i>World J Surg</i> , 2007	Pathology (NL, liver injury)	26.5%	31%	-	0.8% (60 d)
van den Esschert <i>et al</i> <sup>[52]</sup>	<i>J Gastrointest Surg</i> , 2009	Pathology (CH, LC)	-	40%	50%	ND
Kishi <i>et al</i> <sup>[53]</sup>	<i>Ann Surg</i> , 2009	Pathology (NL)	20%	-	-	2.0% (30 d) 4.7% (60 d) 6.0% (90 d)
Suda <i>et al</i> <sup>[54]</sup>	<i>Am J Surg</i> , 2009	Pathology (NL, HCCa, GBCa, ICCa)	40%	-	-	8.1% (ND)
Vauthey <i>et al</i> <sup>[55]</sup>	<i>HPB</i> , 2010	Pathology (NL, liver injury, LC)	20%	30%	40%	ND
Gulielmi <i>et al</i> <sup>[56]</sup>	<i>Dig Surg</i> , 2012	Pathology (NL, steatosis, LC)	20%	30%	40%	ND
Hwang <i>et al</i> <sup>[16]</sup>	<i>J Gastrointest Surg</i> , 2015	Pathology (CH, LC)	-	35%	30%	0.8% (90 d)
Ribero <i>et al</i> <sup>[57]</sup>	<i>J Am Coll Surg</i> , 2016	Pathology (NL, HCCa alone)	30%	-	-	11% (90 d)
Our series	<i>Hepatogastroenterology</i> (in press)	ATIII, ICG15, GSA	35%-95%			2.3% (90 d)

LV: Liver volume; NL: Normal liver; CH: Chronic hepatitis; LC: Liver cirrhosis; HCC: Hepatocellular carcinoma; GBCa: Gallbladder cancer; ICCa: Intrahepatic cholangiocellular carcinoma; HCCa: Hilar cholangiocellular carcinoma; ATIII: Anti-thrombin III; ICG15: Indocyanine green retention rate at 15 min; GSA: 99m Tc-galactosyl human serum albumin scintigraphy; ND: Not describe.



**Figure 3** The results of the linear regression analysis of the reduction in liver volume and the Sapporo score. A: Linear regression analysis of the reduction in liver volume and the Sapporo score; Closed circles: Grade IV and V postoperative liver failure/dysfunction Open circles: Complication-free cases; B: Relationships between the Sapporo score, resectable liver volume, and residual liver volume.

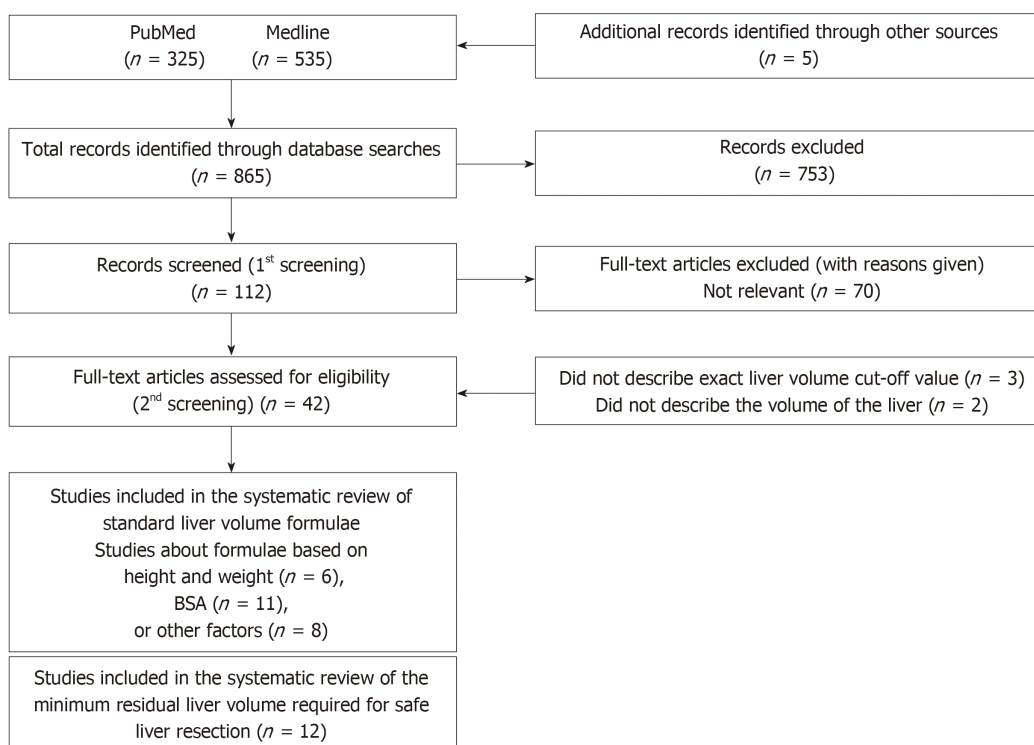


Figure 4 A PRISMA flow diagram.

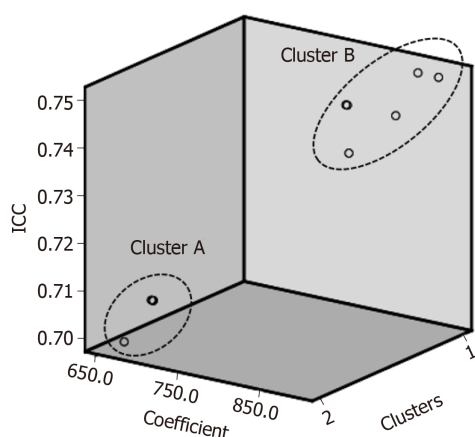


Figure 5 Three-dimensional scatterplot of simple standard liver volume formulae and their intraclass correlation coefficients. ICC: Intraclass correlation coefficients.

## ARTICLE HIGHLIGHTS

### Research background

Various minimum residual liver volume (RLV) has been presented. In addition, many formulas of standard liver volume (SLV) were also established.

### Research motivation

When we planned hepatectomy for malignant tumors, we had not proven which methods were the best reliable assessment to estimate minimum RLV and SLV.

### Research objectives

Aim of this study was to review previous SLV formulae and the methods used to evaluate the minimum RLV, and explore the association between liver volume and mortality.

### Research methods

A systematic review was performed (No. CRD42019123642). We developed an SLV formula using data for 86 consecutive patients who underwent hepatectomy at our institution between

July 2009 and August 2011.

### Research results

Our formula:  $SLV\ (mL) = 822.7 \times BSA - 183.2$  ( $R^2 = 0.419$  and  $R = 0.644$ ,  $P < 0.001$ ). We retrieved 25 studies relating to SLV formulae and 12 studies about the RLV required for safe liver resection. The minimum RLV for normal and damaged livers ranged from 20%-40% and 30%-50%, respectively. The Sapporo score indicated that the minimum RLV ranges from 35%-95% depending on liver function.

### Research conclusions

We reviewed SLV formulae and the minimum RLV required for safe liver resection. Although several SLV formulae have been presented, we created two simple SLV formulae that could be applied to the clinical setting. The Sapporo score is the only liver function-based method for estimating the minimum RLV.

### Research perspectives

The Sapporo score should be validated by large study with prospective registration. The common SLV, which is  $cSLV\ (mL) = 710$  or  $770 \times$  body surface area, needs to verify in the specific population.

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## Pancreatic stents to prevent post-endoscopic retrograde cholangiopancreatography pancreatitis: A meta-analysis

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### Abstract

#### BACKGROUND

Endoscopic retrograde cholangiopancreatography (ERCP) plays a major role in the investigation and treatment of pancreaticobiliary diseases. However, post-ERCP pancreatitis (PEP) is a severe adverse effect. Prior meta-analyses have shown that prophylactic PS was useful for preventing PEP. However, abstract reports and patients who underwent endoscopic ampullectomy were included in the previous analyses. In addition, two meta-analyses involved non-randomized controlled trials (RCTs). The efficacy of PS for preventing severe PEP was different in each meta-analysis. Therefore, we performed the current meta-analysis, which included only full-text articles, and added new findings.

#### AIM

To reveal the efficacy of prophylactic pancreatic stent (PS) placement for preventing PEP.

#### METHODS

We searched the MEDLINE, Cochrane Library and PubMed databases for related RCTs. Among the reports retrieved, 11 studies were included in this meta-analysis. All full-text articles were published between 1993 and 2016. A total of 1475 patients were enrolled in the included studies; of these patients, 734 had a PS inserted, and 741 did not have a PS inserted. PEP and severe PEP occurrence were evaluated in this meta-analysis.

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## RESULTS

PEP was observed in all studies and occurred in 39 (5.3%) patients who received a PS. On the other hand, PEP occurred in 141 (19%) patients who did not receive a PS. The occurrence of PEP was significantly lower in the patients who underwent PS placement than in the patients who did not receive a PS (OR = 0.32; 95%CI: 0.23-0.45;  $P < 0.001$ ). In addition, the occurrence of severe PEP was evaluated. Notably, the occurrence of severe PEP was not observed in the stent group; however, the occurrence of severe PEP was observed in 8 (1.3%) patients who did not have a PS inserted. Severe PEP occurred significantly less often in the stent group than in the no stent group (OR = 0.24; 95%CI: 0.06-0.94;  $P = 0.04$ ).

## CONCLUSION

In conclusion, prophylactic PS placement is useful for preventing PEP and severe PEP.

**Key words:** Endoscopic retrograde cholangiopancreatography; Pancreatic stent; Post-endoscopic retrograde cholangiopancreatography pancreatitis; Meta-analysis

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**Core tip:** Endoscopic retrograde cholangiopancreatography (ERCP) plays a major role in the investigation and treatment of pancreaticobiliary diseases. However, post-ERCP pancreatitis (PEP) is a severe adverse effect. To prevent PEP, prophylactic pancreatic stent (PS) placement was recommended in some randomized controlled trials (RCTs). We performed this meta-analysis that included only RCTs with full-text articles to evaluate the efficacy of prophylactic PS for preventing PEP. As a result, the rates of PEP and severe PEP occurrence were statistically lower in the stent group than in the no stent group. Prophylactic PS was efficient in preventing PEP.

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## INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) occupies an important place in the endoscopic treatment and investigation of pancreatic and biliary diseases. However, post-ERCP pancreatitis (PEP) is a severe adverse event. Several past studies have reported that the occurrence of PEP was observed in 0.4%–5.6% of patients<sup>[1-8]</sup>. Additionally, the fatality rate of PEP was 0%–0.1%<sup>[4,6-8]</sup>.

The risk factors shown to influence PEP occurrence in past reports were previous history of pancreatitis or PEP, two or more pancreatography procedures, sphincter of Oddi dysfunction (SOD), age younger than fifty years, female sex, difficulty of biliary cannulation, biliary sphincter balloon dilation, and precut sphincterotomy<sup>[7-15]</sup>. However, the usefulness of pancreatic stent (PS) placement for PEP has been reported in these high-risk patients<sup>[16-55]</sup>. Several prospective randomized controlled trials (RCTs) were discussed in these reports. Some RCTs showed the efficacy of PS placement in preventing PEP<sup>[19,20,22,26,27,30,45,51-53,55]</sup>. In addition, six meta-analyses were performed on this topic. The insertion of a PS was recommended in all of the meta-analyses<sup>[35,38,56-59]</sup>. However, the RCTs involved in these meta-analyses were varied. In addition, two meta-analyses involved non-RCTs<sup>[38,59]</sup>. In a study included in the two meta-analyses, the no stent group was not randomized<sup>[31]</sup>. Therefore, we performed a meta-analysis limited to full-text articles and excluding any RCTs of special cases (for example, ampullectomy cases, only abstracts, *etc.*). In addition, we included new RCTs in this meta-analysis.

## MATERIALS AND METHODS

### Literature search

We conducted a meta-analysis data search according to PRISMA statement guidelines<sup>[60]</sup>. MS and TT performed literature retrieval using the MEDLINE, PubMed, Cochrane Library databases. The retrieval was limited to reports written in English. The following keywords were used for the search: “pancreatic stent” and “post-ERCP pancreatitis”.

### Study selection

The studies that met the following criteria were selected: (1) RCTs comparing patients who received a PS for the prevention PEP and patients who did not receive a PS during ERCP; (2) Full-length articles; and (3) Articles written in English. We excluded studies that met the following criteria: (1) Case reports; (2) Case series; (3) Retrospective case control studies; and (4) Studies on endoscopic ampullectomy, because the procedure considerably changes the form of the Vater papilla. Moreover, we performed a manual search of reports cited in the extracted articles to discover any additional reports.

### Data extraction

The data extracted were as follows (Tables 1 and 2): (1) Study data (first author, year of publication, country); (2) Patient characteristics (age, sex, number of patients who received a PS, number of patients who did not receive a PS); and (3) Factors related to ERCP procedures (type of PS, success rate of PS insertion, occurrence of PEP, severity of PEP, severity criteria of PEP).

### Evaluation of bias

The publication bias for the obtained data was assessed using funnel plots.

### Statistical analysis

The meta-analysis was performed using The EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan)<sup>[61]</sup>. The homogeneity of each study was judged by determining the  $I^2$  value. An  $I^2$  value  $\leq 25\%$  was considered to have no statistical heterogeneity. An  $I^2$  value of 25%-50% was treated as low statistical heterogeneity, and an  $I^2$  value of 50%-75% was treated as moderate statistical heterogeneity. An  $I^2$  value  $> 75\%$  was considered to have high statistical heterogeneity. A fixed-effects model was used if extracted studies had low heterogeneity. A random-effects model was used if the extracted studies were heterogeneous. A  $P$  value  $< 0.05$  indicated a significant difference.

## RESULTS

### Selection of eligible studies

A total of 369 articles were identified by searching MEDLINE, Cochrane Library and PubMed. Of these reports, 80 studies were excluded because of duplication. In addition, 279 studies were excluded according to the selection criteria described above, as determined from the title and abstract. Finally, 11 studies were included in this meta-analysis (Figure 1).

All of these studies were RCTs published between 1993 and 2016. A total of 1475 patients were included in the studies, and of whom, 734 patients underwent insertion of PS, and 741 patients did not have a PS inserted. In some studies, proteinase inhibitors or antibiotics were administered as other prophylaxis; however, rectal indomethacin was not used in any study. All patient characteristics are shown in Table 1, and ERCP-related procedures are shown in Table 2.

### The definition of PEP and severity of PEP

In the RCTs, with the exception of two studies by Smithline *et al*<sup>[55]</sup> and Fazel *et al*<sup>[52]</sup>, PEP was defined according to Cotton's criteria<sup>[62]</sup>. In these RCTs, new abdominal pain after ERCP with elevated serum amylase no less than three times the normal upper limit in 24 h was diagnosed as PEP. In the study by Smithline *et al*<sup>[55]</sup>, abdominal pain with elevated serum lipase or amylase no less than two times the normal upper limit was diagnosed as PEP. In the study by Fazel *et al*<sup>[52]</sup>, epigastric and umbilical pain with elevated serum amylase no less than two times the normal upper limit was diagnosed as PEP.

The severity of PEP was classified according to Cotton's criteria in almost all RCTs<sup>[62]</sup> (Table 2). In the criteria, mild pancreatitis was defined as an extension of planned hospitalization of two to three days. Moderate pancreatitis was defined as an



Table 1 Patient characteristics of selected studies

Ref.	Country	Sample number		Mean age		Sex (male / female)		Patients
		Stent	No stent	Stent	No stent	Stent	No stent	
Smithline <i>et al</i> <sup>[55]</sup> , 1993	United States	43	50	46	47	19/81	22/78	SOD, CBD < 10 mm
Tarnasky <i>et al</i> <sup>[51]</sup> , 1998	United States	41	39	45.7	46.4	NA	NA	SOD
Fazel <i>et al</i> <sup>[52]</sup> , 2003	United States	38	36	45.8	43.6	4/32	6/32	SOD, difficult cannulation
Sofuni <i>et al</i> <sup>[19]</sup> , 2007	Japan	98	103	67.0	66.0	60/38	64/38	NA
Tsuchiya <i>et al</i> <sup>[20]</sup> , 2007	Japan	32	32	65.0	69.0	19/13	22/10	NA
Ito <i>et al</i> <sup>[22]</sup> , 2010	Japan	35	35	68	70	19/16	20/15	Difficult cannulation
Sofuni <i>et al</i> <sup>[27]</sup> , 2011	Japan	213	213	NA	NA	NA	NA	Risk factors, such as SOD, history of pancreatitis
Pan <i>et al</i> <sup>[26]</sup> , 2011	China	20	20	61.0	57.0	9/11	10/10	High-risk patients
Kawaguchi <i>et al</i> <sup>[30]</sup> , 2012	Japan	60	60	66.0	68.0	27/33	25/35	SOD, previous PEP
Lee <i>et al</i> <sup>[53]</sup> , 2012	South Korea	50	51	57.3	57.9	17/33	21/30	Difficult cannulation
Yin <i>et al</i> <sup>[45]</sup> , 2016	China	104	102	57.2	57.4	59/45	55/47	High-risk patients

SOD: Sphincter of Oddi dysfunction; CBD: Central bile duct; NA: Not available; PEP: Post-endoscopic retrograde cholangiopancreatography pancreatitis.

extension of planned hospitalization of four to ten days. Severe pancreatitis was defined as an extension of planned hospitalization of more than ten days with or without bleeding or a pseudocyst requiring intervention.

### Meta-analysis

PEP was observed in all studies; it occurred in 39 (5.3%) patients who underwent PS insertion, and on the other hand, it occurred in 141 (19%) patients who did not have a PS inserted. The heterogeneity among the included studies was low ( $I^2 = 31\%$ ,  $P = 0.15$ ); therefore, we selected a fixed-effects model. The occurrence of PEP was significantly lower in patients who received a PS than in the patients who did not receive a PS (OR = 0.32; 95% CI: 0.23-0.45;  $P < 0.001$ ; Figure 2).

We also evaluated severe PEP between the stent group and the no stent group. The occurrence of severe PEP was not observed in the stent group; however, the occurrence of severe PEP was observed in 8 (1.3%) patients who did not undergo PS insertion. Statistical heterogeneity was not seen in the included studies ( $I^2 = 0\%$ ,  $P = 0.99$ ); therefore, a fixed-effects model was chosen. The occurrence of severe PEP was significantly lower in the stent group than in the no stent group (OR = 0.24; 95% CI: 0.06-0.94;  $P = 0.04$ ; Figure 3).

### Publication bias

Egger's test of funnel plot asymmetry showed publication bias ( $P = 0.009$ ; Figure 4). The funnel plot was asymmetric, and we found that negative studies with a smaller number of subjects were missing.

## DISCUSSION

In this meta-analysis, prophylactic PS placement was efficient for preventing PEP. This result is the same as that in each previous RCT that was included in this meta-analysis. In addition, this meta-analysis proved that prophylactic PS placement prevented the occurrence of severe PEP.

In the eleven RCTs in this meta-analysis, ten RCTs indicated that prophylactic PS placement decreased the occurrence of PEP<sup>[19,20,22,26,27,30,45,51-53]</sup>. However, Smithline *et al*<sup>[55]</sup> reported that prophylactic main pancreatic duct stenting is not recommended for the prevention of PEP<sup>[55]</sup>. The different results among the RCTs was influenced by the small sample size. In addition, there were far fewer patients with severe PEP. Therefore, the occurrence of severe PEP was not significantly different between the stent group and the no stent group in any of the included studies. On the other hand, severe PEP was not observed in the stent group in the included RCTs. These results indicated that prophylactic PS might prevent not only total PEP but also severe PEP.

The efficacy of prophylactic PS for preventing severe PEP was not statistically proven in any RCT. However, six meta-analyses were previously performed on

**Table 2** The factors related to the endoscopic retrograde cholangiopancreatography procedures of selected studies

Ref.	ERCP procedure	Pancreatic stent	Success rate (%)	PEP n (%) stent/ no stent	Criteria of PEP severity
Smithline <i>et al</i> <sup>[55]</sup> , 1993	Precut EST	Double-barbed 5 or 7fr, 2 or 2.5 cm	90	Total 6 (14)/9 (18) Mild 5 (12)/5 (10) Moderate 1 (2)/2 (4) Severe 1 (2)/2 (4)	Cotton
Tarnasky <i>et al</i> <sup>[51]</sup> , 1998	EST	5 or 7Fr, 2 or 2.5 cm	NA	Total 1 (2)/10 (26) Mild 0 (0)/5 (13) Moderate 0 (0)/5 (13) Severe 0 (0)/0 (0)	Cotton
Fazel <i>et al</i> <sup>[52]</sup> , 2003	EST	5fr nasopancreatic catheter or Double-barbed 5fr, 2 cm	95	Total 2 (5.3)/10 (28) Mild 2 (5.3)/5 (14) Moderate 0 (0)/2(6) Severe 0 (0)/3 (8)	Cotton
Sofuni <i>et al</i> <sup>[19]</sup> , 2007	EST, EPBD, IDUS, biopsy, sphincter of Oddi manometry, POCS	5Fr, 3 cm with 2 flanges on the duodenal side	97	Total 3 (3)/14 (13.6) Mild 2 (2)/8 (7.8) Moderate 1 (1)/6 (4.6) Severe 0 (0)/0(0)	Cotton
Tsuchiya <i>et al</i> <sup>[20]</sup> , 2007	EST, IDUS, EPBD, sphincter of Oddi manometry	5fr, 3 or 4 cm duodenal pig tail stent without inner flange	100	Total 1 (3.1)/4 (12.5) Mild 1 (3.1)/2 (6.3) Moderate 0 (0)/1 (3.1) Severe 0 (0)/1 (3.1)	Cotton
Ito <i>et al</i> <sup>[22]</sup> , 2010	EST, IDUS, EPBD, biopsy	5fr, 4 cm with a single duodenal pig tail	97	Total 1 (2.9)/8 (23) Mild 1 (2.9)/8 (23) Moderate and severe 0	Cotton
Sofuni <i>et al</i> <sup>[27]</sup> , 2011	EST, EPBD, ENBD, IDUS, biopsy	5Fr, 3 cm with 2 flanges on the duodenal side	88	Total 20 (9.4)/31 (15.2) Mild 16 (7.5)/22 (14.6) Moderate 4 (1.9)/8 (3.8) Severe 0 (0)/1 (0.5)	Cotton
Pan <i>et al</i> <sup>[26]</sup> , 2011	ERCP	5fr single pig tail	NA	Total 4 (20)/14 (70) Mild, moderate, severe NA	Cotton
Kawaguchi <i>et al</i> <sup>[30]</sup> , 2012	Precut EST, pancreatic sphincterotomy, biopsy, IDUS	5fr, 3 cm with two flanges on the duodenal side	100	Total 1 (1.7)/8 (13.3) Mild 1 (1.7)/8 (13.3)	Modified Cotton
Lee <i>et al</i> <sup>[53]</sup> , 2012	EST, precut EST, IDUS, biopsy	Unflanged 3fr, 4, 6, or 8 cm duodenal pig tail stent	96	Total 6 (12)/15 (29.4) Mild 5 (10)/12 (23.5) Moderate 1 (2)/2 (3.9) Severe 0 (0)/1 (2)	Cotton
Yin <i>et al</i> <sup>[45]</sup> , 2016	EST, EPBD	5Fr, 5, 7, or 9 cm	NA	Total 8 (7.7)/18 (17.7) Mild, Moderate, severe NA	NA

PEP: Post-ERCP pancreatitis; EST: Endoscopic sphincterotomy; NA: Not available; EPBD: Endoscopic papillary balloon dilation; IDUS: Intraductal ultrasonography; POCS: Peroral cholangioscopy.

prophylactic PS to prevent PEP. Additionally, two of the six meta-analyses also reported that prophylactic PS did not significantly prevent severe PEP<sup>[56,58]</sup>. As more cases about prophylactic PS were reported, two meta-analyses performed by Mazaki *et al*<sup>[57,59]</sup> proved that prophylactic PS was efficient for preventing severe PEP. The second recent meta-analysis was carried out by Shi *et al*<sup>[35]</sup> and involved only full-text articles and excluded reports with only abstracts. However, the efficacy of prophylactic PS for preventing severe PEP was not shown in the meta-analysis. In the current meta-analysis, we included only full-text articles. As a result, PS was found to be efficient for preventing severe PEP. The addition of new RCTs and exclusion of RCTs on special cases such as ampullectomy<sup>[63]</sup> may have contributed to the definitive results of this meta-analysis.

This study has some limitations. First, all RCTs involved in this meta-analysis were written in English. Second, the type of PS was different in each RCT. Third, publication bias existed in this study. In the future, we hope that the accumulation of a greater number of relevant RCTs will overcome this bias.

In conclusion, prophylactic PS was useful for preventing not only PEP but also severe PEP.

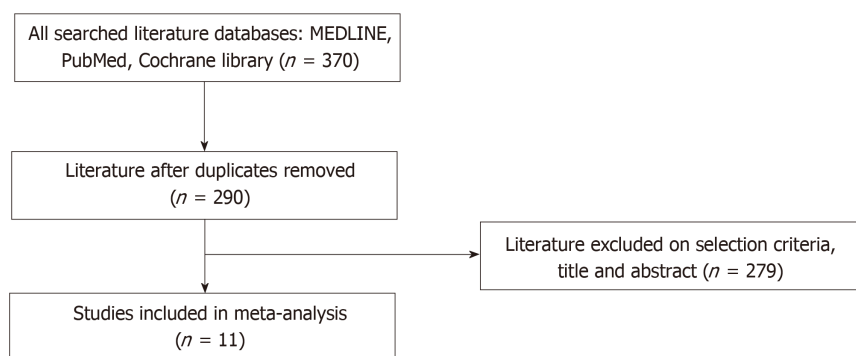


Figure 1 The flowchart of the article selection process.

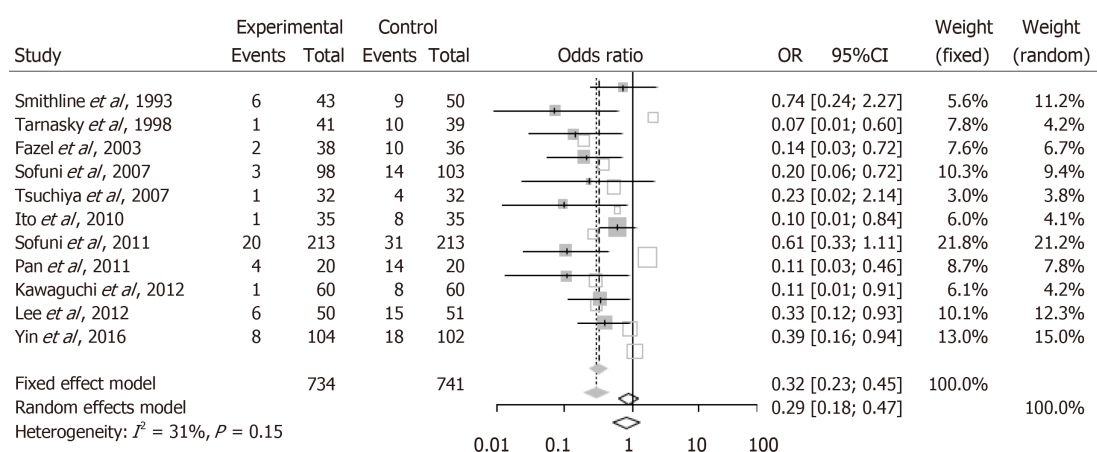


Figure 2 Forest plot of post-endoscopic retrograde cholangiopancreatography pancreatitis.

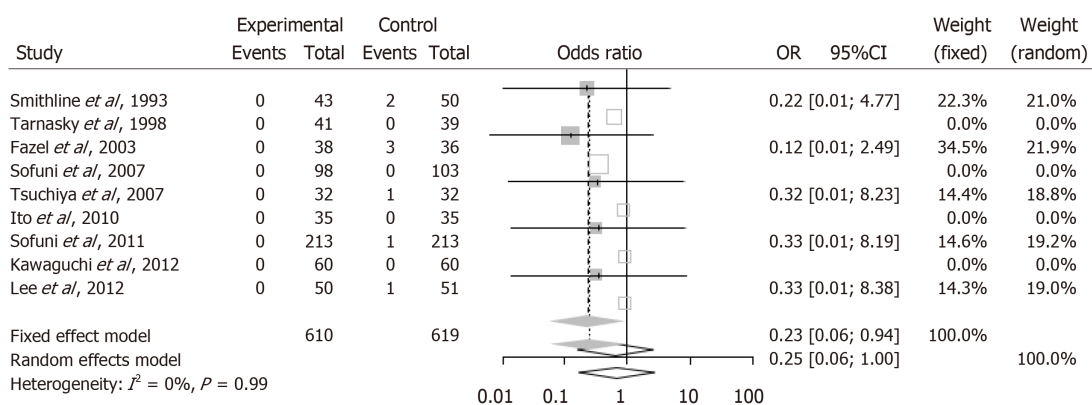


Figure 3 Forest plot of severe post-endoscopic retrograde cholangiopancreatography pancreatitis.

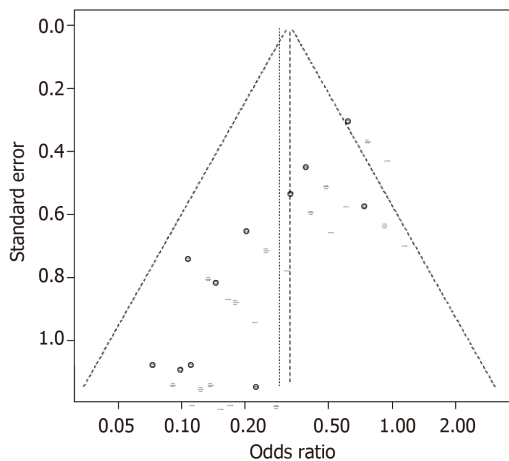


Figure 4 Funnel plot of post-endoscopic retrograde cholangiopancreatography pancreatitis occurrence.

## ARTICLE HIGHLIGHTS

### Research background

Endoscopic retrograde cholangiopancreatography (ERCP) occupies an important place in the endoscopic treatment and investigation of pancreatic and biliary diseases. However, post-ERCP pancreatitis (PEP) is a severe adverse effect. To prevent PEP, prophylactic pancreatic stent (PS) placement has been recommended based on the results of several randomized controlled trials (RCTs).

### Research motivation

Prior meta-analyses have shown that prophylactic PS was useful for preventing PEP. However, abstract reports and patients who underwent endoscopic ampullectomy were included in the previous analyses. The efficacy of PS for preventing severe PEP was different in each meta-analysis. Therefore, we performed the current meta-analysis, which included only full-text articles, and added new findings.

### Research objectives

In this meta-analysis, we evaluated the efficacy of prophylactic PS for the prevention of PEP.

### Research methods

We identified the included RCTs by searching MEDLINE, Cochrane Library and PubMed. Among the retrieved reports, 11 studies were included in this meta-analysis. The occurrence of PEP and severe PEP was evaluated.

### Research results

The rates of PEP and severe PEP occurrence were significantly lower in patients who received a PS than in patients who did not receive a PS.

### Research conclusions

Prophylactic PS was useful not only for preventing PEP but also for preventing severe PEP.

### Research perspectives

This meta-analysis proved that prophylactic PS prevented severe PEP. This result will contribute to a reduction in PEP and severe PEP in patients undergoing ERCP.

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## Prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis using pancreatic stents: A review of efficacy, diameter and length

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### Abstract

Although endoscopic retrograde cholangiopancreatography (ERCP) is an important procedure for the diagnosis and treatment of pancreaticobiliary diseases, post-ERCP pancreatitis (PEP) is the most frequent adverse event that can sometimes be fatal. However, prophylactic pancreatic stent (PS) insertion has been performed to prevent PEP in high-risk patients. In some randomized controlled trials (RCTs) and meta-analyses, the efficacy of prophylactic PS insertion has been shown to prevent PEP. In addition, several types of stents have been used to decrease PEP. In this review, we introduce the details of these RCTs and meta-analyses and reveal the specifications for stent placement, for example, the stent diameter and length and the pancreatic region into which the stent should be inserted.

**Key words:** Endoscopic retrograde cholangiopancreatography; Post-endoscopic retrograde cholangiopancreatography pancreatitis; Prophylactic pancreatic stent

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**Core tip:** Post-endoscopic retrograde cholangiopancreatography pancreatitis (PEP) is the most frequent adverse event that can sometimes be fatal. Pancreatic stent (PS) insertion is recommended to prevent PEP based on some randomized controlled trials (RCTs) and

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meta-analyses. Currently, several types of PS have been used. In this review, we introduce these RCTs and meta-analyses and reveal what stent should be used.

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## INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) is an important procedure for the diagnosis and treatment of pancreaticobiliary diseases but is sometimes a dangerous procedure. Several adverse events related to ERCP have been reported (duodenal perforation, bleeding, etc)<sup>[1-4]</sup>. Among them, post-ERCP pancreatitis (PEP) is the most frequent adverse event and is sometimes fatal. According to past reports, PEP occurs in 0.4%-5.6% of patients<sup>[5-12]</sup>, and the mortality rate of PEP is 0-0.1%<sup>[8,10-12]</sup>. The risk factors of PEP that have been specified in past reports were history of previous PEP, more than two contrast injections into the pancreatic duct, sphincter of Oddi dysfunction (SOD), age less than 50 years, female gender, difficult biliary duct cannulation, biliary sphincter balloon dilation, precut sphincterotomy, and a history of previous pancreatitis<sup>[11-19]</sup>. As prophylaxis for PEP in high-risk patients with these risk factors, pancreatic stent (PS) insertion is a preventative option. In this review, we present our investigations on the efficacy of PS placement for preventing PEP, and we disclose what stent should be selected and how the PS should be inserted.

## SEARCH STRATEGY

The studies included in this review were retrieved from PubMed using the following keywords: "Post-ERCP pancreatitis" and "pancreatic stent". Furthermore, studies written in English were selected. Only randomized controlled trials (RCTs) and meta-analyses that examined the efficacy of PS for preventing PEP were selected for further analysis. Studies that compared different stents (flanged or unflanged, diameter, length) were analyzed to determine which PSs should be used.

## ADAPTATION OF PROPHYLACTIC PS INSERTION

As mentioned above, patients with high risk factors become candidates for prophylactic PS insertion. The patients recommended PS insertion had a history of previous PEP, SOD, difficult biliary duct cannulation, biliary sphincter balloon dilation, precut sphincterotomy or sphincterotomy, pancreatic duct cannulation or contrast agent injection to the pancreatic duct, or endoscopic ampullectomy<sup>[20]</sup>.

## RCTs

In an RCT in 1993, Smithline *et al*<sup>[21]</sup> reported first prophylactic PS insertion for preventing PEP. In the report, the risk factors of PEP were acinarization, precutting, and a history of pancreatitis. The report could not prove the efficacy of PS insertion and did not recommend PS for PEP (PEP rate: Stent group 14% (6/43) *vs* 18% (9/50),  $P = 0.299$ ). However, several additional RCTs were performed, and the total number of RCTs on this topic increased to eleven from 1993 to 2016<sup>[21-31]</sup> (Table 1). Except for the first report written by Smithline, all reports indicated the efficacy of PS insertion for preventing PEP, and severe PEP did not occur in patients who received a PS<sup>[22-31]</sup>. Although a significant difference was not observed, the PEP rate was lower in the stent group than in the no stent group in the report written by Tsuchiya *et al*<sup>[25]</sup> [stent group 1/32 (3.1%) *vs* no stent group 4/32 (12.5%),  $P > 0.05$ ].

**Table 1 Randomized controlled trials of prophylactic pancreatic stent insertion for preventing post-endoscopic retrograde cholangiopancreatography pancreatitis**

Author	Yr	Country	Sample number		Risk factors	PEP <i>n</i> (%)	Criteria for PEP
			Stent	No stent		Stent/no stent	
Smithline <i>et al</i> <sup>[21]</sup>	1993	United States	43	50	Acinarization, pre-cutting, history of pancreatitis	Total 6 (14)/9 (18), <i>P</i> = 0.299; Mild 5 (12)/5 (10), <i>P</i> = NA; Moderate 1 (2)/2 (4), <i>P</i> = NA; Severe 0 (0)/2 (4), <i>P</i> = 0.264	Cotton
Tarnasky <i>et al</i> <sup>[22]</sup>	1998	United States	41	39	SOD	Total 1 (2)/10 (26), <i>P</i> = 0.003; Mild 0 (0)/5 (13), <i>P</i> = NA; Moderate 0 (0)/5 (13), <i>P</i> = NA; Severe 0 (0)/0 (0), <i>P</i> = NA	Cotton
Fazel <i>et al</i> <sup>[23]</sup>	2003	United States	38	36	Difficult cannulation SOD	Total 2 (5.3)/10 (28), <i>P</i> < 0.05; Mild 2 (5.3)/5 (14), <i>P</i> = NA; Moderate 0 (0)/2(6), <i>P</i> = NA; Severe 0 (0)/3 (8), <i>P</i> = NA	Cotton
Sofuni <i>et al</i> <sup>[24]</sup>	2007	Japan	98	103	IDUS, biopsy, EPBD, SOD, POCS, Duodenal diverticulum, acinarization, initial pancreatography, difficulty of cannulation	Total 3 (3)/14 (13.6), <i>P</i> = 0.019; Mild 2 (2)/8 (7.8), <i>P</i> = 0.139; Moderate 1 (1)/6 (4.6), <i>P</i> = 0.156; Severe 0 (0)/0(0), <i>P</i> = NA	Cotton
Tsuchiya <i>et al</i> <sup>[25]</sup>	2007	Japan	32	32	EST, IDUS, EPBD, SOD, pancreatic duct cannulation	Total 1 (3.1)/4 (12.5), <i>P</i> > 0.05; Mild 1 (3.1)/2 (6.3), <i>P</i> = NA; Moderate 0 (0)/1 (3.1), <i>P</i> = NA; Severe 0 (0)/1 (3.1), <i>P</i> = NA	Cotton
Ito <i>et al</i> <sup>[26]</sup>	2010	Japan	35	35	History of pancreatitis, history of PEP, pancreatic duct opacification, EST, IDUS, EPBD, cytology of pancreatic juice, biopsy of pancreatic duct	Total 1 (2.9)/8 (23) (per-protocol) 0 (0)/9 (24), <i>P</i> = 0.0096; Mild 1 (2.9)/8 (23); Moderate and severe 0	Cotton

Sofuni <i>et al</i> <sup>[28]</sup>	2011	Japan	213	213	History of pancreatitis, SOD, pancreatography, EST, precut sphincterotomy, EPBD, CBD tissue sampling, pancreatic duct tissue sampling, biliary drainage without EST, ENBD without EST, IDUS, difficulty of cannulation, long procedural time	(Intention to treat) Total 20 (9.4)/31 (14.6), $P = 0.076$ ; Mild 16 (7.5)/22 (10.3), $P = 0.24$ ; Moderate 4 (1.9)/8 (3.8), $P = 0.389$ ; Severe 0 (0)/1 (0.5), $P = 1.00$ ; (Full analysis set) Total 16 (7.9)/31 (15.2), $P = 0.021$ ; Moderate 12 (5.9)/22 (10.8), $P = 0.77$ ; Mild 4 (1.97)/8 (3.92), $P = 0.952$ ; Severe 0 (0)/1 (0.5), $P = 1.00$	Cotton
Pan <i>et al</i> <sup>[27]</sup>	2011	China	20	20	History of pancreatitis, pancreatic duct cannulation, pancreatography, difficult cannulation, hyperamylase-mia	Total 4 (20)/14 (70), $P < 0.01$ ; Mild, moderate, severe NA	Cotton
Kawaguchi <i>et al</i> <sup>[29]</sup>	2012	Japan	60	60	History of PEP, SOD, difficult cannulation, pre-cutting, pancreatic duct biopsy, IDUS of pancreatic duct	Total 1 (1.7)/8 (13.3), $P = 0.032$ ; Mild 1 (1.7)/8 (13.3), $P = 0.032$	Modified Cotton
Lee <i>et al</i> <sup>[30]</sup>	2012	Korea	50	51	Difficult biliary cannulation, pancreatic cannulation	Total 6 (12)/15 (29.4), $P = 0.031$ ; Mild 5 (10)/12 (23.5), $P = NA$ ; Moderate 1 (2)/2 (3.9), $P = NA$ ; Severe 0 (0)/1 (2), $P = NA$	Cotton
Yin <i>et al</i> <sup>[31]</sup>	2016	China	104	102	History of PEP, cannulation difficulty, periampullary diverticulum	Total 8 (7.7)/18 (17.7), $P = 0.031$ ; Mild, Moderate, severe NA	NA

RCT: Randomized controlled trial; PEP: Post-endoscopic retrograde cholangiopancreatography pancreatitis; SOD: Sphincter of Oddi dysfunction; IDUS: Intraductal ultrasonography; EPBD: Endoscopic papillary balloon dilation; POCS: Peroral cholangioscopy; EST: Endoscopic sphincterotomy; CBD: Common bile duct; ENBD: Endoscopic nasobiliary drainage; NA: Not available.

## PS FOR AMPULLECTOMY

In 2005, Harewood *et al*<sup>[32]</sup> reported on prophylactic PS placement for endoscopic snare excision of the duodenal ampulla. In this study, 19 patients were enrolled, and 10 received a PS. Although the number of participants was small, postprocedure pancreatitis was significantly higher in patients without PS than in patients with PS [33% (3/9) *vs* 0% (0/10),  $P = 0.02$ ].

## META-ANALYSES

Among the eleven RCTs, PEP occurred more in patients without PS than in patients with PS. PS insertion was recommended for preventing PEP. Additionally, severe PEP did not occur in any patient who received a PS in all eleven RCTs. However, the frequency of severe PEP was not significantly different between the stent group and



the no stent group in any of the RCTs. The results of severe PEP referred to the small sample size in each RCT and far fewer patients with severe PEP. These facts indicated that prophylactic PS might prevent not only total PEP but also severe PEP.

The usefulness of prophylactic PS placement for preventing severe PEP was not statistically recognized within each RCT. However, six meta-analyses were previously performed on prophylactic PS placement to prevent PEP<sup>[33-38]</sup> (Table 2). Among them, two of the six meta-analyses reported that prophylactic PS insertion did not statistically prevent severe PEP<sup>[33,35]</sup>. As more cases of prophylactic PS were reported, the second-most recent meta-analysis was conducted by Shi *et al*<sup>[37]</sup>; however, the efficacy of prophylactic PS for preventing severe PEP could not be proven. As a cause, the meta-analysis involved only full text articles and excluded articles with only abstracts, and the number of cases became small. On the other hand, two meta-analyses written by Mazaki *et al*<sup>[34,36]</sup> involved both full-text articles and articles with only abstracts; therefore, the number of cases was large. In the two meta-analyses written by Mazaki *et al*<sup>[34,36]</sup>, the efficacy of prophylactic PS insertion for preventing severe PEP was indicated (2010: Stent group 0/336 *vs* no stent group 7/344,  $P < 0.04$ , 2014: Stent group 0/694 *vs* no stent group 13/718,  $P = 0.01$ ). Furthermore, in the most recent meta-analysis written by Fan *et al*<sup>[38]</sup>, severe PEP was significantly lower in patients with a PS than in patients without a PS (stent group 0/493 *vs* no stent group 13/516,  $P < 0.01$ ).

From a meta-analysis, it became apparent that prophylactic PS might be efficient for preventing not only PEP but also severe PEP.

## WHAT STENT SHOULD BE USED?

As described above, PEP is reduced by PS insertion. However, several forms, diameters, and lengths of PSs exist. What stent should we use (Table 3)?

### Internal flanged or unflanged

In 2018, He *et al*<sup>[39]</sup> compared 5-Fr 3 cm internal unflanged stents with a single pigtail on the duodenal side and 5-Fr 3 cm internal flanged stents with a single pigtail on the duodenal side. The PEP rates were not different between the two types of stents [unflanged stents 5.07% (7/138) *vs* flanged stents 7.97% (11/138),  $P = 0.329$ ]. However, spontaneous PS displacement at 5 d was significantly higher in the internal unflanged stent group than in the internal flanged stent group [unflanged stent 47.72% (63/138) *vs* flanged stent 15.67% (21/134),  $P < 0.001$ ]. Furthermore, spontaneous PS displacement at 14 d was significantly higher in the internal unflanged stent group than in the internal flanged stent group [unflanged stent 84.21% (112/133) *vs* flanged stent 42.65% (58/136),  $P < 0.001$ ]. When the internal unflanged stent with a single pigtail on the duodenal side was used, an additional endoscope insertion to remove the PS was avoided.

### PS diameter

In past reports, the diameter of the PS makes a difference not only in the occurrence of PEP but also in usability. In 2004, Rashdan *et al*<sup>[40]</sup> wrote a retrospective study about prophylactic PS placement in 2940 cases. They described that small-diameter stents (*i.e.*, 3-4-Fr) were more effective than were 5-Fr or 6-Fr stents in preventing PEP [PEP rate: 3-4-Fr stent 8.7% (213/2447) *vs* 5-6-Fr stent 11.0% (54/493),  $P = 0.0471$ ]. However, Zolotarevsky *et al*<sup>[42]</sup> reported that there was no significant difference in the PEP rate between patients who received a 3-Fr PS and patients who received a 5-Fr PS. However, insertion of a 5-Fr stent was faster (9.2 min *vs* 11.1 min,  $P = 0.355$ ), easier [mean modified 5-point Likert scale<sup>[41,42]</sup>: 1.8 (5-Fr) *vs* 3.4 (3-Fr),  $P < 0.01$ ], and required fewer wires [1.5 (5-Fr) *vs* 1.9 (6-Fr),  $P = 0.002$ ] than insertion of a 3-Fr PS<sup>[43]</sup>. Pakh *et al*<sup>[44]</sup> reported that spontaneous passage was more frequent with 4-Fr PSs than with 5-Fr PSs [95.8% (115/137) *vs* 68.7% (134/209),  $P < 0.001$  (by log-rank test)]; therefore, the need for additional endoscopy to retrieve the PS was reduced by using a 4-Fr PS. However, the incidence of PEP was not significantly different between the 4-Fr PS group and the 5-Fr PS group. An additional report stated that insertion of a PS with a diameter  $> 5$ -Fr was effective in preventing PEP (PEP rate:  $> 5$ -Fr  $> 5$  cm 1.4% *vs*  $\leq 5$ -Fr  $\leq 5$  cm 9.4%,  $P = 0.0252$ )<sup>[45]</sup>.

Based on the above results, whether the diameter of PS influences the occurrence of PEP remains controversial. According to past reports, thin stents (*i.e.*, 3-Fr or 4-Fr) should be used with the expectation of spontaneous dislodgment, and a 5-Fr stent should be used in cases that were difficult to insert PS.

### PS length

Few reports have described the length of PSs (Table 3). In 2009, Chahal *et al*<sup>[46]</sup>

**Table 2 Meta-analyses of prophylactic pancreatic stent insertion for preventing post-endoscopic retrograde cholangiopancreatography pancreatitis**

Author	Yr	Number of included studies	Type of included studies	PEP rateStent/no stent	PS insertion for preventing PEP
Singh <i>et al</i> <sup>[33]</sup>	2004	5	Full text Abstract	$n = 206/275$ Total 12/43, $P = 0.001$ Mild to moderate 12/36, $P = 0.001$ ; Severe 0/7, $P = 0.15$	Recommended
Mazaki <i>et al</i> <sup>[34]</sup>	2010	8	Full text Abstract	$n = 336/344$ Total 19/64, $P < 0.001$ ; Mild to moderate 19/55, $P < 0.001$ ; Severe 0/7, $P < 0.04$	Recommended
Choudhary <i>et al</i> <sup>[35]</sup>	2011	8	Full text Abstract	$n = 322/334$ Total 16/66, $P < 0.00001$	Recommended
Mazaki <i>et al</i> <sup>[36]</sup>	2014	14	Full text Abstract	$n = 751/781$ Total 49/133, $P < 0.001$ ; Mild to moderate 49/120, $P < 0.001$ ; Severe 0/13, $P = 0.01$	Recommended
Shi <i>et al</i> <sup>[37]</sup>	2014	10	Full text	$n = 561/584$ ; Total 34/117, $P < 0.001$ ; Mild 24/70, $P < 0.001$ ; Moderate 6/24, $P = 0.004$ ; Severe 0/6, $P = 0.077$	Recommended
Fan <i>et al</i> <sup>[38]</sup>	2015	15	Full text Abstract	$n = 1233/1277$ Total 49/133, $P < 0.00001$ ; Mild 49/120, $P < 0.00001$ ; Severe 0/13, $P < 0.00001$	Recommended

PS: Pancreatic stent; PEP: Post-endoscopic retrograde cholangiopancreatography pancreatitis.

compared the occurrence of PEP between 5-Fr, 3 cm long unflanged PSs and 3-Fr, 8 cm or longer unflanged PSs. PEP was less frequent in the 5-Fr, 3 cm stent group than in the 3-Fr, long-stent group [PEP rate: 3-Fr 8 cm 14% (18/133) *vs* 5-Fr 3 cm 9% (11/116),  $P = 0.30$ ]. However, significant differences between these two groups were not observed. Fujisawa *et al*<sup>[47]</sup> compared PS lengths (unflanged straight stent, 5-Fr at 3 cm *vs* 5-Fr at 5 cm) and reported that the PEP rate and the median time until stent dislodgement were both lower in the 3 cm group than in the 5 cm group (PEP rate: 3 cm 2.0% *vs* 5 cm 8.8%,  $P = 0.035$ , median period until spontaneous PS dislodgement: 3 cm 2 d *vs* 5 cm 4 d,  $P < 0.001$ ). In this report, earlier stent dislodgement of the 3 cm PS might contribute to preventing PS obstruction-induced PEP. However, Olsson *et al*<sup>[45]</sup> reported that a PS with a length  $> 5$  cm and a diameter  $> 5$  Fr is the most effective in preventing PEP. In this report, the frequency of PEP was not significantly different between patients who received a PS  $\leq 5$  cm and patients who received a PS  $> 5$  cm.

These results regarding the influence of PS length on PEP varied, and we propose two explanations for these inconsistencies. Perhaps the diameters of PS were not matched, except for in the second report written by Fujisawa *et al*<sup>[47]</sup>; although in this report the pancreatic region into which the PS was inserted was not investigated, and only PS length was investigated. Pancreas size differs among people; therefore, both a 3 cm and 5 cm stent can be inserted into the pancreatic head depending on the patient. However, spontaneous dislodgement could contribute to preventing PEP if both a 3 cm and 5 cm PS were inserted in or near to the pancreatic head.

#### Location in the pancreas of PS insertion

As described in the previous section, the PEP rate was compared between patients who received a PS  $\leq 5$  cm and patients who received a PS  $> 5$  cm in a report written by Olsson *et al*<sup>[45]</sup>. In comparison, the PEP rate was not significantly different between the two groups. In patients who received a PS  $> 5$  cm, the stent might reach the pancreatic body or the tail. However, the pancreatic regions into which the stents were inserted were not described.

However, Sugimoto *et al*<sup>[48]</sup> compared hyperamylasemia and the PEP rate between

Table 3 Comparison of stent type

Author, yr	Stent type	n	Results
Flanged or unflanged			
He <i>et al</i> <sup>[39]</sup> , 2018	Internal unflanged 5-Fr 3 cm stent with a single pigtail on the duodenal side <i>vs</i> internal flanged 5-Fr 3 cm stent with a single pigtail on the duodenal side	138/138	Spontaneous migration was more frequent with the internal unflanged stent (migration at five days: 47.72% <i>vs</i> 15.67%, <i>P</i> < 0.001, migration at 14 d 84.21% <i>vs</i> 42.65%, <i>P</i> < 0.001)
Comparison of stent diameter			
Rashdan <i>et al</i> <sup>[40]</sup> , 2004	3-4-Fr, 3-8 cm without internal flange <i>vs</i> 5-6-Fr, NA, with internal flange	2447/493	The 3-4-Fr stent was more effective in preventing PEP than the 5-6-Fr stent (PEP rate: 3-4-Fr stent 8.7% (213/2447) <i>vs</i> 5-6-Fr 11.0% (54/493), <i>P</i> = 0.0471)
Zolotarevsky <i>et al</i> <sup>[43]</sup> , 2011	5-Fr 5 cm <i>vs</i> 3-Fr 6 cm	38/40	PEP rates did not differ. 5-Fr PS placement was easier [mean modified 5-point Likert scale <sup>[40,41]</sup> : 1.8 (5-Fr) <i>vs</i> 3.4 (3-Fr), <i>P</i> < 0.01], faster [9.2 (5-Fr) <i>vs</i> 11.1 minutes (3-Fr), <i>P</i> = 0.355], and required fewer wires [1.5 (5-Fr) <i>vs</i> 1.9 (6-Fr), <i>P</i> = 0.002]
Pahk <i>et al</i> <sup>[44]</sup> , 2011	4-Fr <i>vs</i> 5-Fr, both stents were 2 to 11 cm, unflanged	137/209	PEP rates did not differ. Spontaneous migration was more frequent with the 4-Fr stent [95.8% (115/137) <i>vs</i> 68.7% (134/209), <i>P</i> < 0.001 (by log-rank test)]
Olsson <i>et al</i> <sup>[45]</sup> , 2016	≤ 5-Fr, ≤ 5 cm <i>vs</i> > 5-Fr, > 5 cm	241 (≤ 5-Fr)/135 (> 5-Fr)	The > 5-Fr, > 5 cm stent was more effective in preventing PEP (> 5-Fr, > 5 cm 1.4% <i>vs</i> ≤ 5-Fr, ≤ 5 cm 9.4%, <i>P</i> = 0.0252)
Comparison of stent length			
Chahal <i>et al</i> <sup>[46]</sup> , 2009	5-Fr 3 cm, unflanged <i>vs</i> 3-Fr 8 cm or longer, unflanged	116/133	Spontaneous migration was more frequent with the 5-Fr 3 cm stent (5-Fr 98% <i>vs</i> 3-Fr 88%, <i>P</i> = 0.0001). Failure of PS placement was observed more often in the longer 3-Fr stent group (5-Fr 0/116 <i>vs</i> 3-Fr 11/133, <i>P</i> = 0.0003). PEP rates did not differ
Fujisawa <i>et al</i> <sup>[47]</sup> , 2016	5-Fr 3 cm <i>vs</i> 5-Fr 5 cm, both stents were unflanged and straight	98/102	The 5-Fr 3 cm stent was more efficient for preventing PEP (3 cm 2.0% <i>vs</i> 5 cm 8.8%, <i>P</i> = 0.035). The period until spontaneous dislodgement was significantly shorter for the 3 cm stent than for the 5 cm stent (3 cm 2 d <i>vs</i> 5 cm 4 d, <i>P</i> < 0.001)
Part of the pancreas in which the stent was inserted			
Sugimoto <i>et al</i> <sup>[48]</sup> , 2018	Pancreatic head <i>vs</i> pancreatic body or tail	131/16	After ERCP, the level of the pancreatic isozyme of serum amylase was higher in the head group than in the body/tail group [head group 138.5 (7.0-2086) IU/L <i>vs</i> body/tail group 78.5 (5.0-1266.5) IU/L, <i>P</i> < 0.03]

ERCP: Endoscopic retrograde cholangiopancreatography; PEP: Post-ERCP pancreatitis.

patients who had a PS inserted into the pancreatic head (the head group) and patients who had a PS inserted into the pancreatic body or tail (the body/tail group). Although a significant difference was not observed, the PEP rate was lower in the body/tail group than in the head group [0% (0/16) *vs* 9.2% (12/131), *P* = 0.363]; PEP was not observed in the body/tail group. Furthermore, after ERCP, the level of the pancreatic isozyme of serum amylase was significantly higher in the head group than in the body/tail group [138.5 (7.0-2086) IU/L *vs* 78.5 (5.0-1266.5) IU/L, *P* = 0.03]. Proteinase activation, which exacerbates pancreatitis, is induced by difficult pancreatic duct drainage<sup>[49]</sup>; therefore, stent placement up to the pancreatic body or tail contributes to greater pancreatic drainage than stent placement in the pancreatic

head does.

## CONCLUSION

The results of several RCTs and meta-analyses have revealed that PS is efficient for preventing PEP. However, PEP can occur in patients who underwent stent placement. Currently, the main argument is which PS should be used. Additional endoscopic insertion to remove the PS could be avoided by using an internal unflanged PS. The diameter of PS is controversial because thin stents easily migrate, and thick stents are easily inserted in some cases. With respect to the length of the stent, a 3 cm stent may be more efficient than a 5 cm stent in preventing PEP. However, the risk of PEP may be altered according to the pancreatic region into which the PS is inserted.

Overall, there remain few cases in which a prophylactic PS was utilized; therefore, the accumulation of additional cases is necessary.

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## Significance of multivisceral resections in oncologic surgery: A systematic review of the literature

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### Abstract

#### BACKGROUND

Multivisceral resections (MVR) are often necessary to reach clear resections margins but are associated with relevant morbidity and mortality. Factors associated with favorable oncologic outcomes and elevated morbidity rates are not clearly defined.

#### AIM

To systematically review the literature on oncologic long-term outcomes and morbidity and mortality in cancer surgery a systematic review of the literature was performed.

#### METHODS

PubMed was searched for relevant articles (published from 2000 to 2018). Retrieved abstracts were independently screened for relevance and data were extracted from selected studies by two researchers.

#### RESULTS

Included were 37 studies with 3112 patients receiving MVR for colorectal cancer (1095 for colon cancer, 1357 for rectal cancer, and in 660 patients origin was not specified). The most common resected organs were the small intestine, bladder and reproductive organs. Median postoperative morbidity rate was 37.9% (range: 7% to 76.6%) and median postoperative mortality rate was 1.3% (range: 0% to 10%). The median conversion rate for laparoscopic MVR was 7.9% (range: 4.5% to 33%). The median blood loss was lower after laparoscopic MVR compared to the open approach (60 mL vs 638 mL). Lymph-node harvest after laparoscopic MVR was comparable. Report on survival rates was heterogeneous, but the 5-year overall-survival rate ranged from 36.7% to 90%, being worst in recurrent rectal

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cancer patients with a median 5-year overall survival of 23%. R0 -resection, primary disease setting and no lymph-node or lymphovascular involvement were the strongest predictors for long-term survival. The presence of true malignant adhesions was not exclusively associated with poorer prognosis. Included were 16 studies with 1.600 patients receiving MVR for gastric cancer. The rate of morbidity ranged from 11.8% to 59.8%, and the main postoperative complications were pancreatic fistulas and pancreatitis, anastomotic leakage, cardiopulmonary events and post-operative bleedings. Total mortality was between 0% and 13.6% with an R0 -resection achieved in 38.4% to 100% of patients. Patients after R0 resection had 5-year overall survival rates of 24.1% to 37.8%.

## CONCLUSION

MVR provides, in a selected subset of patients, the possibility for good long-term results with acceptable morbidity rates. Unlikelihood of achieving R0 -status, lymphovascular- and lymph -node involvement, recurrent disease setting and the presence of metastatic disease should be regarded as relative contraindications for MVR.

**Key words:** Colorectal cancer; Gastric cancer; Primary; Recurrent; Multivisceral resection; Hyperthermic intraperitoneal chemotherapy; Morbidity

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**Core tip:** Multivisceral resections constitute a huge challenge for an interdisciplinary team. Proper patient selection, combined perioperative systemic treatment and en-bloc resection of adherent organs can provide acceptable morbidity-, mortality- and long-term survival rates.

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## INTRODUCTION

Patients with locally advanced primary and recurrent cancers constitute a challenge for the interdisciplinary treatment team because the only chance for cure and prolonged survival is complete resection of the tumor with clear margins. Invasion of adjacent organs occurs in 10%-20% of patients suffering from colorectal cancer and gastric cancer. The prerequisite for long- and short-term results is completeness of surgical resection. This aggressive surgical concept is accompanied by pre- and postoperative systemic treatment schedules, consisting of chemotherapy, radiotherapy and chemoradiotherapy. Due to the lack of sufficient and reliable pre-operative data the decision in favor of multivisceral resections (MVR) is often made intraoperatively. MVR is defined as the *en-bloc* resection of the tumor and the adjacent organs including reproductive organs and organs of the urinary tract. MVR should therefore always be taken into account if macroscopic complete resection is achievable. Adherence of the primary or recurrent tumor to adjacent structures does not necessarily predict true malignant invasion. Winter *et al*<sup>[1]</sup> stated that up to two-third of cases are postoperatively classified as inflammatory adhesions rather than true malignant invasion. Furthermore, lysis of adhesions or separation of the adjacent organ from the tumor dramatically increases the risk of recurrence and should be avoided. The significance of palliative MVR for patients with obstruction, fistula and pain is not clearly defined but the data presented in this review suggest that non-curative MVR does not improve patient outcome. Leijssen *et al*<sup>[2]</sup> showed that patients with a T4 -tumor not undergoing MVR had a poorer outcome regarding overall-, disease-free-, and cancer-specific survival. The indication in favor of MVR for patients with metastatic disease is also common in the current literature but the true benefit of MVR for stage IV disease is unclear.

This review aims to systematically evaluate the current literature on outcomes following MVR for colorectal and gastric cancer and for patients undergoing MVR and HIPEC for peritoneal metastasis of gastrointestinal, especially colorectal, origin.

## MATERIALS AND METHODS

A systematic review was conducted with reference to the PRISMA statement and the current methodological literature<sup>[3,4]</sup>. Electronic medical literature databases were screened for appropriate publications from 2000 to 2018. Databases were searched using the following terms: “multivisceral” AND “colon cancer”, “multivisceral” AND “rectal cancer”, “multivisceral” AND “gastric cancer”, “multivisceral AND “cytoreductive surgery”, and “multivisceral” AND “hyperthermic intraperitoneal chemotherapy”. Comments and case reports were excluded. Furthermore, publications that did not report performance of MVR, morbidity and mortality rates, oncologic outcome and publications that included unspecified cancer types were also not included in this systematic review.

For the search terms “multivisceral” AND “colon cancer” and “multivisceral” AND “rectal cancer” 211 records were provided. After the abstracts were screened (level 1 screening) independently by two reviewers 165 publications excluded (Figure 1).

For the search terms “multivisceral” AND “gastric cancer” 93 records were provided. After the abstracts were screened (level 1 screening) independently by two reviewers 71 publications excluded.

After level 2 screening, 37 publications for “Multivisceral resection for colon cancer and rectal cancer”, 16 publications for “Multivisceral resection for gastric cancer and 3 publications for “Multivisceral resections with hyperthermic intraperitoneal chemotherapy” were included.

MVR were defined as resection of more than two organs.

## RESULTS

MVR for colon cancer and rectal cancer ( $n = 37$ ).

### Study design

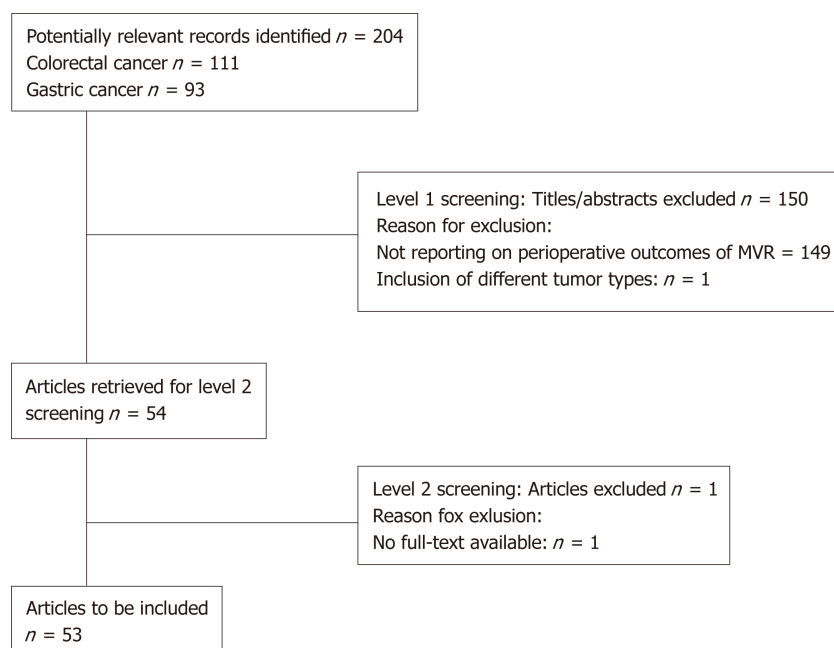
After full-text screening 37 studies were selected that met the inclusion criteria. Of these 37 included studies, 36 were retrospective.

### Demographics

In total 3112 patients underwent MVR for colon and rectal cancer (1095 for colon cancer, 1357 for rectal cancer and in 660 patient's origin of primary tumor was not specified (Table 1). Of the 36 studies ten included patients with recurrent colon and rectal cancer. The remainder dealt only with primary colon and rectal cancer. Included studies were published after 1999 to the present time and all but one was retrospective. In total five publications presented patient- and treatment-related data after minimally-invasive MVR. The decision for or against suspected MVR, according to preoperative imaging modalities like CT, MRI, EUS and PET-CT, was made intraoperatively. Every verified adhesion of the primary tumor to adjacent structures was classified as a cT4b -situation. All but seven publications did not report the true pT4b -rate. There were 17 studies that included patient with Stage IV disease. Another seven studies did not specify whether or not patients with metastatic disease were included.

### Pathological features

In the event of adhesion of adjacent structures to the primary tumor, these adhesions should definitely not be separated intraoperatively. For the surgeon it is not possible to distinguish between inflammatory and malignant adhesions. Hunter *et al*<sup>[5]</sup> showed that patients with adherent colon cancer and lysis of adhesion, had a local recurrence rate of 69% and a 5-year overall survival rate of only 23%. Of the included studies, 30 publications report the histopathologically confirmed malignant invasion rate. The true pT4b -rate varied from 23% to 77%. Three publications performed multivariate analysis in order to determine whether true malignant invasion into adjacent structures is of predictive value for overall- and progression-free survival<sup>[6-8]</sup>. Rosander *et al*<sup>[7]</sup> and Lehnert *et al*<sup>[8]</sup> did not find malignant invasion to be a predictive factor in multivariate analysis. Rosander *et al*<sup>[7]</sup> found female sex, adjuvant chemotherapy, low tumor stage and R0-resection to be associated with better overall survival. On the other hand, Lehnert *et al*<sup>[8]</sup> found intraoperative blood loss, age older



**Figure 1** Preferred reporting items for systematic reviews and meta-analyses diagram.

than 64 years and UICC stage to be predictive. Contrary to the aforementioned results Chen *et al*<sup>[6]</sup> found adhesion pattern (inflammatory *vs* malignant) to be highly significantly associated with reduced overall survival for both, colon and rectal cancer patients.

Concerning resection status, 27 studies report R0 rates, ranging from 65% to 100%. In the vast majority of publications R0 *vs* R1 -status was of significant prognostic impact (Table 2). Data show a trend towards decreased R0 -rates in patients undergoing MVR for recurrent cancers, especially rectal cancer. Nielsen *et al*<sup>[9]</sup>, Rottoli *et al*<sup>[10]</sup> and Vermaas *et al*<sup>[11]</sup> reported resection status in primary and recurrent rectal cancers and showed decreased R0 -rates for recurrent rectal cancer without being statistically significant (66% *vs* 38%; 71% *vs* 56% and 82% *vs* 58%).

### Morbidity and mortality

There was heterogeneity in reporting total complication rate, degree of complications and specification of different complications, so that the focus was set on complications, which were reported in the vast majority of publications. The post-operative morbidity rates ranged from 7%<sup>[12]</sup> to 76.6%<sup>[13]</sup>. Only one study reported that the occurrence of perioperative complications was an independent predictor of shorter overall survival (HR 3.53)<sup>[14]</sup>.

**Anastomotic insufficiency:** Twelve studies did not report occurrence of anastomotic insufficiency (AI). The remainder reported AI-rates ranging from 0.8%<sup>[15]</sup> to 19%<sup>[16]</sup>. There was no structured report on management of AI in the studies included.

**Surgical site infection:** Surgical site infections (SSI) were one of the most common complications ranging from 2.5%<sup>[15]</sup> to 53%<sup>[13]</sup>. The differentiation into superficial and deep SSI was inconsistently used in the studies included. Kumamoto *et al*<sup>[15]</sup> reported the lowest rate of SSI including 118 patients undergoing minimally-invasive MVR. The other studies, looking at minimal-invasive MVR, reported SSI -rates ranging from 12%-17%. The study by Kim *et al*<sup>[17]</sup> found no statistically significant difference in the occurrence of SSI between the open and the minimally-invasive group.

**Intraabdominal abscess:** Intraabdominal abscess (IAA) formation was not reported in 17 studies. The remainder reported IAA rates ranging from 1%<sup>[18]</sup> to 21%<sup>[19]</sup>. Documentation of IAA management was again inconsistently reported in the included studies.

**Re-operation:** The rate of necessary surgical re-intervention was again not reported in 17 studies. In the remaining studies the re-operation rate ranged from 0%<sup>[14]</sup> to 20%<sup>[19]</sup>.

**Mortality:** In total 15 studies reported mortality rates of 0% and the median mortality rate was 1.3%. The highest reported perioperative mortality rate, namely 10% was



**Table 1 Patient demographics**

Study/Yr	n	Disease	Site
Cukier <i>et al</i> <sup>[24]</sup> , 2012	33	Primary	Colon
Hallet <i>et al</i> <sup>[20]</sup> , 2014	15	Recurrent	Colon
Kumamoto <i>et al</i> <sup>[15]</sup> , 2017	118	Primary	Colon
Leijssen <i>et al</i> <sup>[2]</sup> , 2018	103	Primary	Colon
López-Cano <i>et al</i> <sup>[49]</sup> , 2010	113	Primary	Colon
Rosander <i>et al</i> <sup>[7]</sup> , 2018	121	Primary	Colon
Takahashi <i>et al</i> <sup>[12]</sup> , 2017	84	Primary	Colon
Tei <i>et al</i> <sup>[23]</sup> , 2018	29	Primary	Colon
Chen <i>et al</i> <sup>[6]</sup> , 2011	287; Colon (152); Rectum (135)	Primary recurrent	Colorectal
Eveno <i>et al</i> <sup>[58]</sup> , 2014	152; Colon (81); Rectum (71)	Primary	Colorectal
Fujisawa <i>et al</i> <sup>[29]</sup> , 2002	35; Colon (19); Rectum (17)	Primary recurrent	Colorectal
Hoffmann <i>et al</i> <sup>[21]</sup> , 2012	78; Colon (52); Rectum (26)	Primary	Colorectal
Gezen <i>et al</i> <sup>[18]</sup> , 2012	90; Colon (43); Rectum (47)	Primary	Colorectal
Kim <i>et al</i> <sup>[17]</sup> , 2012	54; Colon (32); Rectum (22)	Primary	Colorectal
Laurence <i>et al</i> <sup>[56]</sup> , 2017	660; Colon/Rectum not specified	Primary	Colorectal
Lehnert <i>et al</i> <sup>[8]</sup> , 2002	201; Colon (139); Rectum (62)	Primary	Colorectal
Li <i>et al</i> <sup>[16]</sup> , 2011	72; Colon (28); Rectum (44)	Primary	Colorectal
Park <i>et al</i> <sup>[53]</sup> , 2011	54; Colon (23); Rectum (31)	Primary	Colorectal
Rizzuto <i>et al</i> <sup>[57]</sup> , 2016	22; Colon (16); Rectum (6)	Primary	Colorectal
Winter <i>et al</i> <sup>[1]</sup> , 2007	63; Colon (46); Rectum (17)	Primary	Colorectal
Bannura <i>et al</i> <sup>[55]</sup> , 2006	30	Primary	Rectal
Crawshaw <i>et al</i> <sup>[25]</sup> , 2015	61	Primary recurrent	Rectal
Derici <i>et al</i> <sup>[48]</sup> , 2008	57	Primary	Rectal
Dinaux <i>et al</i> <sup>[50]</sup> , 2018	29	Primary	Rectal
Dosokey <i>et al</i> <sup>[30]</sup> , 2017	34	Primary	Rectal
Gannon <i>et al</i> <sup>[28]</sup> , 2007	72	Primary recurrent	Rectal
Harris <i>et al</i> <sup>[19]</sup> , 2011	42	Primary	Rectal
Ishiguro <i>et al</i> <sup>[54]</sup> , 2009	93	Primary	Rectal
Mañas <i>et al</i> <sup>[13]</sup> , 2014	30	Primary	Rectal
Nielsen <i>et al</i> <sup>[9]</sup> , 2012	90	Primary recurrent	Rectal
Pellino <i>et al</i> <sup>[14]</sup> , 2018	82	Primary	Rectal
Rottoli <i>et al</i> <sup>[10]</sup> , 2017	46	Primary recurrent	Rectal
Sanfilippo <i>et al</i> <sup>[51]</sup> , 2001	32	Primary	Rectal
Shin <i>et al</i> <sup>[22]</sup> , 2016	22	Primary	Rectal
Smith <i>et al</i> <sup>[47]</sup> , 2012	124	Primary	Rectal
Vermaas <i>et al</i> <sup>[11]</sup> , 2007	35	Primary recurrent	Rectal

reported in the study by Manas *et al*<sup>[13]</sup>.

### Long-term outcomes

**Table 3** shows overall (OS)- and disease-free survival (DFS) rates and depicts factors associated with decreased OS and DFS after MVR for rectal and colon cancers. 5-year OS rate ranged from 36.7%<sup>[13]</sup> to 90%<sup>[20]</sup>, but the proportion of included patients with metastatic disease differed between those two studies (20% *vs* 0%).

**Local and distant recurrences:** The local control rate expressed by the local recurrence rate were reported in 27 publications and ranged from 1.8% to 66.7%<sup>[15]</sup>. The aforementioned study and Rosander *et al*<sup>[7]</sup> showed higher rates of local recurrences after R1 -resection. Distant recurrence rates varied from 10.9%<sup>[2]</sup> to 45.5%<sup>[17]</sup>. Patients with metastatic disease, receiving MVR, were also included in the vast majority of publications and the rate of patients with Stage-IV disease varied from 0% to 49%<sup>[21]</sup>.

### Operative approach

**Laparoscopic *vs* open surgery:** Five publications focused on the perioperative and long-term results of minimally-invasive (laparoscopic and/or robotic) MVR (**Table 4**).

**Table 2 Patient- and treatment- associated parameters after multivisceral resection for colon and rectal cancers**

Study	Resection margin (R0 vs R1)	Local and distant recurrence	Most common resected organs	Lymph node involvement	Age	Blood loss(mL)	Pre-operative (Chemo)-radiation	Complications (AI; SSI; IAA) (Re-OP)	Prognostic factors/conclusions
Cukier <i>et al</i> <sup>[24]</sup>	R0: 100%	LR: 6%; DR: 18%	Small bowel (56%); Bladder/Ureter (54%)	N0: 79% N1: 21%	64	NR	RCTX:100%	6%; 18%; NR (9%)	No statistical difference in terms of disease-free survival when analyzing subgroups stratified by nodal-status ypN0 vs ypN1: ( $P = 0.29$ )
Hallet <i>et al</i> <sup>[20]</sup>	R0: 87%	LR: 13%; DR: 13%	Colon (87%) Small bowel (47%) Bladder (40%)	N0: 70% N1: 30%	60.2	1500	RCTX:100%	NR	Neoadjuvant RCTX for recurrent colon cancer is feasible; no addition of toxicity (radiation plus MVR)
Kumamoto <i>et al</i> <sup>[15]</sup>	R0: 95%	LR: R0: 1.8% R1: 66.7%; DR: NR	Small bowel (14%) Bladder (12%) Colorectum (11%)	N0: 62% N1: 28% N2: 10%	64	48	CTX: 4.4%	(0.8%; 2.5%; 0.8%) (0%)	R1-resection and N+ status predictors of poor prognosis Laparoscopic approach: Feasible, low conversion, low R1-rate
Leijssen <i>et al</i> <sup>[2]</sup>	R0: 89%	LR: 14.5%; DR: 10.9%	Small intestine (31%); Reproductive organs (9%); Bladder (7%)	NR	69	NR	NR	(1.8%; 3.6%; NR) (2%)	Patients with T4-cancer not undergoing MVR had a significantly poorer outcome regarding overall-, disease-free and cancer-specific survival
López-Cano <i>et al</i> <sup>[49]</sup>	R0: 85%	LR: 23%; DR: 19%	Small intestine (42%) Oophorectomy (28%) Bladder (19%)	N0: 35% N1: 32% N2: 34%	71	NR	0%	(NR; 10%; NR) (8%)	Poorly differentiated tumors and stage IV were associated with a poor survival; significant predictors of disease progression: Venous invasion (RR 2.34) and four or more positive lymph nodes (RR 3.99)

Rosander <i>et al</i> <sup>[7]</sup>	R0: 93%	LR: R0: 7% R1: 33% DR: 14%	Bowel (45%) Ovaries (24%) Bladder (partial/total): 22%/19% Uterus/Vagina (17%)	N0: 71% N1: 19% N2: 10%	67	NR	CTX: 27% RT: 1% RCTX: 5%	(8%; 7%; 7%) (14%)	Female sex, low tumor stage, and adjuvant CTX, and N - but not tumor infiltration per se, were independently associated with better overall survival
Takahashi <i>et al</i> <sup>[12]</sup>	R0: 96%	LR: 2%	Bowel (38%); Uterus/Ovaries (5%); Bladder (11%)	NR	68.5- 71.5	Lap. completion: 50; Conversion: 366; Lap overall: 57.5; open: 321	CTX: open: 25% lap: 6%	(4%; NR; NR) (NR)	Overall- and disease-free survival (multivariate) was shorter in the males; operative approach did not affect overall- and disease-free survival
Tei <i>et al</i> <sup>[23]</sup>	R0: 93%-100%	LR: NR; DR: 24%	Small intestine (38%); Bladder (17%); Ovaries (14%)	N0: 48% N1: 24% N2: 28%	70	60-220	NR	(3%; 17%; 10%) (3%)	S-MVR and M-MVR do not differ significantly in terms of blood loss, operative time and number of harvested lymph nodes. No difference in occurrence of complications
Chen <i>et al</i> <sup>[6]</sup>	NR	NR	Colon cancer: small bowel (40%); Rectal cancer: Bladder (36%)	NR	NR	NR	NR	NR	Multivariate analysis showed that adhesion pattern was independently associated with overall survival among both colon ( $P = 0.00001$ ) and rectal ( $P = 0.0002$ ) cancer patients
Eveno <i>et al</i> <sup>[58]</sup>	R0: 90%	NR	Vagina (25%); Small bowel (23%); Bladder (20%); Ovaries/Uterus (each 19%)	N0: 55% N1: 25% N2: 19%	63	NR	RT: 8%; CT: 2%; RCTX: 27%	(3%, 4%; NR) (9%)	Patients with resection of multiple organs had a better survival rate than patients with single organ resection ( $P = 0.0469$ )
Fujisawa <i>et al</i> <sup>[29]</sup>	NR	NR	Bladder (partial/total): 54%/34%	NR	59	NR	0%	NR	Complication rate was higher in pat; undergoing cystectomy <i>vs</i> partial cystectomy (58.3% <i>vs</i> 10.5%)

Hoffmann <i>et al</i> <sup>[21]</sup>	R0: 95%	LR: 2%	53%: 1 add. Organ 27%: 2 add; organs	NR	69	NR	RCTX (rectal): 35%	(9%; 9%; NR) (19%)	No significant differences in overall survival: Colon <i>vs</i> rectal cancer ( <i>P</i> = 0.839); lap <i>vs</i> open ( <i>P</i> = 0.610); emergency <i>vs</i> planned ( <i>P</i> = 0.674), pN0 <i>vs</i> pN1 ( <i>P</i> = 0.658)
Gezen <i>et al</i> <sup>[18]</sup>	R0: 91%	NR	Ovaries: 27%; Bladder: 26%; Small bowel: 21%; Uterus: 19%	NR	59	450 (non-MVR: 250)	NR	(2%; 3%; 1%) (2%)	MVR do not alter the rates of sphincter-saving procedures, morbidity and 30-d mortality
Kim <i>et al</i> <sup>[17]</sup>	R0: 71%	LR: 7.7% (lap) and 27.3% (open) <i>P</i> = 0.144 DR: 15.4% (lap) <i>vs</i> 45.5% (open) <i>P</i> = 0.091	Small bowel: 10%; Bladder: 10%; Seminal vesicle: 13%; Prostate: 6%	NR	68	lap: 269; open: 638	RCTX: 50% of rectal cancer patients	(12%; 8%; NR) (NR)	No adverse long-term oncologic outcomes of laparoscopic MVR were observed
Laurence <i>et al</i> <sup>[56]</sup>	NR	NR	NR	NR	64	NR	RT: 62%	NR	Female gender, tumor grade 2, MVR were significant protective factors of mortality
Lehnert <i>et al</i> <sup>[8]</sup>	R0: 65% R1: 9% R2: 26%	LR: 7% DR: 13% Both: 4%	Small bowel: 29%; Bladder: 24%; Uterus: 13%	NR	64	< 1000 mL: 37%; 1000-2000 mL: 13%; > 2000 mL: 10%	RT/CT/RCT X: 40% of R0 resected patients	(5%; 9%; 1%) (5%)	Intraoperative blood loss, age older than 64 and UICC stage but not histologic tumor infiltration <i>vs</i> inflammation were prognostic factors
Li <i>et al</i> <sup>[16]</sup>	NR	LR at 5 years: 15% DR: 14%	Bladder (partial/total): 56%/19%	NR	67	Partial cystectomy: 0; Urologic reconstruction: 1700	0%	(19%; 25; 6%) (4%)	Negative prognostic factors: Age older than 70 years; receiving palliative resection and not involvement of the bladder dome
Park <i>et al</i> <sup>[53]</sup>	NR	NR	Small bowel: 24%; Ovary: 17%; Bladder: 14%	NR	64	NR	NR	(6%; 11%; 9%) (NR)	MVR was associated with a two times higher complications rate compared to standard resections
Rizzuto <i>et al</i> <sup>[57]</sup>	R0: 91%	NR	Small bowel: 36%; Bladder: 27%; Vagina/Uterus/Ovaries: Each 22%	N0: 50% N+: 50%	62	NR	RCTX: 28%	(11%; 14%; 5%) (NR)	Patients with rectal cancer and occlusive disease had worse prognosis

Winter <i>et al</i> <sup>[1]</sup>	R0: 89%	LR: 14%	Bladder (partial): 84%	N0: 65% N1: 35%	63	NR	RCTX: 37%	(3%; NR; NR) (NR)	Bladder reconstruction is achievable in most patients; margin- and node-negative patients benefit the most
Banamura <i>et al</i> <sup>[56]</sup>	NR	LR: 13%; DR: 23%; Both: 20%	APR: 30%; PPE: 70%	NR	57	NR	RCTX: 20%	(3%; 27%; NR) (NR)	PPE showed prolonged operative time, higher postoperative complications, a trend towards a poor prognosis in recurrence and survival
Crawshaw <i>et al</i> <sup>[25]</sup>	R0: 87%	LR: 16%	Bladder: 49%; Vagina: 38%; Prostate: 31%; Uterus: 31%; Ovaries: 20%; Small bowel: 10%	NR	62	800	RCTX: 90%	(NR; 7%; 12%) (NR)	Sphincter perseveration did not affect oncologic outcomes
Derici <i>et al</i> <sup>[48]</sup>	R0: 75%	LR: 18%	Adnexa: 47%; Uterus: 32%; Bladder: 30%	NR	60	NR	RCTX: 51%	(7%; 19%; NR) (NR)	Lymph node status pN0 ( $P = 0.007$ ) and R0 resection ( $P = 0.005$ ) were independently significant factors in the multivariate analysis for overall survival
Dinaux <i>et al</i> <sup>[50]</sup>	R0: 100%	LR: 3%; DR: 21%	Bladder: 28%; Prostate: 21%; Ovaries: 20%; Uterus: 20%	NR	55	NR	CTX: 100%; RCTX: 97%	(3%; 14%; 3%) (NR)	Chance of overall mortality significantly increased for patients; who underwent MVR, for administration of adjuvant CTX, for Pn+ and ypN+ status
Dosokey <i>et al</i> <sup>[30]</sup>	NR	LR: 3% DR: 11%	Vagina: 50%; Prostate: 30%; Bladder: 33%	NR	66	549	CTX: 97% RT: 92%	(16%; NR; NR) (NR)	Patients with APR only had a longer 5 yr overall survival and a longer disease-free survival compared to patients undergoing MVR



Gannon <i>et al</i> <sup>[28]</sup>	R0: 90%	Primary: LR: 9%, LR + DR: 13%, DR: 22%; Recurrent: LR: 4%, LR + DR: 48%, DR: 15%	TPE: 47% SLE: 47% PPE: 33%	NR	52	NR	RCTX: 85%	(NR; 4%; 11%) (4%)	A significant difference in 5-yr disease-free survival was found between primary and recurrent tumors (52% vs 13%, $P < 0.01$ )
Harris <i>et al</i> <sup>[19]</sup>	R0: 93%	LR: 7%	Bladder+ Prostate: 55% Uterus: 24%	N0: 52% N1: 29% N2: 17% N3: 2%	62	NR	RCTX: 74%	(5%; 5%; 21%) (20%)	Association with worse overall survival in multivariate analysis: Metastatic disease, pT4N1 stage, vascular invasion
Ishiguro <i>et al</i> <sup>[54]</sup>	R0: 98%	LR: 9% DR: 25%	Uterus+ Bladder+ Rectum: 89%	N0: 57% N+: 43%	55	NR	RCTX: 14%	(4%; 23%; 8%) (9%)	Patients with positive lateral pelvic lymph node had a higher probability to recur and a decreased 5-yr over all survival
Mañas <i>et al</i> <sup>[13]</sup>	R0: 73%	LR: 37% DR: 35%	Uterus/Ovaries (each): 53%; Vagina: 27%; Seminal vesicle: 23%	N0: 40% N1: 27% N2: 34%	68	NR	RCTX: 20%	(13%; 53%; 10%) (NR)	Multivariate analysis showed that nodal involvement was independent predictor of poor survival (> 4 pos; nodes RR: 9.06 ( $P = 0.006$ ))
Nielsen <i>et al</i> <sup>[9]</sup>	Primary: R0: 66% Recurrent: R0: 38%	NR	TPE with sacrectomy: 22%	NR	63	NR	RT: 65%	(4%; 20%; 7%) (NR)	There was no statistically significant difference in overall survival between primary and recurrent disease when comparing R0 resections
Pellino <i>et al</i> <sup>[14]</sup>	R0: 77%	LR: 16% DR: 22%	Not clearly specified	N0: 13% N1: 29% N2: 43%	62	NR	RT: 54%	(NR; 37%; 10%) (10%)	Perioperative complications were independent predictors of shorter survival (HR 3.53)

Rottoli <i>et al</i> <sup>[10]</sup>	Primary: R0 71%, Recurrent: R0: 56%	Primary: LR: 18% DR: 29% Both: 7%; Recurrent: LR: 22% DR: 33% Both: 17%	Sacrectomy: Primary: 18% Recurrent: 22%)	N0: 41% N1: 15% N2: 37%	57	Primary: 600 Recurrent: 750	65% (not specified)	NR	The long-term disease-free survival of patients undergoing pelvic exenteration is significantly worse when the procedure is performed for recurrent rectal cancer, regardless of the tumor involvement of the resection margins
Sanfilippo <i>et al</i> <sup>[51]</sup>	NR	LR: 20% DR: 44%	Vagina: 66%; Bladder/Prostate: 14%; Bladder/Vagina: 6%; Vagina/Uterus/Ovaries: 6%	N0: 72% N1: 9% N2: 9%	55	NR	RCTX: 100%	(NR; 19%; 6%) (9%)	No significant association with pelvic control rate and age, sex, cN-stage, tumor distance from the anal verge, clinical tumor length, tumor circumference, tumor mobility, obstruction, grade, neoadjuvant CTX, and MVR
Shin <i>et al</i> <sup>[22]</sup>	R0: 100%	LR: 4%	Prostate: 36%; Vagina: 23%; Small bowel: 14%; Bladder wall: 14%	N0: 41% N1: 46% N2: 14%	54	225	RCTX: 82%	(NR; 17%; 17%) (13%)	Robotic MVR including resection of lateral pelvic lymph nodes is feasible with acceptable morbidity and no conversion
Smith <i>et al</i> <sup>[47]</sup>	R0: 85%	LR: 19%	Vagina: 52%; Uterus: 23%; Bladder: 11%	N0: 60% N+: 40%	63	NR	RCTX: 73% RT: 2%	(6%; 19%; 6%) (at least 1%)	5-yr overall survival in stage I-III: Tumor category (T3-4 vs T0-2: HR 2.80), Node category (N1-2 vs N0: HR 1.75), Involved resection margin: HR = 2.19), lymphovascular invasion (L0 vs L1: HR 1.56)

Vermaas <i>et al</i> <sup>[11]</sup>	Primary: R0: 82%; Recurrent: R0: 58%	LR at 5-yr: Primary: 12%; Recurrent: 40%	TPE: 83% TPE an sacral bone: 11%; TPE with coccygeal bone: 6%	N0: 37% N1: 6% N2: 6%	58	NR	RT: 97%	(NR; 26%; NR) (9%)	Patients with recurrent rectal cancers have a higher rate of complications, a high distant metastasis rate and a poor overall survival
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CTX: Chemotherapy; MVR: Multivisceral resection; S-MVR: Single-port MVR; M-MVR: Multi-port MVR; HR: Hazard ratio; RR: Relative risk; APR: Abdominoperitoneal resection; PPE: Posterior pelvic exenteration; RCTX: Chemoradiotherapy; TPE: Total pelvic exenteration; LR: Local recurrence; DR: Distant recurrence; AI: Anastomotic insufficiency; SSI: Surgical site infections; IAA: Intraabdominal abscess; RT: Radiotherapy; NR: Not reported.

Completeness of surgical resection was not impaired by minimally-invasive MVR and the included studies showed no reduction in lymph -node harvest as compared to open surgery. The conversion rate to open surgery varied from 4.5%<sup>[22]</sup> to 33%<sup>[23]</sup>. The most common reasons for conversion were involvement of the small intestine, intraperitoneal adhesions and the need for urologic reconstructive procedures. The minimally-invasive approach offered a reduced length of stay, significantly reduced blood loss but prolonged operative time.

### Chemoradiotherapy

The number of patients receiving any kind of preoperative therapy, including chemotherapy, radiotherapy and combined chemoradiotherapy, was mentioned in 31 studies. Preoperative chemotherapy was received by 129 (4%) patients, 591 (19%) patients underwent preoperative radiotherapy and 423 (14%) patients were given preoperative combined chemoradiotherapy. Two studies reported on applications of chemoradiotherapy in primary and recurrent colon cancers<sup>[20,24]</sup>. Cukier *et al*<sup>[24]</sup> reported that perioperative complication rates were not negatively impacted by chemoradiotherapy. The same results were obtained by Hallet *et al*<sup>[20]</sup> who stated that the addition of neoadjuvant chemoradiotherapy prior to MVR for recurrent adherent colon cancer did not elevate toxicity-or complication rates.

Six studies reported on patients receiving intraoperative radiotherapy (IORT)<sup>[11,22,24-27]</sup>. All studies exclusively included patients with primary and or recurrent rectal cancer. Indications for application of IORT were a minimal circumferential free resection margin equal to or less than 2 mm in the study from Vermaas *et al*<sup>[11]</sup> and the concern for close and/or involved radial margins in the study by Gannon *et al*<sup>[28]</sup>. Only 12 patients in the study by Vermaas *et al*<sup>[11]</sup> received IORT but no improvement in overall survival was seen.

### Primary vs recurrent rectal cancer

In total seven publications included primary as well as recurrent rectal cancers<sup>[6,9-11,26,28,29]</sup>. The studies by Gannon *et al*<sup>[28]</sup> Nielsen *et al*<sup>[9]</sup> and Vermaas *et al*<sup>[11]</sup> included 197 patients and only Gannon *et al*<sup>[28]</sup> reported that the disease setting was the only significant prognostic factor in favor of primary rectal cancers. This is in line with the results published by Rottoli *et al*<sup>[10]</sup> who also found the recurrent disease setting to be a negative prognostic factor.

MVR for gastric cancer ( $n = 16$ ).

### Study design

A total of 93 articles were identified using the aforementioned search algorithm (Figure 1). After full-text screening 16 studies were selected that met the inclusion criteria.

### Demographics

We identified 16 studies published between 1998 and 2019 describing MVR for a total of 1600 patients with locally advanced gastric cancer (Table 5). One publication reported patient- and treatment-related data after minimally-invasive MVR, whereas the other authors either performed open surgery or did not mention whether an open or laparoscopic approach was chosen<sup>[31]</sup>. The decision for or against suspected MVR, according to preoperative imaging modalities like CT, MRI, EUS and PET-CT, was made intraoperatively. Every verified adhesion of the primary tumor to adjacent structures was classified as a cT4b -situation. Together with a gastrectomy, mainly surrounding organs like spleen, pancreas or colon were resected. More rarely, the gallbladder or parts of the small bowel or the liver had to be removed.

**Table 3** Morbidity, mortality and survival rates after multivisceral resection for colon and rectal cancer

Study	Follow-up (mo)	Morbidity (%)	Mortality (%)	Survival <sup>†</sup>	Stage IV disease (%)	True pT4b (%)
Cukier <i>et al</i> <sup>[24]</sup>	36	36	0	3-yr OS: 85.9%; 3-yr DFS: 73.7%	0	67
Hallet <i>et al</i> <sup>[20]</sup>	54	33.3	0	90%; 5-yr DFS: 63.5%	0	50
Kumamoto <i>et al</i> <sup>[15]</sup>	32	17.8	0.8	87%	12	45
Leijssen <i>et al</i> <sup>[2]</sup>	48.5	25	0	5-yr OS (pT3): 63%; 5-yr OS (pT4): 70%	0	24
López-Cano <i>et al</i> <sup>[49]</sup>	74.9	47.8	7.1	48%; 5-yr DFS: 46.3 mo	20	65
Rosander <i>et al</i> <sup>[7]</sup>	28	37% (≥ Grade III)	5	60.8% for the infiltration group; 86.9% for the inflammation group	0	63
Takahashi <i>et al</i> <sup>[12]</sup>	48.4	LAP: 7 OPEN: 36	0	3-ys OS (open): 79.8%; (lap): 92.8%	25	50
Tei <i>et al</i> <sup>[23]</sup>	34	37.9	0	3-yr OS Stage II-III (S-MVR/M-MVR): 81.8%/80.0% 3-yr DFS Stage II-III (S-MVR/M-MVR): 58.3%/70.0%	28	34
Chen <i>et al</i> <sup>[6]</sup>	NR	11.5	NR	59% (Colon/inflammation) 39% (Colon/invasion) 63% (Rectum/inflammation); 42% (Rectum/invasion)	54	55
Eveno <i>et al</i> <sup>[58]</sup>	48	12	1.3	77%; 3-yr OS (without stage IV disease): 89%; 5-yr DFS: 58%	13	65
Fujisawa <i>et al</i> <sup>[29]</sup>	42 (mean)	NR	NR	3-yr OS (colon/bladder sparing): 90%; (colon/nonsparing): 67%; 3 yr OS (rectal/bladder sparing): 50%; (rectal/nonsparing): 67%	NR	NR
Hoffmann <i>et al</i> <sup>[21]</sup>	NR	34.6	7.7	55% (if curative)	49	63
Gezen <i>et al</i> <sup>[18]</sup>	25 (mean)	24.4	4.4	69.4%	12	34
Kim <i>et al</i> <sup>[17]</sup>	35/40 (mean)	LAP: 21 OPEN: 44	0	LAP: 60.5%; OPEN 48%	33	44
Laurence <i>et al</i> <sup>[56]</sup>	NR	NR	NR	52.7%	3	NR
Lehnert <i>et al</i> <sup>[8]</sup>	71	33	7.5	51%	5	50
Li <i>et al</i> <sup>[16]</sup>	64.3	61	5.6	50%; 59%: if curative	21	47
Park <i>et al</i> <sup>[53]</sup>	NR	35.2	3.1	58%	0	44
Rizzuto <i>et al</i> <sup>[57]</sup>	NR	55	0	3-yr OS (non-occlusive): 58.4%; (occlusive): 33.3%	0	77
Winter <i>et al</i> <sup>[11]</sup>	84	18	1.5	57%; 61% (R0); 17% (R1) 77% (R0, N0); 28% (R0, N+)	NR	54
Banmura <i>et al</i> <sup>[56]</sup>	32	50	0	Local recurrence rate: 30%	33	63
Crawshaw <i>et al</i> <sup>[25]</sup>	27.8	57.4	0	49.2%; 5-yr DFS: 45.3%	0	39
Derici <i>et al</i> <sup>[48]</sup>	40.4 (mean)	38.6	3.5	49%; 3-yr OS: 81.6%	0	58
Dinaux <i>et al</i> <sup>[50]</sup>	38.2	72.4	0	OS: 45 mo	0	24
Dosokey <i>et al</i> <sup>[30]</sup>	32 (mean)	39	0	67%; 5-yr DFS: 79%	0	NR

Gannon <i>et al</i> <sup>[28]</sup>	40	43	0	48%; Primary: 65% Recurrent: 22%; 5-yr DFS: 38%; Primary: 52% Recurrent: 13%	NR	NR
Harris <i>et al</i> <sup>[19]</sup>	30	50	0	5-yr OS (R0): 48%; R1/R2: 33%	14	52
Ishiguro <i>et al</i> <sup>[54]</sup>	40	39.8	2.2	52%; 5-yr DFS: 46%	NR	49
Mañas <i>et al</i> <sup>[13]</sup>	28.8	76.6	10	36.7%	20	67
Nielsen <i>et al</i> <sup>[9]</sup>	12	51	2.2	5-yr OS (primary): 46%; (recurrent):17%	0	NR
Pellino <i>et al</i> <sup>[14]</sup>	NR	54.9	2.4	67%	NR	70
Rottoli <i>et al</i> <sup>[10]</sup>	32.5/56.6	33 Primary: 32% Recurrent: 33%	4	5-yr DFS (primary): 46% (recurrent): 24%	NR	NR
Sanfilippo <i>et al</i> <sup>[51]</sup>	NR	25	NR	4-yr OS: 69%	0	44
Shin <i>et al</i> <sup>[22]</sup>	30	41.7	0	80%	27	23
Smith <i>et al</i> <sup>[47]</sup>	NR	47.6	0.8	53.3%; M0: 59%	20	44
Vermaas <i>et al</i> <sup>[11]</sup>	28 (mean)	69; Primary: 61; Recurrent: 83	3	52% (primary); 3-yr OS (recurrent): 32%	NR	43

<sup>1</sup>if not specified 5-yr OS is reported. S-MVR: Single-port laparoscopic multivisceral resection; M-MVR: Multi-port laparoscopic multivisceral resection; NR: Not reported.

### Pathological features

Prior clinically suspected T4-tumor was confirmed in 14%<sup>[32]</sup>-89.0%<sup>[33]</sup> of histopathological samples. Involvement of lymph nodes was described in 38.8%<sup>[33]</sup>-89.3%<sup>[34]</sup> of patients.

### Morbidity and mortality

The rate of morbidity ranged from 11.8%<sup>[35]</sup> to 59.8%<sup>[31]</sup> of patients who underwent gastrectomy and MVR (Table 6). Main postoperative complications were pancreatic fistulas and pancreatitis, anastomotic leakage, cardiopulmonary events and post-operative bleedings. Total mortality lay between 0%<sup>[35]</sup> and 13.6%<sup>[33]</sup>. R0-resections were achieved in 38.4%<sup>[34]</sup>-100%<sup>[36]</sup> of patients.

**Anastomotic insufficiency:** Ten studies did not report the occurrence of anastomotic insufficiency (AI). The remainder reported AI -rates ranging from 0%<sup>[37,38]</sup> to 19.4%<sup>[31]</sup>. There was no structured report on management of AI in the studies included.

**Re-operation:** The rate of re-operation was only mentioned in 4 publications and ranged from 0%<sup>[37,38]</sup> to 13.8%<sup>[31]</sup>.

### Long-term outcomes

Patients after R0 resection had 5 year overall survival rates of 24.1%<sup>[38]</sup> to 37.8%<sup>[35]</sup>. In the multivariate analysis, mostly incomplete resection status<sup>[34,39-42]</sup> as well as lymph node involvement<sup>[31,34,36,39,40,42-45]</sup> were found to be negative prognostic factors for survival. Further negative prognostic factors were metastasized stage<sup>[35,39]</sup>, advanced age<sup>[44]</sup> the number of resected organs<sup>[31,42,44,46]</sup>, no adjuvant chemotherapy<sup>[31]</sup> and white race<sup>[31]</sup>.

## DISCUSSION

MVR for locally advanced and adherent colorectal and gastric cancers seems to be a feasible approach that is associated with an acceptable morbidity - and mortality -rate and in a subset of patients good oncologic long-term results can be obtained<sup>[15,20,25,42,44,47]</sup>. Due to the reduced sensitivity and specificity of preoperative imaging for prediction of true malignant adhesion, the decision in favor of performing MVR is made intraoperatively in the vast majority of cases<sup>[1]</sup>. It is virtually impossible for the surgeon to differentiate between inflammatory and true malignant adhesions, so that every adherence to the tumor must be considered malignant and the appropriate operative strategy has to be applied. Data on intraoperative lysis of adhesions to the primary tumor, which were proven malignant by histopathological examination, revealed devastating overall survival rates and high local recurrence rates (Hunter *et al*<sup>[5]</sup>). In this review the true pT4b -rate varied from 23% to 77% and data on the impact of malignant invasion are heterogeneous with two studies<sup>[7,8]</sup> reporting no impact on overall-survival if malignant adhesions were detected and one



**Table 4 Patient- and treatment- associated parameters of minimal-invasive multivisceral resection for colon and rectal cancer**

Study	Resection margin (R0 vs R1)	Lymph-node harvest (n)	Conversion rate	Reason for conversion	Blood loss (mL)	Operative time (min)	LOS (d)
Kumamoto <i>et al</i> <sup>[15]</sup>	R0: 95%	26	6.8%	Excessive tumor fixation (n = 4); Suspicion of invasion to the duodenum (n = 2); Intraoperative adhesion (n = 2)	49	254	11
Takahashi <i>et al</i> <sup>[12]</sup>	R0: 96%	34 Open: 33	12%	The conversion rate was highest in cases involving the urinary tract (40%)	50; Open: 321	279; Open: 255	14; Open: 22.5
Tei <i>et al</i> <sup>[23]</sup>	R0: S-MVR: 100%; M-MVR: 93%	S-MVR: 30; M-MVR: 25	S-MVR M-MVR: 14%; M-MVR Open: 33%	Small intestine involvement	S-MVR: 60; M-MVR: 220	S-MVR: 222; M-MVR: 255	S-MVR: 11; M-MVR: 18
Kim <i>et al</i> <sup>[17]</sup>	R0: 71%	34; Open: 40	7.9%	NR	268; Open: 637	330; Open: 257	21.9; Open: 21
Shin <i>et al</i> <sup>[22]</sup>	R0: 100%	20	4.5%	Unable to tolerate Trendelenburg position and intraoperative adhesions	225	421	4.5

LOS: Length of hospital stay; S-MVR: Single-port multivisceral resection; M-MVR: Multi-port MVR.

study reporting the opposite<sup>[6]</sup>. It seems it is not the presence of proven malignant infiltration into adherent adjacent organs but the presence other tumor- and treatment-associated factors that are of prognostic importance. This review emphasized the importance of microscopic complete surgical resection, as one of the most predictive factors for overall- and recurrence-free survival<sup>[15,48]</sup>. These results are further highlighted by the results presented by Nielsen *et al*<sup>[9]</sup> comparing primary and recurrent rectal cancers. The authors stated that no statistically significant difference in overall survival was seen regarding the disease setting when comparing R0-resections. The remaining studies dealing with primary versus recurrent rectal cancer found the disease setting to be of significant prognostic impact<sup>[10,28]</sup>. Patient selection for MVR in the recurrent disease setting should be made on a case-by-case basis, because achievement of R0 -resection in these patients can also produce acceptable long-term results. The intraoperative assessment of truly preventing an R1 -resection is virtually not possible, but nevertheless palliative MVR should not be performed as shown by the data from Leijssen *et al*<sup>[2]</sup>. Authors reported for patients with proven T4 -cancers not undergoing MVR the highest local recurrence rate, namely 21.5% (compared to patients undergoing MVR: 14.5%) and the worst 5-year OS-and DFS rates (46.3% *vs* 52.7% *vs* 70% and 74.1%, respectively).

Apart from the completeness of surgical resection factors like lymph -node and lymphovascular involvement seem to be predictive for survival. López-Cano *et al*<sup>[49]</sup>, Smith *et al*<sup>[47]</sup> and Harris *et al*<sup>[19]</sup> showed that lymphatic spread was associated with worse prognosis. Cukier *et al*<sup>[24]</sup> and Dinaux *et al*<sup>[50]</sup> discussed the significance of the ypN -stage. Cukier *et al*<sup>[24]</sup> reported no statistical difference in terms of DFS when comparing ypN0 and ypN1 patients. Contrarily, Dinaux *et al*<sup>[50]</sup> showed that ypN+ status was significantly associated with overall mortality. Hoffmann *et al*<sup>[21]</sup> found no difference in terms of OS for pN0 versus pN1 patients after MVR for primary colorectal cancers.

The role of neoadjuvant and adjuvant chemo- (radio-) therapy in short- and long-term results was hardly assessable due to the heterogeneity of data provided. The study by Sanfilippo *et al*<sup>[26]</sup> showed no significant association between application of neoadjuvant chemotherapy and local pelvic control rate. Dinaux *et al*<sup>[50]</sup> even found the performance of adjuvant chemotherapy to be significantly associated with overall mortality.

The significance of minimally-invasive MVR was highlighted in a couple of studies (Table 4). The laparoscopic approach for standard -resections for colon - and gastric cancer has already become accepted with low morbidity rates and comparable oncologic long-term results. The acceptance of laparoscopic or robotic MVR is low but the minimally-invasive approach seems to harbor some advantages over the open

**Table 5 Patient- and treatment- associated parameters after multivisceral resection for gastric cancer**

Study	Resection margin (R0 vs R1)	Most common resected organs	Lymph node involvement	Age	Blood transfusion	Complications (AI) (Re-OP)	Other prognostic factors
Carboni <i>et al</i> <sup>[39]</sup> , 2005	R0 61.5%; R1 27.7%; R2 10.8%	Spleen: 48%; Pancreas: 43%; Colon: 25%	86.2%	61	NR	(1.5%) (1.5%)	Lymph-node involvement and metastatic disease
Colen <i>et al</i> <sup>[37]</sup> , 2004	NR	Spleen: 62%; Pancreas 57%; Colon: 24%	NR	67.5	NR	0% (NR)	NR
D'Amato <i>et al</i> <sup>[38]</sup> , 2004	R0: 69%	Pancreas: 62%; Colon: 12%	NR	NR	NR	(0%) (NR)	NR
Jeong <i>et al</i> <sup>[43]</sup> , 2009	R0: 78.3%; R+: 21.7%	Spleen: 47%; Pancreas: 61%; Colon: 24%	N+: 90.1%	59	NR	(6.7%) (11%)	Lymph-node and lymphovascular involvement
Kim <i>et al</i> <sup>[35]</sup> , 2009	R0: 43%; R1: 15%; R2: 74%	Spleen: 38%; Pancreas: 29%; Colon: 56%	NR	NR	NR	(2.9%) (0%)	histologic type, M stage, peritoneal metastasis, curability and treatment groups
Martin <i>et al</i> <sup>[36]</sup> , 2002	R0: 100%	Spleen: 67%; Pancreas: 19%; Colon: 6%; Liver: 4% Gallbladder: 7%	N0: 35% N+: 65%	66	NR	(NR) (NR)	Lymph-node involvement and > pT3
Oñate-Ocaña <i>et al</i> <sup>[32]</sup> , 2008	R0: 58.1%; R1: 18.9%; R2: 23%	Spleen: 68%; Pancreas: 26%; Colon: 12%; Liver: 9%	NR	NR	NR	(NR) (NR)	NR
Ozer <i>et al</i> <sup>[44]</sup> , 2009	NR	Pancreas: 54%; Colon: 32%; Liver: 18%	NR	58	NR	(8.9%) (NR)	Advanced age, lymph node involvement, and resection of more than 1 additional organ were significant prognostic factors for survival.
Persiani <i>et al</i> <sup>[46]</sup> , 2008	R0: 320; R1: 39; R2: 29%	Spleen: 84%; Pancreas: 25%; Colon: 10%	NR	63.4	NR	(NR) (NR)	Splenectomy, D2 lymphadenectomy, and age greater than 64 yr were the only factors predictive of overall morbidity
Shchepotin <i>et al</i> <sup>[33]</sup> , 1998	NR	Spleen: 43%; Pancreas: 69%; Colon: 45% Liver: 29%	N+: 38.8%	NR	NR	(3.7%) (NR)	NR
Isozaki <i>et al</i> <sup>[45]</sup> , 2000	NR	Pancreas + Spleen: 36%; Pancreatoduodenectomy: 7%	N0 = 13%; N1 = 36%; N2 = 25%; N3 = 12%	NR	NR	(NR) (NR)	Location of the tumor, lymph node metastasis, histological depth of invasion, and extent of lymph node dissection
Molina <i>et al</i> <sup>[40]</sup> , 2019	R0: 94%	Pancreas (49%); Spleen (34%) Liver (29%).	N+: 80%	64,5	NR	(NR) (NR)	Lymph-node involvement and R1-status
Mita <i>et al</i> <sup>[42]</sup> , 2017	R0: 82.5%; R1: 17.5%	Spleen 29.1%; Pancreas: 46.6%; Colon: 13.6%; Liver: 11.7%	N+: 84.5%	70	NR	(NR) (NR)	Resection status
Vladov <i>et al</i> <sup>[38]</sup> , 2015	R0: 75%	Spleen: 76.7%; Pancreas: 40%; Colon: 18.3%; Liver 15%	NR	NR	NR	(NR) (NR)	NR

Tran <i>et al</i> <sup>[31]</sup> , 2015	R1: 15.5	Spleen: 48%; Pancreas: 27% Liver 14% Colon: 13%	N0: 34.5%	64	NR	(11.5%) (13.8%)	MVR with pancreatectomy, was significantly associated with decreased survival, along with T-stage, N stage, perineural invasion, and
Pacelli <i>et al</i> <sup>[34]</sup> , 2013	R0: 38.4%	Pancreas 46; Colon 43	N+: 89.3%	NR	NR	(7%) (NR)	Lymph-node involvement and incomplete resection

MVR: Multivisceral resection; NR: Not reported; AI: Anastomotic insufficiency.

approach. Table 4 sums up the most important studies, highlighting the fact that minimally-invasive MVR is associated with a reduced operative time, reduced blood loss and transfusion requirement. The conversion rates were low by a comparable lymph-node harvest. Prior to scheduling patients for minimal-invasive MVR, relative contraindications like excessive small bowel- and urologic tract involvement should receive attention.

Our analysis of the so far published results of MVR for patients with locally advanced gastric cancer shows 5-year survival rates of 24.1%-37.8% for patients with an R0-resection, while the rate of morbidity was 11.8% to 59.8% and the rate of mortality 0-15%. The authors of these studies therefore consider MVR for locally advanced gastric cancer to be a potentially beneficial procedure, especially if there is a possibility of curative resection.

Comparable results can also be found for MVR of other abdominal tumor entities such as neuroendocrine tumors or gastrointestinal stroma tumors<sup>[51]</sup>. Similar approaches were also investigated for locally advanced pancreatic adenocarcinoma and colorectal cancer. With the acceptance of higher rates of morbidity and longer operating times MVR for locally advanced pancreatic adenocarcinoma may lead to a long-term survival comparable to that for standard resections of the pancreas<sup>[52]</sup>.

In conclusion, the main limitation of this review is the mainly retrospective studies included and the heterogeneity in reporting short- and long-term outcomes. Nevertheless, MVR for primary cancers are of significant importance in oncologic surgery providing acceptable morbidity- and mortality rates with good long-term survival for selected patients. Negative selection criteria are incomplete surgical resection, recurrent rectal cancer, and lymph-node and lymphovascular involvement. Stage-IV disease should be regarded as a relative contraindication for MVR.

**Table 6** Morbidity, mortality and survival rates after multivisceral resection for gastric cancer

Study	n	Follow-up (mo)	Morbidity (%)	Mortality (%)	Survival	Stage IV (%)	True pT4b (%)
Carboni <i>et al</i> <sup>[39]</sup> , 2005	65	13	27.7	12.3	OS: 21.8 mo	46	80
Colen <i>et al</i> <sup>[37]</sup> , 2004	21	NR	39	10	OS: 30 mo	NR	38
D'Amato <i>et al</i> <sup>[38]</sup> , 2004	52	NR	34.6	1.9	OS: 31 mo	NR	NR
Jeong <i>et al</i> <sup>[43]</sup> , 2009	71	17.6	26.8	NR	3-yr OS: 36.4%	76	63
Kim <i>et al</i> <sup>[35]</sup> , 2009	34	NR	11.8	0	OS: 37.8 mo	38	NR
Martin <i>et al</i> <sup>[36]</sup> , 2002	268	NR	39.2	NR	OS: 63 mo	NR	21
Oñate-Ocaña <i>et al</i> <sup>[32]</sup> , 2008	74	NR	26.9	NR	OS: 30.5 mo	NR	14-38
Ozer <i>et al</i> <sup>[44]</sup> , 2009	56	10.8	37.5	12.5	3-yr OS: 53.3%	62	66
Persiani <i>et al</i> <sup>[46]</sup> , 2008	51	NR	16.2	2.3	NR	79	19.6
Shchepotin <i>et al</i> <sup>[33]</sup> , 1998	353	NR	31.2	13.6	5-yr OS: 25%	NR	89.0
Isozaki <i>et al</i> <sup>[45]</sup> , 2000	86	NR	NR	NR	5-yr OS: 35%	NR	53
Molina <i>et al</i> <sup>[40]</sup> , 2019	35	31	46	3	5-yr OS: 34%	NR	40
Mita <i>et al</i> <sup>[42]</sup> , 2017	103	23.0	37.9	1.0	3-yr OS: 42.1%	0	57
Vladov <i>et al</i> <sup>[38]</sup> , 2015	60	NR	28.3	6.7	5-yr OS: 24.1%	NR	70
Tran <i>et al</i> <sup>[31]</sup> , 2015	159	NR	59.8	4.3	5-yr OS: MVR with pancreatectomy: 20%; MVR without: 36%	0	67
Pacelli <i>et al</i> <sup>[34]</sup> , 2013	112	18.7	33.9	3.6	5-yr OS: 27.2%	NR	88

OS: Overall survival; NR: Not reported; MVR: Multivisceral resection.

## ARTICLE HIGHLIGHTS

### Research background

Multivisceral resections (MVR) still constitute a challenge for the interdisciplinary team. The indications to perform MVR are not clearly defined.

### Research motivation

Motivation was generated by the fact that there are no recommendations regarding MVR.

### Research objectives

In order to define indications and factors associated with beneficial oncologic outcomes and reduced perioperative morbidity and mortality this systematic review was conducted.

### Research methods

We performed a PubMed-search from 2000 to 2018 including articles reporting on MVR in patients with colon-, rectal- and gastric cancer.

### Research results

Available data shows that MVR from locally advanced colorectal and gastric cancer is a feasible option which is associated with acceptable morbidity- and mortality-rates. Oncologic outcome is favorable when clear resection margins can be obtained.

### Research conclusions

Patients who are clinically fit and preoperative imaging does not reveal obvious contraindication for radical surgery, the option of MVR should not be abandoned. Clear resection margins are the main goal of aggressive surgical approach.

## Research perspectives

Perspectives are to evaluate more patient- and treatment-specific parameters in order to define more clearly patients who are likely to benefit from this approach.

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# Relationship between perioperative anaemia and outcomes in older people with hip fractures: A systematic review and meta-analysis protocol

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## Abstract

### BACKGROUND

Hip fractures are common with increasing age and is associated with decline in mobility. Both the fracture and the surgery can lead to blood loss, resulting in anaemia. However, it is uncertain at which time point haemoglobin is most strongly associated with different clinical outcomes after hip fracture. Our hypothesis is perioperative anaemia (admission, postoperative and discharge) during hip fracture surgery is associated with poor clinical outcomes.

### AIM

To determine the effects of perioperative anaemia during hip fracture surgery on mortality, functional status and other clinical outcomes.

### METHODS

Electronic databases will be searched to identify studies evaluating perioperative anaemia and outcomes of hip fracture surgery. Reference lists of included studies will also be searched to identify additional published studies. Eligibility criteria are as follows: Population: People who underwent hip fracture surgery; Exposure: Perioperative anaemia; Comparison: No anaemia before or after hip fracture surgery; Outcome: Mortality, hospital length of stay, postoperative complications, hospital readmission, change of discharge destination, quality of

supplementary file.

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life and functional status. Risk of bias assessment will be assessed using the Cochrane Collaboration's tool for randomized controlled trials and the modified version of the Epidemiological Appraisal Instrument for observational studies. Data will be pooled for meta-analysis if deemed appropriate.

## CONCLUSION

This review seeks to clarify outcomes associated with perioperative anaemia at various time-points around hip fracture surgery. These findings will potentially inform evidence-based clinical practice on interventions in those with anaemia.

**Key words:** Anaemia; Haemoglobin; Hip fracture; Length of stay; Mortality; Outcomes; Perioperative; Readmission

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**Core tip:** Hip fracture is a growing public health problem because of population aging. Recovery from hip fracture can be slow and complicated by morbidities and decline in functional abilities. Perioperative anaemia is common with hip fractures. However, it is uncertain at which time point haemoglobin level is most strongly associated with different clinical outcomes after hip fracture surgery. Better understanding of the relationship between perioperative haemoglobin and mortality, length of hospital stay, functional status, postoperative complications, hospital readmission and admission to residential care after discharge, is required.

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## INTRODUCTION

Mobility is vital to older people, especially for maintaining functional independence and good quality of life. Older people have identified that living in their own home as long as possible is a priority for them. However, sustaining a hip fracture is a serious life-changing event for many older people, which disrupts their ability to walk.

Hip fractures in the older population are associated with adverse outcomes which may include prolonged hospitalisation, decline in functional status, long-term institutionalisation and excessive mortality<sup>[1-3]</sup>. For example, less than 50% of patients regain their prior level of mobility at one year after hip fracture, and nearly 20% become immobile<sup>[4]</sup>. The loss of independence after hip fractures result in older people needing long-term residential care. In a meta-analysis of seventy-five studies involving more than 64000 subjects from multiple countries, the overall mortality at one year was 24.5% and this increased to 34.5% at 2 years<sup>[5]</sup>. Therefore, the healthcare burden of hip fractures is significant and strategies are needed to mitigate these adverse outcomes.

One potential way to improve outcomes after hip fractures is to better manage anaemia in patients with hip fractures. Hip fractures are associated with significant blood loss, either from the fracture itself or from the surgery to repair it<sup>[6]</sup>. In the general population, anaemia is present in 10% of women and 11% of men over the age of 65 years<sup>[7]</sup>. This prevalence of anaemia is higher in older people who had hip fractures. It is present in approximately 50% at the time of hospital admission<sup>[8]</sup>, increasing to more than 90% following hip fracture surgery<sup>[8]</sup>. Anaemia, independent of other health conditions, places older people at risk of adverse health outcomes. The increased risk of mortality among those with anaemia is well documented<sup>[9,10]</sup>. However, there is conflicting data about whether anaemia is an independent risk factor for poor postoperative outcome or a marker of severity of comorbid diseases in patients with hip fractures<sup>[9,11]</sup>.

Preoperative anaemia is recognised as a risk factor for mortality, longer length of stay and poorer functional status after hip fracture surgery<sup>[12]</sup>. It is also recognised as one of the most important risk factor for blood transfusions<sup>[12,13]</sup>. In a systematic

review published in 2015, preoperative anaemia was associated with a 64% increase in risk of mortality after hip fractures<sup>[14]</sup>. One of the limitations of this systematic review is that several studies published after 2015 were not included.

In a few studies, the effects of postoperative haemoglobin on clinical outcomes have shown mixed results<sup>[15,16]</sup>. In addition, little is known about the effects of anaemia prior to hospital discharge on outcomes. To date, only a small number of studies have examined the association between anaemia on discharge with outcomes<sup>[8,17,18]</sup>. Therefore, a more robust review is required to evaluate the relationship between perioperative anaemia and clinical outcomes in hip fracture surgery.

The primary aim of this systematic review is to determine the relationship between perioperative (preoperative, postoperative and discharge) anaemia and mortality after hip fracture surgery. Secondary aims are to evaluate the relationship between perioperative anaemia and other clinical outcomes such as hospital length of stay, postoperative complications, hospital readmission, rate of permanent transfer to residential care after discharge, and functional status in terms of mobility or disability.

## MATERIALS AND METHODS

This systematic review and meta-analysis will be performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocol (PRISMA-P) statement<sup>[19]</sup>.

### Definitions

Anaemia refers to a reduced number of circulating red blood cells and is usually based on haemoglobin measurements. Anaemia can occur at various time point of the fracture, either preoperatively (prior to surgery) or postoperatively (up to 7 days after surgery). There is also interest in haemoglobin just before discharge following the index surgery. These measurements are collectively referred to as perioperative haemoglobin.

### Search strategy

We will search for relevant articles in the English language using MEDLINE/PubMed, CINAHL, SCOPUS, EMBASE databases and Cochrane Library from inception until August 2018. The search strategy is provided in [Table 1](#). We will perform a manual search of additional references of articles.

### Eligibility criteria

**Population:** The population of interest is people with hip fractures undergoing surgery. Examples of surgery for hip fractures include sliding hip screw, intramedullary nail and arthroplasty.

**Exposure:** Studies evaluating the effects of perioperative anaemia which are defined as (1) at the time of admission or before surgery; (2) after surgery (within 7 days); and (3) prior to hospital discharge (as defined by the authors) will be included. Anaemia may be defined according to the World Health Organization criteria as haemoglobin concentration less than 120 g/L for women and less than 130 g/L for men<sup>[20]</sup>. For the purpose of this review, moderate and severe anaemia were defined as haemoglobin of 80-100 g/L and less than 80 g/L, respectively, for both sexes.

**Comparator/control:** Participants who had hip fracture surgery without anaemia, at different time points.

**Outcomes:** This review will consider studies that include any of the following outcomes (1) mortality up to 12 months post-surgery; (2) hospital length of stay; (3) postoperative complications; (4) hospital readmission; (5) rate of permanent transfer to residential care after discharge; (6) quality of life; (7) mobility or disability.

**Study design:** All peer-reviewed full-text studies or doctoral dissertations are eligible for initial review. Observational studies designed as longitudinal cohorts, case-control or cross-sectional studies and experimental studies designed as randomized controlled or non-randomized trials will be eligible for inclusion in this review.

**Exclusion:** This study will exclude case series, case reports and studies published in a language other than English. Studies reporting outcomes of cohorts with (1) acetabulum and fractures of the femoral shaft distal to the subtrochanteric region, (2) high-energy traumatic fracture; (3) pathological fracture and (4) non-surgical management of hip fracture will also be excluded.

### Study selection process

Initially, two reviewers (KSK, MWK) will screen the titles and abstracts of all search records independently. After screening, full texts of all potentially eligible studies will be retrieved and examined according to the abovementioned eligibility criteria.



Table 1 Search syntaxes

Database	Search syntax
PubMed	("anaemia" [All Fields] OR "anaemia" [MeSH Terms] OR "haemoglobin" [All Fields]) OR ("haemoglobin" [MeSH Terms] AND ("hip fractures" [MeSH Terms] OR "hip" [All Fields] AND "fractures" [All Fields]))
CINAHL	"hip fracture" AND (anaemia or haemoglobin)
Embase	"hip fracture" and (anaemia or haemoglobin)
Scopus	Hip fracture AND (anaemia or haemoglobin)

Disagreements at both screening levels (title/abstract and full text) will be adjudicated by a third reviewer (SY). A PRISMA-P flow chart will outline the study selection process and reasons for exclusion.

### Data extraction

Data will be extracted by two independent reviewers (KSK, MWK) using a standard data abstraction form (Supplementary material). After determination of the study eligibility, information will be extracted from each study regarding study identification (first author, year of publication, number and location where recruitment took place), study design characteristics (sample size, follow-up duration, inclusion and exclusion criteria, quality assessments), patient population (age, gender, medical comorbidities) and haemoglobin levels (before, after surgery or prior to discharge). Data on the following outcomes will be recorded: mortality up to 12 mo, hospital length of stay, postoperative complications, hospital readmission up to 12 mo, rate of permanent transfer to residential care after discharge, quality of life, mobility or disability.

### Quality assessment

The quality assessment for all studies will be assessed independently by two reviewers (KSK, MWK). The Cochrane Collaboration's tool will be used for assessing risk of bias among RCT studies<sup>[21]</sup>. This tool addresses six domains of bias: (1) Sequence generation; (2) Allocation concealment; (3) Blinding of personnel and participants; (4) Completeness of data; (5) Selective reporting; and (6) Other source of bias not covered in the other domains. Based on empirical and theoretical considerations, RCTs with inadequate random sequence generation, allocation concealment, incomplete outcome data, selective reporting, or with other sources of bias will be considered as high risk of bias<sup>[21]</sup>. When sufficient information was not provided on these three domains of bias to allow a definite judgement, we will consider the risk of bias as unclear. When a study is potentially free of these biases, we will consider the risk as low.

The quality of observational studies will be assessed using the Epidemiological Appraisal Instrument (EAI)<sup>[22]</sup>, a validated and reliable tool. This instrument addresses five domains of bias risk: Reporting, subject selection, measurement quality, data analysis, and generalisation of results. Each of the 43 questions in the EAI was scored as yes (= 2), partial (= 1), no or unable to determine (= 0) with the highest possible score being 86. Each total score will be stratified by quartiles. Quartile 1 (Q1) will be 70-86 (the highest quality), quartile 2 (Q2) will be 46-69, quartile 3 (Q3) will be 24-45 and quartile 4 (Q4) will be 0-23 (the lowest quality). Any disagreement regarding the quality of a study will be resolved by a third reviewer (SY).

### Data synthesis

Detailed description of all included studies will be tabulated. Study identification (first author, year of publication, number and location where recruitment occurred), study design and characteristics (observational or experimental, sample size, duration of follow-up), patient population (age, gender), haemoglobin at different time points and clinical outcomes (mortality at different time points, hospital length of stay, hospital readmission at different time points, postoperative complications, rate of admission to residential care after discharge, quality of life, mobility or ability to perform activities of daily living) will be qualitatively described.

### Statistical analysis

We will use RevMan 5.3 to conduct the meta-analyses. Meta-analyses of pooled data will not be performed for secondary outcomes or when the number of studies were small or highly heterogenous. The summary effect measures may include hazard ratios (HR), relative risk (RR) or odds ratios (OR). When data are available to be

pooled together, we will use a random-effects model to estimate of effect size. Where possible, we will aggregate each included study's outcome data as HR, RR, or OR with the associated 95%CI as these are assumed to measure the same underlying effect<sup>[23]</sup>. When the effect size estimate was not reported in the paper, the RR or OR and associated 95%CI will be calculated using the raw data available. In the first instance, the unadjusted effect sizes for each outcome (permitting age and sex adjustment) will be pooled together. In the second instance, the unadjusted and most adjusted effect sizes for each outcome will be pooled together.

## RESULTS

### *Heterogeneity and publication bias*

Heterogeneity among included studies will be evaluated using the  $I^2$  statistic, which will describe the proportion of variability in effect size estimates that is due to the difference between studies rather than by chance<sup>[21]</sup>. According to the Cochrane Handbook for Systematic Reviews<sup>[24]</sup>,  $I^2$  of 0% to 60% can be considered as not important to moderate, while  $I^2 > 60\%$  indicates substantial heterogeneity.

Funnel plots will be used to assess for any publication bias (eyeball test). Egger's test will be used to identify any funnel plot asymmetry arising from publication bias if present<sup>[24]</sup>.

## DISCUSSION

This systematic review aims to add to the existing literature by aggregating data on specific outcomes after hip fracture surgery in relation to perioperative anaemia. Our review is broader in scope and considers many more clinical outcomes compared with previous systematic reviews that have predominantly focused on postoperative mortality. Understanding the relationship between perioperative anaemia in hip fracture surgery and clinical outcomes is important from a clinical perspective because clinicians find it challenging to know when to transfuse with RBC. Therefore, this review will inform the evidence-based recommendations for this area of clinical practice.

It is common when undertaking such reviews and meta-analysis that gaps in methodology will be identified and as part of quality improvement, strategies to address these design gaps will be identified. Additionally, areas where knowledge gaps remain may be identified to guide future research directions for the benefit of the clinical care of people with hip fractures.

It can be hypothesized that this review will encounter several limitations. This review may not be able to generalize the findings because studies may potentially define anaemia by different haemoglobin cut-offs leading to variation in interpretation. Therefore, the proposed review may be limited by the pooling together of perioperative anaemia studies with varying levels of validity and heterogeneity. Another limitation concerns the length of stay and functional status endpoints, and it is possible that different studies may have utilized different methods to determine these outcomes, each with varying levels of validity.

In conclusion, given that the links between anaemia and clinical outcomes at different time-points before or after hip fracture surgery are complex and the lack of a comprehensive systematic review in this area, the proposed review will help to provide a summary of the available evidence. These findings will assist the development of future clinical practice and policy in this field.

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## ARTICLE HIGHLIGHTS

### *Research background*

Perioperative anaemia in hip fracture is a common problem that can influence clinical outcomes. However, it is uncertain which outcomes will be affected and if anaemia before or after surgery will have different effects.

### Research motivation

A better understanding of how perioperative anaemia influences clinical outcomes after hip fracture surgery will help to develop more timely interventions.

### Research objectives

To determine the effects of perioperative anaemia during hip fracture surgery on mortality, hospital length of stay, postoperative complications, hospital readmission, change of discharge destination, quality of life and functional status.

### Research methods

Electronic databases will be searched for studies evaluating perioperative anaemia and outcomes of hip fracture surgery. Data on study characteristics, patient demographics, timing of anaemia and clinical outcomes will be extracted. Comparison will be made between participants with anaemia and those without. Data will be pooled for meta-analysis for the primary outcome.

### Research conclusions

This systematic review seeks to clarify the outcomes associated with perioperative anaemia at various time-points among patients who had hip fracture surgery. An evaluation of the outcomes associated with perioperative anaemia in hip fracture surgery will potentially inform evidence-based clinical practice on the effectiveness and timing of interventions in those with reduced haemoglobin.

### Research perspectives

In presence of small studies evaluating perioperative anaemia among older people having hip fracture surgery, a systematic review and meta-analysis will provide important directions for future research and clinical practice in this field. This protocol will provide an important methodological foundation for the systematic review.

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# Treatment options for rumination syndrome: A systematic review

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## Abstract

### BACKGROUND

Rumination syndrome (RS) is characterized by recurrent effortless postprandial regurgitation of recently ingested food from the stomach to the oral cavity and has been associated with quality of life impairment and malnutrition. There is a general lack of consensus on the most appropriate treatment options for RS.

### AIM

To summarize the literature on treatment options for RS.

### METHODS

We conducted a systematic review according to PRISMA guidelines. We searched Medline (1946 to February 2019), EMBASE (1947 to February 2019), PsycINFO (1806 to February 2019) and Cochrane central register of controlled trials for articles discussing treatment options for adult patients (> 18 years) with RS. All relevant articles were accessed in full text. We extracted data on study designs, patient profiles, duration of symptoms, follow up periods, date, diagnostic criteria, interventions and outcomes. Risk of bias assessment was carried out independently by 3 reviewers *via* Cochrane Risk of Bias tool and Newcastle Ottawa Scale for randomized controlled trials and Cohort studies respectively.

### RESULTS

Twelve articles were identified. A total of 254 patients were included in the analysis, with a mean age of 36.1 (range 18-89). 185 patients (72.8%) were females. 5 studies looked into behavioral therapies, primarily diaphragmatic breathing (DB) 2 studies looked at baclofen, 1 fundoplication and 1 supportive lifestyle changes. 3 studies looked at a combination of therapies involving pharmacological, behavioral and psychotherapies.

### CONCLUSION



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Although evidence for treatment options is still limited, the strongest evidence point towards the use of DB and Baclofen, and both should be considered depending on their availabilities.

**Key words:** Rumination; Rumination syndrome; Diaphragmatic breathing; Treatment; Systematic review

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**Core tip:** Rumination syndrome (RS) is a relatively common but underdiagnosed gastroenterological condition. Due to recent advances in research, we have decided to perform the first systematic review on treatment options for RS. Our results show that diaphragmatic breathing has the strongest data for efficacy in this condition.

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## INTRODUCTION

Rumination syndrome (RS) is characterized by recurrent effortless postprandial regurgitation of recently ingested food from the stomach to the oral cavity<sup>[1,2]</sup>. Although rumination was historically described mainly in children or adults with impaired mental development, it is now recognized in adults regardless of mental state<sup>[2,3]</sup>. Although not considered to be life-threatening, RS has been associated with quality of life impairment and even malnutrition<sup>[2]</sup>.

Currently, the diagnosis of RS in adults is based on careful history and subsequently applying the Rome IV criteria<sup>[4]</sup>, often also supported by postprandial High Resolution Impedance Manometry (HRIM) findings of reflux episodes associated with a preceding abdominal pressure increase of > 30 mmHg<sup>[5]</sup>. These findings also allow RS to be discriminated from gastroesophageal reflux disease (GERD), a common competing diagnosis in these patients, often resulting in several years of delay before the diagnosis of RS is made.

There is a general lack of consensus on the most appropriate treatment options for RS. Therefore, the aim of this systematic review is to summarize the literature on studies that looked into treatment options for adult RS patients, and ascertain what is most evidence-based approach in treating them.

## MATERIALS AND METHODS

### Literature search

We followed PRISMA guidelines and the medical literature was searched using OVID within the databases Medline (1946 to February 2019), EMBASE (1947 to February 2019), PsycINFO (1806 to February 2019) and Cochrane central register of controlled trials. Searches were based on controlled vocabulary including medical subject heading terms (MeSH) where possible (e.g., “rumination syndrome” and “eructation”). In addition, a combination of keywords, free text terms and database-specific subject headings for rumination, RS, eructation, postprandial regurgitation, feeding disorder, treatment, therapy, behavioral therapy, non-pharmacological treatment were included. In case of multiple reports of one trial were found, we selected the most updated one.

Abstracts of the papers identified by the initial search were evaluated by the lead reviewer (AO) for appropriateness to the study question, and all potentially relevant papers were obtained and evaluated in detail. In order to identify potentially eligible studies published only in abstract form, conference proceedings (Digestive Diseases Week, American College of Gastroenterology, United European Gastroenterology Week, Federation of Neurogastroenterology and Motility) between 2001 and 2019 were also hand-searched.

The bibliographies of studies included in the final analysis as well as relevant

reviews were also screened for additional relevant articles. The website Clinical-Trials.gov was also searched to look for trials not included in the mentioned databases.

### Data extraction and analysis

We included studies evaluating management of adults over 18 with a diagnosis of RS. Articles involving pediatrics or patients with eating disorders, and those with no mention of treatment strategies or without identifiable outcomes were excluded. Editorials, case reports of single cases, letters, qualitative studies, clinical guidelines and narrative reviews were also excluded. Articles were restricted to English language. Titles and abstracts were then screened by 3 independent reviewers (Ong AML, Wang YT, Tay SW) onto a Microsoft (Richmond, VA) Excel spreadsheet. Using a standardized form, the 3 reviewers independently extracted data and assessed study risk of bias and quality using the Cochrane Risk of Bias tool<sup>[6]</sup> for randomized controlled trials (RCTs) and Newcastle Ottawa Scale (NOS)<sup>[7]</sup> for cohort studies. A trial was judged with low risk of bias when all six domains of the Cochrane risk of bias tool were classified as low risk of bias for RCTs. Studies that achieved at least six stars for the NOS were considered studies of high quality<sup>[7]</sup>. No attempts at assessing study quality was made for studies with case series. Any disagreements were resolved by consensus. No studies in the search were discarded because of assessed quality.

Data on study design, location, patient profile, duration of symptoms, follow up periods, date, diagnostic criteria, intervention, outcome, and follow-up were extracted. Due to significant heterogeneity among studies such as study design, treatment and outcome measurements, no head to head comparisons or meta-analysis was performed.

## RESULTS

We retrieved 298 articles based on our search criteria (Figure 1). After excluding duplicates ( $n = 85$ ), pediatric studies ( $n = 111$ ), studies involving eating disorders ( $n = 27$ ), singular case reports ( $n = 22$ ) and studies without mention of treatment strategies ( $n = 41$ ), we arrived at 12 studies for analysis (Figure 1). These studies consist of 2 RCTs, 1 prospective cohort, 5 prospective observational, 2 mixed retro-spective/prospective observational and 2 retrospective observational studies.

A total of 254 patients were included in the analysis, with a mean age of 36.1 (range 18-89). 185 patients (72.8%) were females. 5 studies looked into behavioral therapies, primarily diaphragmatic breathing (DB), where 2 studies were done with electromyography (EMG) guidance<sup>[8,9]</sup>, 1 study was done with HRiM guidance<sup>[10]</sup> and the other 2 without any visual guidance<sup>[11,12]</sup>. 2 studies looked at the utility of baclofen<sup>[13,14]</sup>, 1 looked at utility of fundoplication<sup>[15]</sup> and 1 looked at supportive lifestyle changes<sup>[16]</sup>. 3 studies looked at a combination of therapies involving pharmacological, behavioral and psychotherapies<sup>[2,17,18]</sup>. Characteristics of the studies can be found in Table 1.

### Assessment of bias for studies

Using the Cochrane risk of bias tool<sup>[6]</sup>, the RCT by Barbal<sup>[8]</sup> had low risk of bias for the following domains: Allocation, missing outcome data, outcome measures, selection of reported results. However, there were some concerns of bias *via* deviations from intended interventions as study patients may be aware, they were given placebo. Also, the interaction with a health care professional may improve symptoms and adherence to treatment. There was also no mention of compliance rates in treatment as well as some concerns of bias in outcome measurement due to lack of blinding of outcome assessors. For the RCT by Pauwels<sup>[14]</sup>, there was low risk of bias for the following domains: Allocation, deviation from intended intervention, missing outcome data, outcome measurement and selection of reported results. The cohort study by Barba<sup>[9]</sup> scored 6 on the NOS with a star given for the following domains: representativeness, selection, ascertainment of exposure, demonstration of outcome not present at start, adequate follow up duration and adequacy of follow up.

## DISCUSSION

We performed a systematic review looking at adult patients diagnosed with RS, and identified 12 articles evaluating the efficacy of various treatment modalities for RS and ranked them in order of level of evidence (Table 2).

The studies with the strongest evidence were the 2 RCTs looking at EMG-guided biofeedback and Baclofen. In the former<sup>[8]</sup>, 12 patients who underwent 3 biofeedback

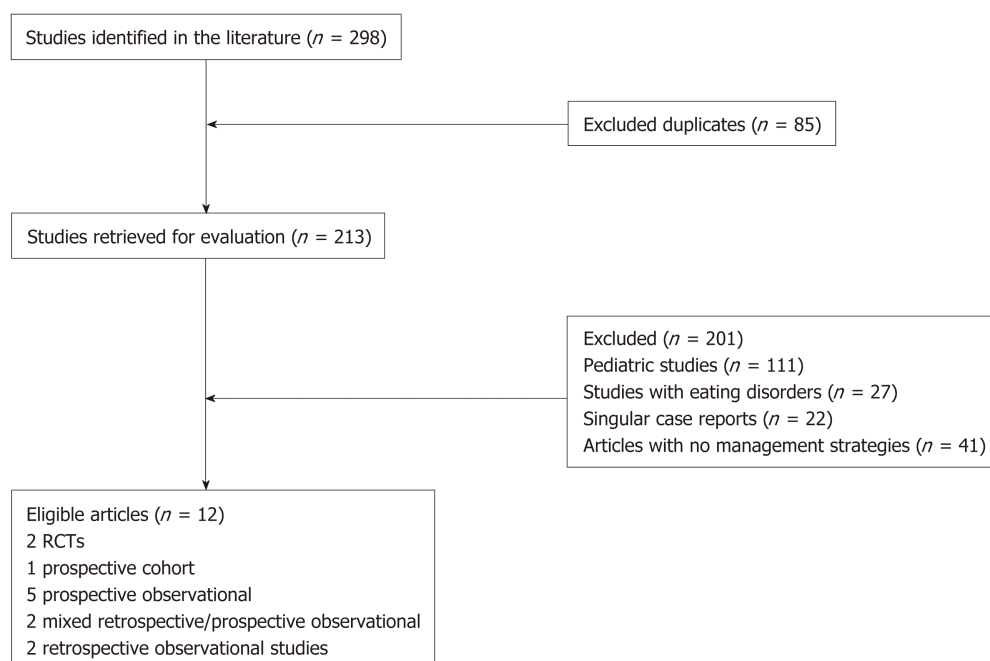


Figure 1 Flow diagram of assessment of studies identified.

sessions had 74% reduction in rumination symptoms compared to 1% reduction in the placebo group with oral simethicone. The improvements with biofeedback appeared sustainable in the long-term with improvement of symptoms at each subsequent follow up. The study when assessed for quality based on the Cochrane Risk of Bias tool<sup>[6]</sup> had generally low risk of bias, although there were some concerns about the lack of blinding of outcome assessors as well as the placebo group being aware they were in the placebo arm. The other RCT<sup>[14]</sup> showed that Baclofen at 5-10 mg three times daily reduced the number of symptoms of regurgitation *via* event markers on HRIM and overall treatment evaluation via questionnaire where 63% of patients improved on Baclofen compared to 26% of patients on placebo treatment ( $P < 0.0001$ ). The study quality was assessed to also have low risk of bias apart from having a heterogeneous population of RS and supragastric belching, although this more accurately mimics real-world situations where there is significant overlap in presentation for these 2 conditions.

The exercises that were part of the biofeedback protocols<sup>[8,9]</sup> were essentially abdominal breathing exercises otherwise known as DB<sup>[19]</sup>. DB was further supported by other non-RCT studies included in this analysis. There were variations on how these were performed, from using EMG guidance<sup>[8]</sup>, HRiM guidance<sup>[10]</sup> or just delivered without visual aids<sup>[11,12]</sup>. Some of the studies showed that even a single session of DB training can improve symptoms, but as these symptoms tend to recur over time, compliance to home exercises is likely important to maintain sustainability of response.

Many of the studies analyzed performed physiological tests prior to the treatment and post-treatment, thus allowing an insight into possible mechanisms of the origin of symptoms in these patients. Although the exact pathogenesis of RS is still unknown, the primary initiating mechanism is commonly a post-prandial gastric pressurization<sup>[10,11]</sup> that possibly results from anterior abdominal muscle contractions<sup>[9]</sup>. However, a low esophageal sphincter (LES) pressure is also required to facilitate the upward movement of gastric contents as Halland<sup>[10]</sup> showed that high intragastric pressure waves led to rumination episodes only when accompanied by reduction in esophagogastric junction (EGJ) pressure. Furthermore, post-prandially patients demonstrated contraction of intercostals muscles to facilitate a negative intra-thoracic pressure<sup>[9]</sup>. It is likely this combination of increased intra-abdominal pressure coupled with negative intra-thoracic pressure and a permissive EGJ that allows rumination to take place. The significance of a low LES pressure has also been highlighted in some of the studies. Patients with low LES baseline pressures were shown to have a poorer outcome to treatment for RS<sup>[17]</sup>. Reasons for this low LES pressure can be a learned prolonged postprandial voluntary relaxation of the diaphragmatic crura or increased TLESRs<sup>[20]</sup>. Other suggested possibilities include increased abdominal pressure displacing the EGJ proximally away from the crura thus losing the crural contribution

Table 1 Characteristics of studies included in analysis

Study	Site	Type of study	n	Fem (%)	Age (yr) (range)	Diagnostic criteria	Physiological tests done	Treatment	Description of treatment	Primary outcome	Main Results	Proposed mechanism of action	Follow up period (mo)
Barba <i>et al</i> <sup>[8]</sup>	Spain	RCT, Placebo controlled	12	7 (58)	Median 42 (19-69)	Rome 3 rumination syndrome	EMG <sup>+</sup> activity of abdominal-thoracic muscles, done PRE and POST	EMG <sup>+</sup> guided biofeedback	Pre-meals, patients were trained to control the activity of the abdominal-thoracic muscles under visual control of EMG <sup>+</sup> recordings displayed on a monitor. Specifically, they were instructed to voluntarily reduce the activity of intercostal and anterior abdominal muscles and to increase the activity of the diaphragm. After each biofeedback session, patients were instructed to perform the same exercises daily at home for 5 min before and after breakfast, lunch, and dinner. At the end of the treatment period, patients were encouraged to continue practicing these same exercises over time. 3 such sessions performed over 10 d	Reduction in rumination episodes measured over 10 d, patient reported	Regurgitation episodes decreased by $74 \pm 6\%$ in the biofeedback group ( $n = 12$ ) but only by $1 \pm 14\%$ in the placebo group ( $n = 11$ ; $P < 0.001$ ). Biofeedback significantly reduced the activity of the abdominothoracic muscles, whereas the placebo had no effect; Number of daily rumination episodes decreased to $7.7 \pm 1.9$ immediately after biofeedback, $3.0 \pm 1.1$ by 1 mo, $1.2 \pm 0.5$ by 3 mo, and $0.7 \pm 0.4$ by 6 mo ( $P < 0.001$ )	Modified basal postprandial muscular tone; Possibly increase awareness in patients to suppress rumination	6 mo
Pauwels <i>et al</i> <sup>[14]</sup>	Belgium	RCT, Placebo controlled	10	6 (60)	Mean 42 (18-61)	Rome 4 rumination syndrome and/or supra-gastric belching	Oesophageal HRIM <sup>+</sup> done PRE and POST	Baclofen	5 mg tds first week then increased to 10 mg tds second week, followed by 1 wk washout period, before 2 wk crossover to alternative treatment	Number of symptoms of regurgitation via event marker on HRIM <sup>+</sup> and overall treatment evaluation (OTE)	Median number of times that the "regurgitation" marker was pushed significantly lower in baclofen group compared to placebo [ $4 (0-14)$ vs $6 (0-19)$ , $P = 0.04$ ] Patients reported significantly better OTE ratings after baclofen compared to placebo [mean score $1 (0-2)$ vs $0 (-1-1)$ , $P = 0.03$ ]. On baclofen treatment, 63% of patients improved on TLESRs was significantly lower after baclofen compared to placebo [ $4 (1-8)$ vs $7 (3-12)$ , $P = 0.017$ ].	Increased LES <sup>+</sup> pressure: Postprandial LES <sup>+</sup> pressure significantly higher in the baclofen arm compared to placebo [ $17.79 (12.72-22.68)$ vs $13.06 (7.16-16.91)$ mm Hg ( $P = 0.0002$ )]. Borderline negative correlation between postprandial LES pressure and the number of rumination episodes in the baclofen condition ( $P = 0.056$ , $r = -0.54$ ). Reduced TLESR <sup>+</sup> : Postprandial TLESRs was significantly lower after baclofen compared to placebo [ $4 (1-8)$ vs $7 (3-12)$ , $P = 0.017$ ].	No long term follows up

Barba <i>et al</i> <sup>[9]</sup>	Spain	Prospective cohort with controls	24	17 (71)	14-76	Rome 3 rumination syndrome	EMG <sup>+</sup> activity, done PRE and POST treatment	EMG <sup>+</sup> guided biofeedback	Pre-meals, patients were trained to control the activity of the abdominal-thoracic muscles under visual control of EMG <sup>+</sup> recordings displayed on a monitor. Specifically, they were instructed to voluntarily reduce the activity of intercostal and anterior abdominal muscles and to increase the activity of the diaphragm. After each biofeedback session, patients were instructed to perform the same exercises daily at home for 5 min before and after breakfast, lunch, and dinner. At the end of the treatment period, patients were encouraged to continue practicing these same exercises over time. 3 such sessions performed over 10 d	Not defined	Post-biofeedback session, patients experienced a decrease in the number of regurgitation events (8 recorded <i>vs</i> 18 in the basal challenge test; $P < 0.001$ ). The improvement observed during the first biofeedback session was strengthened by the following biofeedback sessions. Regurgitation events had decreased by 70% ( $P < 0.001$ ). By the end of the 3 biofeedback sessions, postprandial abdominal symptoms were reduced (1.6 score; $P < 0.001$ <i>vs</i> basal). Further reductions in the number of rumination events during the 6-mo observation period while controls had no changes	6 mo
Halland <i>et al</i> <sup>[10]</sup>	United States	Prospective observational	16	9 (56)	Mean 37	Rome 3 rumination	Oesophageal HRM <sup>+</sup> done PRE, during and POST treatment	HRM <sup>+</sup> guided biofeedback therapy	Behavioral therapy delivered by a single subspecialist gastroenterologist where he placed his hand on the patient's abdomen and instructed patients in diaphragmatic breathing, which entails abdominal rather than chest motion. Patients were also instructed to observe the HRM <sup>+</sup> monitor to observe the impact of DB on reduction in gastric pressurizations and regurgitation.	Not defined	Rumination episodes reduced from a median of 5 (2-10) to 1 (0-2) ( $P < 0.001$ ) during, and 3 (1-5) after ( $P < 0.001$ <i>vs</i> during) diaphragmatic breathing. Diaphragmatic breathing increased ECG pressure ( $P < 0.001$ ) and restored a negative gastroesophageal pressure gradient [20 mmHg (80-7)] by reducing postprandial intragastric pressure. DB may also alter vagal activity and reduce TLESR whilst increasing LES tone	Nil



O'Brien <i>et al</i> <sup>[2]</sup>	United States	Retrospective and Prospective observational	36	29 (81)	Mean 27	Not elaborated	All had oesophageal manometry, 20 had pH studies. Tests done PRE treatment	Various	6 prokinetics 7 antacids 3 behavioural therapy (e.g. biofeedback); 2 psychotherapy; 2 combined behavioural and psychotherapy	Not defined	12/16 patients reported subjective improvement, but not broken down to individual treatment options. No therapy deemed effective enough compared to another	N/A	Mean 25 (7-74)
Soykan <i>et al</i> <sup>[18]</sup>	United States	Retrospective and Prospective observational	10	6 (60)	Mean 28.5 (16-63)	Rome 2 for rumination syndrome	All had oesophageal manometry, electro-gastrography, gastric emptying study. All done PRE treatment	Various	5 biofeedback; 2 prokinetics; 1 prokinetic and acid blockade; 1 leuprolide acetate and antacid; 1 no treatment	Not defined	all 5 undergoing biofeedback improved, 1 taking prokinetic improved	N/A	Mean 31.2 (6-72)
Vijay-vargiya <i>et al</i> <sup>[12]</sup>	United States	Retrospective observational	57	54 (95)	Mean 30.3 (14-62)	Rome 3 for rumination syndrome and rectal evacuation disorder	11 oesophageal manometry, 45 gastric emptying, 3 pH studies, 6 barium oesophagogram, 12 SPECT*. All done PRE treatment	Diaphragmatic breathing	<i>Via</i> behavioural psychologist with instructions on diaphragmatic breathing to abort or control regurgitation	Not defined	Not reported	N/A	N/A
Tucker <i>et al</i> <sup>[11]</sup>	United Kingdom	Prospective observational	46	34 (74)	18-68	HRM* criteria (Rommel)	All had oesophageal HRM* PRE treatment	Diaphragmatic breathing	All patients received a 20 min behavioural intervention immediately after HRM* investigation. This included a description of the abnormal findings, cause of symptoms and explanation of the rationale for behavioural therapy. Behavioural instruction was focused on deep muscle relaxation and diaphragmatic breathing	Not defined	Complete improvement in rumination in 20/46 (43%). Partial improvement in 13 (28%)	N/A	Median 5 (3-11)

Lee <i>et al</i> <sup>[17]</sup>	South Korea	Prospective observational	21	8 (38.1%)	Mean 41.9	Modified Rome 2 for rumination syndrome	All had oesophageal HRM <sup>†</sup> , pH study and gastric emptying tests PRE treatment	Various	All given levosulpride 25 mg TDS <sup>†</sup> ; supportive psychotherapy, education and reassurance given monthly, with 15 min sessions over a minimum of 6 mo via therapists experienced in eating disorders	Not defined	8 (38.1%) showed improvement, 47.6% unchanged while 3 (14.3%) worsened. Those who improved were statistically more likely to have undergone treatment for > 6 mo and less likely to have low mean LES <sup>†</sup> pressure	N/A	Mean 19 (15-24)
Oelschlager <i>et al</i> <sup>[13]</sup>	United States	Prospective observational	5	4 (80%)	Mean 40.6 (18-61)	Rome 2 for rumination syndrome	All had oesophageal manometry and pH studies PRE treatment	Fundoplication	1 laparoscopic, 4 open Nissen fundoplication	Not defined	all had resolution of symptoms; 3/5 had pathological acid exposure, 4/5 had hypotensive LES <sup>†</sup> , 3/5 had hiatal hernias	Restoration of LES <sup>†</sup> dysfunction	Median 6 mo, 2 wk - 1 yr
Blondeau <i>et al</i> <sup>[13]</sup>	Belgium	Prospective observational	12	8 (67)	45 (18-89)	Clinical diagnosis	All had oesophageal HRM <sup>†</sup> PRE and POST treatment	Baclofen	10 mg TDS <sup>†</sup> for a week	Not defined	Patients on baclofen recorded significantly fewer symptoms during the study [6 (2-22); <i>P</i> 0.001]. The number of symptom markers for regurgitation and reduction of compulsive was significantly reduced from 9 (0-11) to 1 (0-13) ( <i>P</i> 0.001); The total number of flow events was significantly reduced from 473 to 282 (39.2%) during baclofen treatment ( <i>P</i> 0.02)	Increase in LES <sup>†</sup> function and reduction in TLESR <sup>†</sup> ; Possible central mechanism of action to reduce sensitivity of stomach during distension and reduction of compulsive behaviour of straining: The number of TLESR <sup>†</sup> s during the postprandial period was significantly reduced from 15 (9-19) in baseline conditions to 7 (6-15) during baclofen treatment ( <i>P</i> 0.03). The number of strains was reduced from 32 (17-48) in baseline conditions to 17 (2-70) during baclofen treatment ( <i>P</i> 0.1).	No long term follows up
Johnson <i>et al</i> <sup>[16]</sup>	United States	Retrospective observational	5	3 (60)	Mean 26.8 (18-43)	Clinical diagnosis	1 barium oesophagram; 1 gastric emptying test; all done PRE	Lifestyle changes	All advised to eat slowly, chew completely, avoid food triggers, regular exercises, weight reduction, stress management strategies	Not defined	All 5 had complete cessation of symptoms	reduction in behavioural and cognitive processes that may develop and maintain symptoms; improvement in coping mechanisms for symptoms	Mean 34.4 (22-43)

EMG: Electromyography; HRM: High resolution impedance manometry; LES: Lower esophageal sphincter; TLESR: Transient lower esophageal sphincter relaxation; TDS: Three times daily; HRM: High resolution manometry; SPECT: Single photon emission computed tomography.

Table 2 Summary of treatment options for rumination syndrome

Treatment	Strength of evidence	Treatment outcome
Diaphragmatic Breathing	RCT <sup>[8]</sup>	Regurgitation episodes decreased by 74% in the biofeedback group compared to 1% in placebo ( $P < 0.001$ )
	Prospective cohort with controls <sup>[9]</sup>	Regurgitation events decreased by 70% ( $P < 0.001$ ).
	Prospective observational <sup>[10]</sup>	Median rumination episodes reduced from 5 (2–10) to 1 (0–2) ( $P < 0.001$ )
	Retrospective observational <sup>[12]</sup>	Not reported
	Prospective observational <sup>[11]</sup>	Complete improvement in rumination in 43%. Partial improvement in 28%
Baclofen	RCT <sup>[14]</sup>	Median regurgitation events lower with baclofen compared to placebo [4 (0–14) <i>vs</i> 6 (0–19), $P = 0.04$ ]
	Prospective observational <sup>[13]</sup>	Median regurgitation events significantly reduced from 9 (0–11) to 1 (0–13) ( $P 0.01$ )
Surgery	Prospective observational <sup>[15]</sup>	100% (5/5) resolution of symptoms
Psychotherapy	Prospective observational <sup>[17]</sup>	38.1% showed improvement. 47.6% unchanged.
	Retrospective observational <sup>[16]</sup>	100% (5/5) resolution of symptoms

to the EGJ. An unrecognized central mechanism may also be involved since healthy adults are not able to induce rumination<sup>[21]</sup>.

The physiology tests also allow us to understand the rationale for these treatment options, especially in DB, where the evidence appears strongest in terms of quantity and quality. In the study by Halland<sup>[10]</sup>, they demonstrated that DB may improve crural function *via* several mechanisms. DB can directly augment the tone of the LES by voluntary contraction of the crural diaphragm. DB can also prevent the increased intra-gastric pressure from displacing the EGJ proximally, thus not allowing a permissive EGJ during such episodes. Also, DB may alter vagal activity and prevent TLESRs from happening and thus maintain a more prolonged high pressure LES tone. DB also likely competes with the need for the learned behavior of gastric straining, and this abolishes the trigger to ruminate when performed post meals<sup>[22]</sup>. Barba<sup>[8]</sup> showed that EMG guided-biofeedback, of which DB was part of the intervention, significantly reduced the activity of the abdominothoracic muscles, whereas the placebo had no effect, and this correlated with reduction of rumination symptoms. Based on their EMG findings pre and post, they postulate that patients with RS have an abnormal level of abdominothoracic muscular tone. They then showed it was possible to specifically target the relevant muscles and unlearn this coordinated abdominothoracic maneuver which generates rumination (*e.g.*, reducing activity of intercostals and anterior abdominal muscle while increasing activity of diaphragm reduces rumination symptoms).

Other studies not involving DB also shed light on mechanisms of RS and its treatment. 2 studies<sup>[13,14]</sup> showed that baclofen reduced the number of rumination episodes possibly by reduction in TLESRs and increasing postprandial LES pressure which were both significantly different in the intervention group compared to placebo. These mechanisms are similar in those postulated to take place post-DB. Baclofen may have other mechanisms of action as well, as shown by<sup>[13]</sup> where baclofen reduced voluntary gastric straining events and the authors postulate that this could be either related to central mechanisms of reducing compulsive behaviours of straining or by reducing the mechanosensitivity of the stomach as studies<sup>[23]</sup> have shown that patients with RS often have increased gastric sensitivity to distension.

There are likely psychosocial and cognitive processes in play that initiate and perpetuate symptoms in RS as evident by patients reporting onset of symptoms following acute illness<sup>[24]</sup>, surgeries<sup>[18]</sup>, psychological stress<sup>[18]</sup> and major life events<sup>[11,18,24]</sup>. Comorbid psychiatric disturbances such as depression, anxiety and somatoform disorders<sup>[12,18]</sup> were frequently found in RS patients, and it is not entirely clear whether these are causes or consequences of RS. Pediatric studies<sup>[25]</sup> have shown that successfully treating psychiatric disorders, when present, is helpful for RS. It is not unreasonable to think that the same applies to adult patients as patients with psychiatric disorders may have a lack of motivation which interferes with compliance to behavioral treatments<sup>[12]</sup>, and therefore needs to be addressed. Behavioral treatments targeting stress reduction and improving coping mechanisms to symptoms have also been shown to be helpful in reducing symptoms in RS<sup>[16]</sup>. A single open label study looked at 21 adults with RS<sup>[17]</sup> and looked the effect of supportive

psychotherapy together with a prokinetic levosulpiride. Only 38% of patients showed improvement, so perhaps psychotherapy itself is not efficacious but possibly, a more targeted form of psychotherapy in association with behavioral treatments may be effective. As such, investigators are currently actively recruiting patients and looking into using a form of Cognitive Behavioral Therapy to treat this condition (<https://clinicaltrials.gov/ct2/show/NCT03113682>).

Interestingly, it has been suggested that refractory cases of rumination be treated with surgery such as fundoplication<sup>[15]</sup>. In this study, all 5 patients had complete cessation of symptoms post-surgery, although 4 out of 5 patients had a hypotensive LES while 3 out of 5 had hiatal hernias and pathological acid exposure, thus improvement in their symptoms could have been due to improvement in their GERD. It is therefore not recommended to treat RS patients with surgery without concomitant GERD or structural abnormalities at this point without further evidence. However, this study showed the likely contribution of an incompetent LES in the overall picture manifestation of RS.

There were some limitations to our analysis. We only included studies in English, and we also excluded pediatric studies since our focus was on adult patients. However, some of the results from pediatric studies could still be relevant in understanding the efficacy of RS treatment. Even though most of the studies included some form of physiological testing, the studies were heterogenous and tests such as gastric emptying studies or 24-h pH impedance studies were often not performed. Thus, the diagnosis of GERD and gastroparesis may be missed in some of these patients labeled as RS, and therefore caution needs to be exercised in interpreting some of the study results. It was difficult to make strong conclusions based on the strength of the data as only 3 studies were controlled and only 2 were randomized interventions. In view of the limited literature available in this field, we retained observational studies despite knowing that they were prone to bias, and thus our recommendations are not based on strong evidence, but rather a summary of what is available in the literature (Tables 2 and 3).

In conclusion, RS may present similarly to other conditions such as GERD and gastroparesis and is likely under-recognized, therefore clinicians need to be aware of this syndrome in their differential diagnosis. Although evidence for treatment options is still limited, the strongest evidence points towards the use of DB and Baclofen, and both should be considered depending on their availabilities. Most of the studies analyze are limited by the small sample sizes and variability in delivery of biofeedback. Therefore, further studies are needed to tackle these knowledge gaps.

**Table 3 Suggested approach in treatment of rumination syndrome**

Condition	Treatment
Initial treatment	Extensive explanation of condition and underlying mechanism together with reassurance of benign nature of condition <sup>[2,20]</sup>
	Diaphragmatic breathing by trained personnel (with EMG guidance or HRiM if available)
	If no response to diaphragmatic breathing after ensuring compliance, Baclofen 5-10 mg three times daily
For refractory cases	Consider alternative diagnosis (GERD, gastroparesis, functional dyspepsia, supragastric belching) and treat appropriately
	Since both DB and baclofen appear to be effective and work via different mechanisms, we postulate that a switching to the other therapy or a combination of these therapies could be useful in cases refractory to either treatments
	Address psychological illness, if present. Consider adjunctive psychological therapies to correct cognitive processes that may perpetuate symptoms

EMG: Electromyography; GERD: Gastroesophageal reflux disease; DB: Diaphragmatic breathing.

## ARTICLE HIGHLIGHTS

### Research background

Rumination syndrome (RS) is a relatively common yet underdiagnosed condition.

### Research motivation

There is no consensus on how to treat patients diagnosed with rumination syndrome.

### Research objectives

Our objectives are to systematically review the literature on the efficacy of treatment options for adults with RS.

### Research methods

We conducted a systematic review according to PRISMA guidelines. We searched Medline (1946 to February 2019), EMBASE (1947 to February 2019), PsycINFO (1806 to February 2019) and Cochrane central register of controlled trials for articles discussing treatment options for adult patients (> 18 years) with RS. All relevant articles were accessed in full text. We extracted data on study designs, patient profiles, duration of symptoms, follow up periods, date, diagnostic criteria, interventions and outcomes. Risk of bias assessment was carried out independently by 3 reviewers *via* Cochrane Risk of Bias tool and Newcastle Ottawa Scale for RCTs and Cohort studies respectively.

### Research results

12 articles were identified. The strongest evidence pointed towards diaphragmatic breathing (DB), and less so for baclofen. A total of 254 patients were included in the analysis.

### Research conclusions

DB has the strongest evidence for efficacy in adults with RS.

### Research perspectives

The quality of the evidence is still weak. More research needs to be done in this field.

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# Surgery with adjuvant or neoadjuvant treatment vs surgery alone for resectable pancreatic cancer: A network meta-analysis

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## Abstract

### BACKGROUND

Pancreatic cancer is one of the most common and lethal malignancies worldwide. The common treatment options for resectable pancreatic cancer include surgery alone, neoadjuvant chemotherapy (CT), neoadjuvant chemoradiotherapy (CRT), adjuvant CT, and adjuvant CRT. However, the optimal treatment is still controversial.

### AIM

To identify the most effective approach for pancreatic cancer using network meta-analysis.

### METHODS

Eligible studies were searched from PubMed, MEDLINE, EMBASE, Cochrane database, and Google scholar. We searched and included randomized controlled trials reporting on neoadjuvant and adjuvant therapies. For direct comparisons, standard pairwise meta-analysis was performed using the inverse variance DerSimonian-Laird random-effects model. For indirect comparisons, Bayesian network meta-analysis was used to combine direct and indirect evidence. We used relative hazard ratios (HRs) to estimate death difference of different treatments, and relative odds ratios (ORs) for toxic effects. Treatment effects were ranked based on their efficacy for improving survival or reducing toxicity using rankogram. The quality of evidence of estimates from direct comparison and network meta-analysis was evaluated following the GRADE approach.

### RESULTS

We included 13 high quality trials with 1591 participants in this network meta-analysis. Compared with surgery alone [pooled HR = 0.7, 95% confidence

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interval (CI): 0.62-0.79] and surgery with adjuvant CRT (pooled HR = 0.6, 95% CI: 0.54-0.72), surgery with adjuvant CT had a higher rate of overall survival. In contrast, standard pairwise meta-analysis showed a statistically significant survival advantage of surgery with adjuvant CT compared with surgery alone (pooled HR = 0.75, 95% CI: 0.63-0.89;  $P < 0.001$ ). Rankogram showed that surgery with adjuvant CT was most likely to rank the best in terms of overall survival (probability: 94.2%), followed by surgery alone (probability: 5.8%). No significant differences in overall toxicity or haematological toxicity were found between all the therapies. High quality evidence supported surgery with adjuvant CT over surgery alone for increasing overall survival. Moderate quality evidence supported surgery with adjuvant CT over surgery with adjuvant CRT for increasing overall survival.

## CONCLUSION

Surgery with adjuvant CT prolongs overall survival compared with surgery alone and surgery with adjuvant CRT, suggesting surgery with adjuvant CT is the optimal treatment for resectable pancreatic cancer.

**Key words:** Pancreatic cancer; Surgery; Network meta-analysis; Adjuvant therapy; Neoadjuvant therapy

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**Core tip:** No consensus is available in previous studies about the most beneficial treatment option for resectable pancreatic cancer. This is the first network meta-analysis comparing the efficiency of surgery alone, neoadjuvant chemotherapy (CT), neoadjuvant chemoradiotherapy (CRT), adjuvant CT, and adjuvant CRT. We investigated these treatment options in terms of overall survival and toxicity. We found that surgery with adjuvant CT prolonged overall survival compared with surgery alone and surgery with adjuvant CRT. Surgery with adjuvant CT is the optimal treatment for resectable pancreatic cancer.

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## INTRODUCTION

Pancreatic cancer is one of the most common and lethal malignancies<sup>[1]</sup>. Surgical resection is the only potential curative treatment for pancreatic cancer. However, even after radical removal of the tumor (R0), the prognosis remained poor, with the 5-year survival rate being less than 25% and the median survival time being 14-21 mo<sup>[2-4]</sup>. High incidence of both locoregional and distant recurrences is responsible for the poor prognosis. Thus, a multimodal approach is needed to decrease the high recurrence rate as well as increase overall survival<sup>[5,6]</sup>.

Several neoadjuvant or adjuvant therapies have been shown to be beneficial in selected patients. These therapies are neoadjuvant chemotherapy (CT), neoadjuvant chemoradiotherapy (CRT), adjuvant CT, and adjuvant CRT. However, there are debates over which therapy can benefit patients mostly. Regarding neoadjuvant therapy, recent meta-analysis found no significant difference in the overall survival between neoadjuvant CRT and surgery<sup>[7]</sup>. With regard to adjuvant therapy, the benefit of adjuvant therapy for resectable pancreatic cancer is still controversial, especially the impact of adjuvant CRT. Adjuvant CRT using fluorouracil is considered standard of care in the United States. However, the EORTC trial demonstrated no benefit of adjuvant CRT over observation in patients with resected pancreatic cancer (median survival: 1.3 year *vs* 1.0 year)<sup>[8]</sup>. Thus, more powerful and comprehensive evidence is needed to evaluate the best treatment strategy for resectable pancreatic cancer.

There have been several traditional meta-analyses comparing the benefit of neoadjuvant therapy or adjuvant therapy. However, all of the previous meta-analyses

only addressed neoadjuvant therapy<sup>[7,9-11]</sup> or adjuvant therapy alone<sup>[12-14]</sup>. Thus, it is interesting and meaningful for us to perform this network meta-analysis, that is, to compare both neoadjuvant and adjuvant therapies with surgery alone. The advantage of network meta-analysis is that it can compare different treatments without direct clinical trials. That is, if we have only clinical trials comparing A to B and B to C, we can estimate A to C using network meta-analysis. Besides, treatment options can be ranked based on their efficacy for improving survival or reducing toxicity in network meta-analysis.

The aim of this network meta-analysis was to identify the most effective treatment for resectable pancreatic cancer by comparing overall survival and toxic effects after neoadjuvant or adjuvant CT and CRT.

## MATERIALS AND METHODS

The protocol of this network meta-analysis was registered with the prospective register of systematic reviews, PROSPERO (CRD42017057053). This network meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses<sup>[15]</sup> and Cochrane guidelines<sup>[16]</sup>.

### Search strategy

Eligible studies were searched from PubMed, Medline, EMBASE, Cochrane database, and Google scholar, using a combination of following terms “pancreatic cancer”, “pancreatic neoplasm”, “neoadjuvant therapy”, and “adjuvant therapy”. A manual search through published articles was performed additionally. No publication year was restricted in the search. The search was carried out independently by two authors.

### Inclusion and exclusion criteria

The following inclusion criteria were used: (a) Randomized controlled trials; (b) Studies investigating surgery alone, neoadjuvant therapies, or adjuvant therapies for resectable pancreatic cancer; and (c) Studies that had at least one of the following outcomes: Survival and toxicity. Single-arm studies, nonrandomized cohort studies, and studies comparing different ways of adjuvant or neoadjuvant treatment were not included in this network meta-analysis.

### Data extraction and quality assessment

The information on study design, methods, patient characteristics, treatment protocols, and outcome (overall survival and toxicity) was extracted independently by two authors. We extracted reported adjusted hazard ratios (HRs) to measure overall survival. When HRs were not reported, we estimated them from summary statistics (Kaplan-Meier curves) in accordance with practical methods for incorporating summary time-to-event data into meta-analysis<sup>[17]</sup>. If there was no enough information to estimate HRs, median survival durations would be used in this network meta-analysis<sup>[18]</sup>. Only grade 3 or 4 toxicities (overall toxicities and haematological toxicities) were extracted and analyzed in this network meta-analysis. The quality of randomized control study was assessed by the Cochrane Collaboration's tool<sup>[19]</sup>. Data collection and study quality assessment were performed following the Quality of Reporting of Meta-Analyses statement.

### Data synthesis and analysis

The study outcomes were overall survival and toxicity after neoadjuvant or adjuvant therapies. For network meta-analysis of overall survival, the preferred outcome measure was reported HRs, followed by estimated HRs and median survival durations. Relative treatment effects (HRs) in multi-arm trials were converted to arm-specific outcomes<sup>[18]</sup>. For network meta-analysis of toxicity (overall toxicity and haematological toxicities), we used odds ratios (ORs) as outcome measures. ORs were calculated from the summary number of reported toxicity events and summary number of exposure patients in each trial. Since the definition and reporting type of toxicity were diverse in the included studies, we only summarize seven toxicity events [nausea/vomiting, infection/fever, asthenia/fatigue, diarrhea, hematological toxicity (leukopenia, thrombopenia, and anemia)] as overall toxicity.

For direct comparisons, standard pairwise meta-analysis was performed using the inverse variance DerSimonian-Laird random-effects model. Heterogeneity was quantified using *I*-squared statistic. Publication bias was evaluated using the funnel plot. Traditional pairwise meta-analysis was performed using REVIEW MANAGER (version 5.0 for Windows; the Cochrane Collaboration, Oxford, United Kingdom).

For indirect comparisons, we conducted random-effects Bayesian network meta-

analysis using Markov chain Monte Carlo methods in The R Programming Language 3.3.2 [R Core Team (2016), R Foundation for Statistical Computing, Vienna, Austria]. Network meta-analysis assumes “consistency” of treatment effects across all included randomized trials, that is, the direct and indirect estimates are the same effects. Network consistency was evaluated by comparing the direct estimates to the indirect estimates using the node splitting model. We used non-informative uniform and normal prior distributions in network meta-analysis. And we used a thinning interval of 500 for each chain and yielded 5000 iterations to obtain the posterior distributions of model parameters. Convergence of iterations was assessed using Gelman-Rubin-Brooks statistic. Trace plot and density plot were used to assess the convergence of the model. The summary effect of each comparison will be presented as point estimate (HR) and the corresponding 95% confidence interval (CI). The probability of each arm achieving the best rank among all the options was calculated and is presented as rankogram. The efficacy of different treatments was ranked using rankogram.

### Quality of evidence

We evaluated the quality of evidence of estimates from direct comparison and network meta-analysis following the GRADE approach. The quality of evidence has four levels orderly: High, moderate, low, and very low quality. In this approach, the quality of direct evidence from RCTs is high initially and can be rated down based on risk of bias, indirectness, imprecision, inconsistency, or publication bias. The quality of indirect evidence starts at the lowest level of direct evidence that contributes as the preferred loops to the indirect evidence, and can be rated down based on imprecision or intransitivity. Network meta-analysis combines both direct and indirect evidence to reach a more comprehensive result, thus, the quality of evidence from network meta-analysis is assigned with the higher level of the direct and indirect evidence.

## RESULTS

### Characteristic of included studies

We identified 350 potentially relevant articles without duplicates from database searches and manual searches. After initial screening of these records, we excluded 252 articles because they investigated neither neoadjuvant nor adjuvant therapy of pancreatic cancer. We detailedly assessed the remaining 98 articles by abstracts and excluded 68 not reporting randomized control studies. After assessing full texts of the potential eligible 30 articles, we included 14 articles<sup>[8,20-32]</sup> (13 trials) in the network meta-analysis (Figure 1). If a single trial was reported in different publications, we combined the data of the different publications. And if a single outcome in a same trial was reported in different publications, the result of the latest publication would be used. The ESPAC-1 trial<sup>[29]</sup> included three subgroups, as the subgroup with two-by-two factorial design was updated in the following report<sup>[28]</sup>; this subgroup comparison was recognized as ESPAC-plus trial<sup>[28]</sup> and the last two subgroups as ESPAC-1 trial<sup>[29]</sup> in this meta-analysis. Also, we included data from ESPAC-3-v1<sup>[25]</sup> which was not included in the ESPAC-1 trial to avoid duplication.

The methodological quality of the included 13 trials was high (Supplemental Table 1). Four trials did not report sequence and six trials did not report allocation concealment. Although blinding was not reported in any trial, the primary outcome (overall survival) would not be affected by blinding or not, and a low risk of bias was recognized. Finally, we included 13 high quality trials with 1591 participants in this network meta-analysis (Figure 2). A total of 1591 participants were randomized to receive either neoadjuvant CRT with surgery ( $n = 51$ ), surgery alone ( $n = 703$ ), surgery with adjuvant CT ( $n = 665$ ), or surgery with adjuvant CRT ( $n = 172$ ).

The characteristics of the 13 included trials are summarized in Table 1 and Supplemental Table 2. All of the included trials were two-arm studies except the ESPAC-1 plus trial<sup>[28]</sup>, which was a four-arm trial using a two-by-two factorial design. The recruitment period ranged from 3 to 8 years. Both pancreatic adenocarcinoma and invasive ductal pancreatic cancer were included in this meta-analysis. For trials including periampullary carcinoma, the data about periampullary cancer were excluded<sup>[8]</sup>. The median age ranged from 57 to 71.5 years old. Most (> 90%) of included participants had primary tumor stage T1-T3, and most of them had nodal status N0-N1. The schedule of CT or CRT can be recognized briefly in Table 1.

### Direct comparison meta-analysis of overall survival

Standard pairwise meta-analysis of direct comparisons was feasible for the following comparisons: neoadjuvant CRT with surgery *vs* surgery alone (2 trials,  $n = 104$ ), surgery with adjuvant CT *vs* surgery alone (7 trials,  $n = 1080$ ), and surgery with



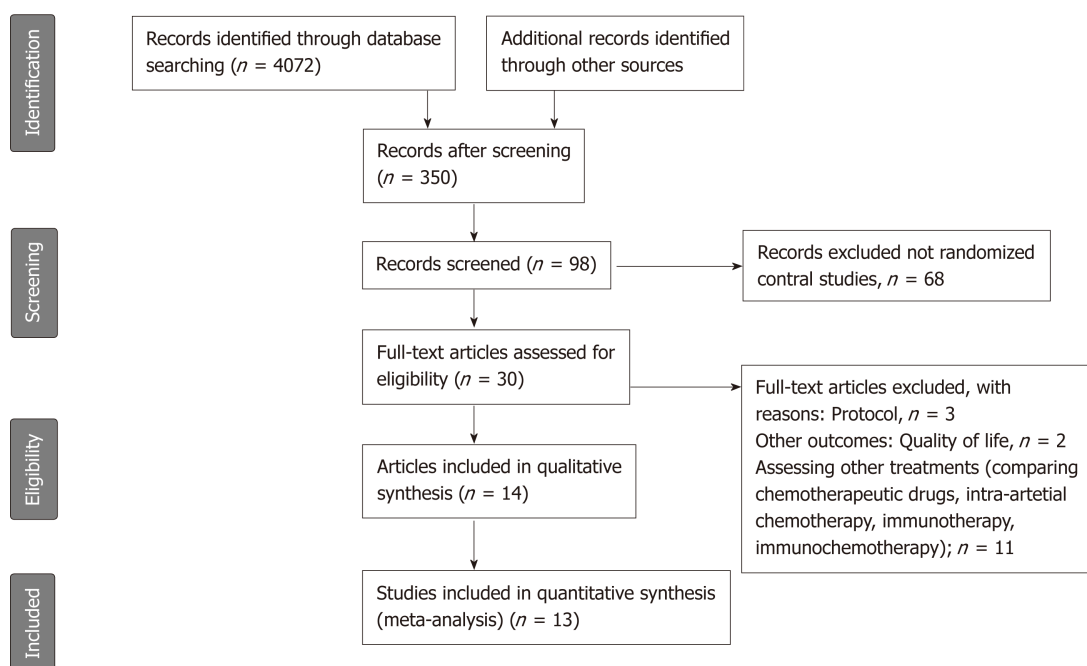


Figure 1 PRISMA flow chart of literature search showing the identification, screening, eligibility, and inclusion phases of the search.

adjuvant CRT *vs* surgery alone (3 trials,  $n = 254$ ), and surgery with adjuvant CRT *vs* surgery with adjuvant CT (1 trial,  $n = 90$ ). Only surgery with adjuvant CT showed a statistically significant survival advantage compared with surgery alone (pooled HR = 0.75, 95% CI: 0.63-0.89;  $P < 0.001$ ) (Figure 3). No statistical difference was found in other direct comparisons. Heterogeneity was found only in the comparison of surgery with adjuvant CRT *vs* surgery alone ( $I^2 = 72\%$ ). No publication bias was found using the funnel plot.

### Network meta-analysis of overall survival

All 12 trials reported information on survival and were included for Bayesian network meta-analysis. Density plot, trace plot, and Brooks-Gelman-Rubin diagnosis plot in Bayesian network meta-analysis of overall survival showed satisfied convergence of network plot model (Supplemental Figure 1). We summarize the result of network meta-analysis of overall survival in Figure 4. Surgery with adjuvant CT showed statistically better overall survival compared with surgery alone (pooled HR = 0.7, 95% CI: 0.62-0.79), which is similar to the results in direct comparison. Surgery with adjuvant CT also statistically improved survival compared with surgery with adjuvant CRT (pooled HR = 0.6, 95% CI: 0.54-0.72). No significant results were found between other comparisons (neoadjuvant CRT with surgery *vs* surgery alone, surgery with adjuvant CRT *vs* surgery alone, surgery with adjuvant CT *vs* neoadjuvant CRT with surgery, and surgery with adjuvant CRT *vs* surgery with adjuvant CT) (Figure 4).

Network meta-analysis results are consistent with the results from traditional pairwise meta-analysis, suggesting no inconsistency between direct and indirect evidence. We also compared the results of direct and corresponding indirect comparison using node-splitting model. No inconsistency was found (surgery with adjuvant CT *vs* surgery alone,  $P = 0.789$ ; surgery with adjuvant CRT *vs* surgery alone,  $P = 0.562$ ; and surgery with adjuvant CT *vs* surgery with adjuvant CRT  $P = 0.205$ ). Heterogeneity between studies was found using the random-effects model ( $I^2_{\text{pair}} = 59.9$ ;  $I^2_{\text{cons}} = 67.6$ ).

Rankogram (Figure 5) summarizes the ranking probability of the four treatment strategies in terms of overall survival. Surgery with adjuvant CT had the highest probability (94.2%) to rank the best in terms of improving overall survival, followed by surgery alone (5.8%), neoadjuvant CRT with surgery (0%), and surgery with adjuvant CRT (0%).

The results of grading the quality of evidence for overall survival are summarized in Table 2. Based on network meta-analysis, high quality evidence supported surgery with adjuvant CT over surgery alone for increasing overall survival. Moderate quality evidence supported surgery with adjuvant CT over surgery with adjuvant CRT for increasing overall survival.

Table 1 Study characteristics of included studies

Study	Arms	Number	Period	Country	Schedule
Casadei <i>et al</i> <sup>[20]</sup> , 2015	NCRT + S	18	2007-2014	Italy	2 cycles of gemcitabine 1000 mg/m <sup>2</sup> on days 1 and 8 every 21 d, then 45 Gy radiation with gemcitabine 50 mg/m <sup>2</sup> twice weekly for 6 wk
Golcher <i>et al</i> <sup>[21]</sup> , 2015	Surgery	20	2003-2009	Germany, Switzerland	8 Gy to 55.8 Gy (tumor) or 50.4 Gy (regional lymph nodes) radiation with gemcitabine 300 mg/m <sup>2</sup> and cisplatin 30 mg/m <sup>2</sup> on days 1, 8, 22, and 29
	NCRT + S	33			
Oettle <i>et al</i> <sup>[22,26]</sup> , 2007, 2013	Surgery	33	1998-2004	Germany, Austria	3 cycles of gemcitabine 1000 mg/m <sup>2</sup> on days 1, 8, and 15 every 4 wk
	S + ACT	179			
Kosuge <i>et al</i> <sup>[27]</sup> , 2006	Surgery	175	1992-2000	Japan	2 courses of cisplatin 80 mg/m <sup>2</sup> on the first day; 5-fluorouracil 500 mg/m <sup>2</sup> daily for the first 5 d
	S + ACT	45			
Ueno <i>et al</i> <sup>[24]</sup> , 2009	Surgery	44	2002-2005	Japan	3 cycles of gemcitabine 1000 mg/m <sup>2</sup> on days 1, 8, and 15 every 4 wk
	S + ACT	58			
Bakkevold <i>et al</i> <sup>[31]</sup> , 1993	Surgery	60	1984-1987	Norway	6 cycles of 5-fluorouracil 500 mg/m <sup>2</sup> , doxorubicin 40 mg/m <sup>2</sup> , and mitomycin C 6 mg/m <sup>2</sup> once every 3 wk
	S + ACT	30			
Smeenk <i>et al</i> <sup>[8]</sup> , 2007 Klinkenbijl <i>et al</i> <sup>[30]</sup> , 1999	Surgery	31	1987-1995	Europe	2 courses of 20 Gy radiotherapy (2 Gy/d, 5 d/wk at weeks 1-2 and 5-6) and 25 mg/kg 5-fluorouracil daily for 5 d
	S + ACDT	110			
Kalser <i>et al</i> <sup>[32]</sup> , 1985	Surgery	108	1974-1982	USA	2 courses of 20 Gy (5 d a week) radiotherapy and 500 mg/m <sup>2</sup> fluorouracil daily for 3 d
	S + ACDT	21			
Van Laethem <i>et al</i> <sup>[23]</sup> , 2010	Surgery	22	2004-2007	France	2 cycles of gemcitabine 1000 mg/m <sup>2</sup> weekly for 3 wk; followed by 50.4 Gy radiotherapy and 300 mg/m <sup>2</sup> gemcitabine weekly for two weeks
	S + ACT	45			
Neoptolemos <i>et al</i> <sup>[25,28,29]</sup> , 2001, 2004, 2009 (ESPAC-1)	S + ACDT	73	1994-2000	Europe	2 courses of 20 Gy radiotherapy and 500 mg/m <sup>2</sup> fluorouracil on days 1-3
	S + ACT	75			
					6 courses of fluorouracil 425 mg/m <sup>2</sup> and folinic acid 20 mg/m <sup>2</sup> daily for 5 d

	S + ACT + ACDT	72			2 courses of 20 Gy radiotherapy and 500 mg/m <sup>2</sup> fluorouracil on days 1-3; then 6 courses of fluorouracil 425 mg/m <sup>2</sup> and folinic acid 20 mg/m <sup>2</sup> daily for 5 d
ESPAC-1 plus	Surgery	69	1994-2000	Europe	2 courses of 20 Gy radiotherapy and 500 mg/m <sup>2</sup> fluorouracil on days 1-3
	S + ACDT	33			
	Surgery	36			6 courses of fluorouracil 425 mg/m <sup>2</sup> and folinic acid 20 mg/m <sup>2</sup> daily for 5 d
	S + ACT	97			
ESPAC-3 (V1)	Surgery	95	1994-2000	Europe	6 courses of fluorouracil 425 mg/m <sup>2</sup> and folinic acid 20 mg/m <sup>2</sup> daily for 5 d
	S + ACT	61			
	Surgery	61			

NCRT + S: Neoadjuvant chemoradiotherapy with surgery; S + ACT: Surgery with adjuvant chemotherapy; S + ACDT: Surgery with adjuvant chemoradiotherapy.

### Network meta-analysis of toxicity

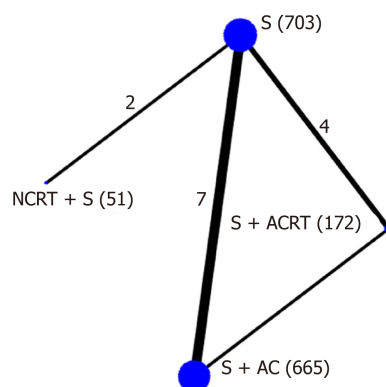
Data on toxicity were available in seven trials. We summarize all the reported toxicity events [nausea/vomiting, infection/Fever, asthenia/Fatigue, diarrhea, and hematological toxicity (leukopenia, thrombopenia, and anemia)] in Supplemental Table 3. Neoadjuvant or adjuvant CT and CRT were well tolerated, and grade 3 or 4 toxicities occurred infrequently. We summarize the result of network meta-analysis on overall toxicity and hematological toxicity in Figure 4. Density plot, trace plot, and Brooks-Gelman-Rubin diagnosis plot showed satisfied convergence of network plot model (Supplemental Figure 2). No significant differences in overall toxicity or hematological toxicity were found between all the comparisons (neoadjuvant CRT with surgery, surgery with adjuvant CRT, and surgery with adjuvant CT) (Figure 4).

## DISCUSSION

This study is the first analysis to compare efficacy of neoadjuvant therapies, adjuvant therapies, and surgery alone for resectable pancreatic cancer together in a single analysis. In our network meta-analysis, we included 13 high quality trials with 1591 participants. We demonstrated three principal findings in our analysis: surgery with adjuvant CT has better survival compared with surgery alone and surgery with adjuvant CRT; neoadjuvant CRT with surgery shows no significant difference in survival compared with surgery alone and adjuvant therapies; and toxicities after CT or CRT are well tolerated and show no significant difference among the treatment strategies included in this meta-analysis.

In our network meta-analysis, high quality evidence confirmed the survival advantage of adjuvant CT over surgery alone. Although overall survival associated with adjuvant CT had been evaluated in several head-to-head comparisons<sup>[22,24,25,27,31]</sup>, the absence of statistical significance led to equivocal conclusions<sup>[24,31]</sup>. Previous meta-analysis also demonstrated a survival difference when comparing surgery alone and surgery with adjuvant CT<sup>[12-14]</sup>. However, the most recent meta-analysis<sup>[13]</sup> was performed in 2007 and only included five randomized control studies. Moreover, it used only median survival time and 5-year survival rate instead of HRs to estimate survival difference, which was less precise. In our study, we estimated the survival difference by combining direct and indirect comparisons of different treatments. Moreover, we used both reported HRs and estimated HRs from all the included studies to minimize the selection bias. Thus, we provided the most powerful and reliable evidence that adjuvant CT is better than surgery alone in increasing overall survival for resectable pancreatic cancer.

The survival difference between adjuvant CT and adjuvant CRT for resectable pancreatic cancer remains controversial. Only a few studies demonstrated the



**Figure 2 Network plot.** Network plot showing the following different treatment strategies for resectable pancreatic cancer: neoadjuvant chemoradiotherapy with surgery (NCRT + S) ( $n = 51$ ), surgery alone (S) ( $n = 703$ ), surgery with adjuvant chemotherapy (S + AC) ( $n = 665$ ), or surgery with adjuvant chemoradiotherapy (S + ACRT) ( $n = 172$ ).

survival difference between adjuvant CT and adjuvant CRT<sup>[23,29]</sup>. A phase II randomized controlled study involving 90 participants compared the toxicity and survival between adjuvant gemcitabine alone and gemcitabine-based CRT, and no significant difference was found in survival due to small sample size<sup>[23]</sup>. The ESPAC-1 trial compared the survival using a two-by-two factorial design (observation, CRT alone, CT alone, or both)<sup>[29]</sup>. However, the trial was not powered to compare these four groups directly, and only found a potential benefit of adjuvant CT but not adjuvant CRT. In our study, moderate quality evidence supported surgery with adjuvant CT over surgery with adjuvant CRT for increasing overall survival. We confirmed the survival benefit of adjuvant CT over surgery with adjuvant CRT for the first time. Pancreatic cancer is a systemic disease and micrometastasis after surgery may be responsible for high recurrence and low survival. Thus, adjuvant CT but not CRT can benefit the survival of pancreatic cancer patients after surgery. However, CRT in the included studies was performed mainly using external beam, and more highly targeted radiotherapy is now available. The survival benefit between highly targeted radiotherapy and adjuvant CT should be reevaluated in the future study.

CT agents for adjuvant CT are diverse. It is still controversial regarding the best CT agents for adjuvant CT. The ESPAC-3 trial demonstrated that fluorouracil plus folinic acid resulted in similar overall survival to gemcitabine in patients after complete resection of pancreatic cancer<sup>[33]</sup>. A recent network meta-analysis showed that adjuvant CT with fluorouracil or gemcitabine provided better overall survival than observation<sup>[34]</sup>. S-1 is another new CT agent for pancreatic cancer. Recent randomized control trials showed that S-1 was superior to gemcitabine, suggesting that S-1 is a new standard care for resected pancreatic cancer<sup>[35-37]</sup>. In our study, CT agents for adjuvant therapy included gemcitabine<sup>[22,24]</sup>, cisplatin<sup>[27]</sup>, 5-fluorouracil plus doxorubicin plus mitomycin C<sup>[31]</sup>, and fluorouracil plus folinic acid<sup>[31]</sup>. We combined all of the adjuvant CT with different CT agents in a single arm in this network meta-analysis, because we assumed that the effect of different CT agents for adjuvant CT was consistent. Besides, we tried to compare the effect difference of adjuvant CT with adjuvant CRT and neoadjuvant CRT, and the effect difference was not affected by different CT agents.

The necessity and survival benefit of neoadjuvant therapy for pancreatic cancer is controversial. Borderline pancreatic cancer recently emerged as a category clinically distinct from resectable or locally advanced disease. Neoadjuvant therapy is currently recommended for borderline resectable disease in the National Comprehensive Cancer Network guidelines<sup>[38,39]</sup>. However, only two reported RCTs assess neoadjuvant CRT for resectable pancreatic cancer so far, and both two RCTs found no survival benefit of neoadjuvant CRT. One of the included RCTs involving 38 participants chose R0 resection as the primary endpoint<sup>[20]</sup>, and another RCT involving 66 patients was terminated early due to slow recruiting<sup>[21]</sup>. Neoadjuvant therapy is also assessed in our network meta-analysis. Only neoadjuvant CRT with surgery was assessed, as no RCTs about neoadjuvant CT can be found. We found no significant result when comparing neoadjuvant CRT with surgery alone, adjuvant CT, and adjuvant CRT. Now, several randomized controlled trials are ongoing to investigate the survival benefit of neoadjuvant CRT for the treatment of borderline and resectable pancreatic cancer<sup>[40-43]</sup>. Our result showed no survival benefit of neoadjuvant CRT. Thus, we should be cautious with using neoadjuvant CRT for resectable pancreatic cancer until other powerful evidence exists.

**Table 2 Pooled hazard ratio of overall survival from direct and network meta-analysis**

Intervention	Direct meta-analysis		Network meta-analysis	
	HR	Evidence	HR	Evidence
Compared to surgery alone				
Neoadjuvant CRT + S	0.96 (0.68, 1.37)	Low <sup>1,2</sup>	1.10 (0.64, 1.90)	Low
S + adjuvant CT	0.75 (0.63, 0.89)	High	0.70 (0.62, 0.79)	High
S + adjuvant CRT	0.88 (0.51, 1.54)	Moderate <sup>3</sup>	1.10 (0.97, 1.30)	Moderate
Compared to neoadjuvant CRT + S				
S + adjuvant CT	-	-	0.63 (0.36, 1.10)	Low
S + adjuvant CRT	-	-	1.00 (0.57, 1.80)	Low
Compared to S + adjuvant CT				
S + adjuvant CRT	0.98(0.59, 1.64)	Low <sup>4</sup>	1.6 (1.40, 1.80)	Moderate

<sup>1</sup>Risk of bias: one of included trial did not report allocation concealment and random sequence generation.

<sup>2</sup>Imprecision: small sample size.

<sup>3</sup>Inconsistency: heterogeneity was found in this comparison ( $I^2 = 72\%$ ).

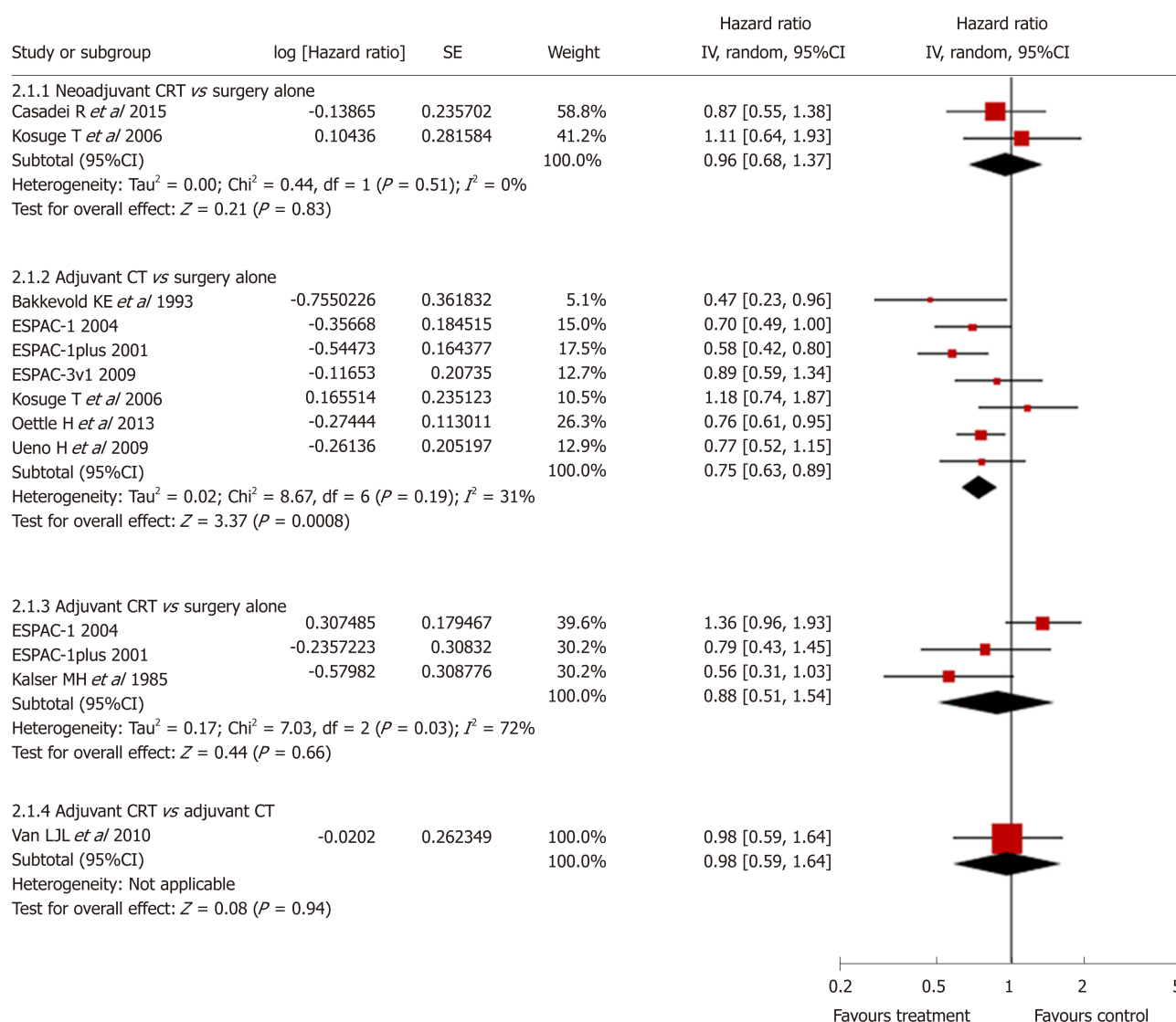
<sup>4</sup>Imprecision: wide confidence interval. HR: Hazard ratio; CRT: Chemoradiotherapy; S: Surgery; CT: Chemotherapy.

Our network meta-analysis has several strengths. It is the first comprehensive analysis of all the major treatment strategies for resectable pancreatic cancer including neoadjuvant therapy, surgery, and adjuvant therapy. We combined both direct and indirect evidence to reach more precise conclusions, which also allowed us to compare therapies indirectly and rank different therapies clearly. Furthermore, we assessed both overall survival and toxicity of all the therapies. Our meta-analysis provides comprehensive and clear evidence for the treatment of resectable pancreatic cancer, which is great important and meaningful in clinical care.

The limitations of this meta-analysis also need to be acknowledged. First of all, the RCTs included in this analysis were conducted over four decades, and changes in CRT schedule, CT agents, schedules, and surgery techniques may affect the results. However, transitivity assumption was met and there was no evidence of statistically significant inconsistency in this network. This may have less effect on the result. Second, we included both neoadjuvant and adjuvant therapies to offer a comprehensive overview. However, we included only a limited number of trials ( $n = 13$ ), and only two trials evaluated neoadjuvant therapies. Thus, although no significant result about overall survival was found when comparing neoadjuvant therapies with other treatments, this conclusion about neoadjuvant therapies should be interpreted with some caution. Finally, since the definition and reporting type of toxicity were diverse in the included studies, we only summarized seven typical toxicity events as overall toxicity. Although some toxicity events may be neglected in this analysis, the results should still provide effective estimates.

In conclusion, our network meta-analysis show that surgery with adjuvant CT prolongs overall survival compared with surgery alone and surgery with adjuvant CRT. Therefore, we recommend surgery with adjuvant CT as the optimal care for resectable pancreatic cancer. Later research should be focused on the best agents for adjuvant CT.

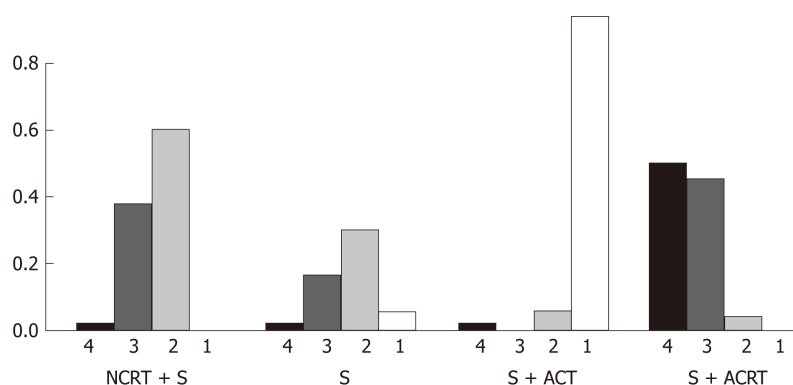




**Figure 3 Forest plot of direct comparison meta-analysis of overall survival.** Squares and horizontal lines correspond to the study-specific HRs and 95% CIs, respectively. The area of the squares correlates with the weight of each enrolled study, and the diamonds represent the summary HRs and 95% CIs. HRs: Hazard ratios; CI: Confidence interval.

A	S	1.1 (0.64, 1.9)	0.7 (0.62, 0.79)	1.1 (0.97, 1.3)
	Neoadjuvant CRT + S	0.63 (0.36, 1.1)	1.0 (0.57, 1.8)	
		S + adjuvant CT	1.6 (1.4, 1.8)	
			S + adjuvant CRT	
B	Neoadjuvant CRT + S	0.68 (0.21, 22)	0.33 (0.0064, 13)	
		S + adjuvant CT	0.50 (0.042, 3.8)	
			S + adjuvant CRT	
C	Neoadjuvant CRT + S	0.67 (0.019, 20)	0.33 (0.0053, 12)	
		S + adjuvant CT	0.49 (0.040, 3.9)	
			S + adjuvant CRT	

**Figure 4 Network meta-analysis of overall survival (A), overall toxicity (B), and haematological toxicity (C).** The column treatment is compared with the row treatment. Overall survival was estimated using pooled hazard ratios and 95% confidence intervals. Toxicity was estimated using pooled odds ratios and 95% confidence intervals.



**Figure 5 Rankogram of overall survival.** The height of column represents the probability of ranking the first (1) second (2), third (3), and fourth (4). NCRT + S: Neoadjuvant chemoradiotherapy with surgery; S: Surgery alone; S + ACT: Surgery with adjuvant chemotherapy; S + ACRT: Surgery with adjuvant chemoradiotherapy.

## ARTICLE HIGHLIGHTS

### Research background

Pancreatic cancer is one of the most common and lethal malignancies worldwide. The common treatment options for resectable pancreatic cancer include surgery alone, neoadjuvant chemotherapy (CT), neoadjuvant chemoradiotherapy (CRT), adjuvant CT, and adjuvant CRT. However, the optimal treatment is still controversial.

### Research motivation

The optimal treatment for resectable pancreatic cancer is still controversial.

### Research objectives

This study aimed to identify the most effective approach for resectable pancreatic cancer using network meta-analysis.

### Research methods

Eligible studies were searched from PubMed, Medline, EMBASE, Cochrane database, and Google scholar. We searched and included randomized controlled trials reporting on neoadjuvant and adjuvant therapies. For direct comparisons, standard pairwise meta-analysis was performed using the inverse variance DerSimonian-Laird random-effects model. For indirect comparisons, Bayesian network meta-analysis was used to combine direct and indirect evidence. We used relative hazard ratios (HRs) to estimate survival difference between different treatments, and relative odds ratios (ORs) for toxic effects. Treatment effects were ranked based on their efficacy for improving survival or reducing toxicity using rankogram. The quality of evidence of estimates from direct comparison and network meta-analysis were evaluated following the GRADE approach.

### Research results

We included 13 high quality trials with 1591 participants in this network meta-analysis. Compared with surgery alone (pooled HR = 0.7, 95%CI: 0.62-0.79) and surgery with adjuvant CRT (pooled HR = 0.6, 95%CI: 0.54-0.72), surgery with adjuvant CT had a higher rate of overall survival. In contrast, standard pairwise meta-analysis only showed a statistically significant survival advantage of surgery with adjuvant CT compared with surgery alone (pooled HR = 0.75, 95%CI: 0.63-0.89;  $P < 0.001$ ). Rankogram showed that surgery with adjuvant CT was most likely to rank the best in terms of overall survival (probability: 94.2%), followed by surgery alone (probability: 5.8%). No significant differences in overall toxicity or haematological toxicity were found between all the therapies. High quality evidence supported surgery with adjuvant CT over surgery alone for increasing overall survival. Moderate quality evidence supported surgery with adjuvant CT over surgery with adjuvant CRT for increasing overall survival.

### Research conclusions

Our network meta-analysis show that surgery with adjuvant CT prolongs overall survival compared with surgery alone and surgery with adjuvant CRT.

### Research perspectives

We recommend surgery with adjuvant CT as the optimal care for resectable pancreatic cancer. Later research should be focused on the best agents for adjuvant CT.

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# Single strain probiotics for dyslipidemia, fatty liver, and obesity: A systematic review and meta-analysis

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## Abstract

### BACKGROUND

A number of non-systematic reviews on the effects or mechanisms of probiotics on improving dyslipidemia, fatty liver, and obesity have been available but inconclusive to determine the independent effects of probiotics on each of the three conditions.

### AIM

To perform a systematic review and meta-analysis on potential benefits of probiotics among individuals with fatty liver or obesity or hyperlipidemia.

### METHODS

A systematic literature search was performed using PubMed and Embase. Adult participants of any gender without major comorbidities who received probiotics were considered following these criteria: (1) Studies on a single genus of probiotics with or without prebiotics; (2) Studies specifying the probiotic dosage into colony-forming units (CFUs); and (3) Studies on food-based probiotics were excluded. The primary outcome measures for fatty liver, obesity, and dyslipidemia were fibrosis score (kPa), body mass index (BMI; kg/m<sup>2</sup>), and serum lipid profiles (mg/dL), respectively. The secondary outcome measures for fatty liver and obesity were liver enzymes (U/L) and subcutaneous fat area (cm<sup>2</sup>).

### RESULTS

A total of 13 articles, published between 1997 and 2018, fulfilled the selection criteria. Three probiotics were included, of which *Lactobacillus* was the most commonly studied (10 studies), followed by *Bifidobacterium* (two studies) and

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*Pediococcus* (one study). Probiotics significantly reduced BMI ( $P = 0.013$ ), total cholesterol ( $P = 0.011$ ), and low-density lipoprotein ( $P = 0.006$ ) while increased high-density lipoprotein ( $P = 0.028$ ); high heterogeneities were observed. Only *Lactobacillus* could decrease triglyceride level ( $P = 0.005$ ) with low heterogeneity. No included studies reported fibrosis score, liver functions, subcutaneous fat outcomes.

## CONCLUSION

Single probiotics, especially *Lactobacillus*, have a potentially beneficial effect on improving obesity and dyslipidemia. Evidence on the fatty liver is limited.

**Key words:** Fatty liver; Obesity; Hyperlipidemia; Dyslipidemia; Probiotics; Non-alcoholic fatty liver disease; Overweight

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**Core tip:** No consensus is available about the benefit of single probiotics on improving dyslipidemia, fatty liver, and obesity. This meta-analysis investigated the effect of single, non-food-based probiotics, with specified dosage and duration, on body mass index, serum lipid profiles, fibrosis score, liver functions, and subcutaneous fat.

**Citation:** Pongpirul K, Janchot K, Dai Y. Single strain probiotics for dyslipidemia, fatty liver, and obesity: A systematic review and meta-analysis. *World J Meta-Anal* 2019; 7(6): 323-338

**URL:** <https://www.wjgnet.com/2308-3840/full/v7/i6/323.htm>

**DOI:** <https://dx.doi.org/10.13105/wjma.v7.i6.323>

## INTRODUCTION

The gut microbiota is a diverse and dynamic collection of micro-organisms that live in the human gastrointestinal tract. They are essential for maintaining the health of the human host in the "symbiosis" state whereas a "dysbiosis" could lead to a number of diseases or worsen health conditions<sup>[1]</sup>. Probiotics are live bacteria and yeasts that are presented either in "functional food" (*i.e.*, fermented food such as yogurt, cheese, miso, kimchi, and kefir) or as supplements in several forms. Probiotics have been claimed to boost the digestive system, support the immune system and reduce the risks associated with metabolic syndrome<sup>[2]</sup>.

There are three main types of fat metabolism disorders: Dyslipidemia, fatty liver, and obesity. Identified as a major risk factor for cardiovascular disease (CVD), dyslipidemia has been the main point of scientific interest affecting clinical practice especially pharmacological intervention<sup>[3]</sup>. Metabolic activity of the gut microbiota has been proposed as an influencer of the human serum lipid content<sup>[3]</sup>; it was estimated that 1% reduction in serum total cholesterol level could yield as high as 3% reduction in CVD risk<sup>[4]</sup>. Probiotics that exhibit a cholesterol reduction effect is of great interest because they are safer and usually cheaper than chemical drugs. Potential mechanisms for the cholesterol reduction effect of probiotics consumption have been discussed in a recent review<sup>[3]</sup>.

Non-alcoholic fatty liver disease (NAFLD) has been the most common chronic liver disease along with the prevalent obesity worldwide. The alteration of gut microbiota has been shown to promote the development of NAFLD by mediating processes of inflammation, insulin resistance, bile acids, as well as choline metabolisms<sup>[5]</sup>. Probiotics are one of the common ways to manipulate the gut microbiota as part of NAFLD management.

The number of overweight (body mass index; BMI 25-29.9 kg/m<sup>2</sup>) or obese (BMI ≥ 30 kg/m<sup>2</sup>) individuals has been rising worldwide<sup>[6]</sup>. The gut microbiota synthesizes short-chain fatty acids and amino acids, ferment otherwise indigestible carbohydrates, and contribute to the energy supplied to the animal and human host<sup>[7,8]</sup>. Evidence on the association between bacterial richness/dysbiosis and weight loss<sup>[9]</sup> suggested that modifying gut microbiota including probiotics administration is a potential target for obesity treatment<sup>[10]</sup>.

Unlike other conventional interventions, the practical uses of probiotics have greatly varied. As mentioned earlier, probiotics could be in functional food or as a

supplement. They could be used as live organisms with the unclear quantified amount; commonly measured in colony-forming units (CFU). Assessment of a single probiotic is scientifically difficult since more than one genus/species/strains are commonly offered simultaneously. Probiotics are usually regarded as supplements, so their therapeutic effects do not require to be supported by robust scientific evidence by the national food and drug authorities. Although conducting a randomized controlled trial (RCT) on this type of complex intervention is relatively more difficult than other interventions, a substantial amount of clinical experiments on probiotics have been prevalent in a variety of healthy and disease-specific study populations.

A number of non-systematic reviews on the effects or mechanisms of probiotics on improving dyslipidemia<sup>[3]</sup>, fatty liver<sup>[5]</sup>, and obesity<sup>[10]</sup> have been available. However, previous reviews could not determine the independent effects of probiotics on each of the three conditions. Also, many reviews could not differentiate the effects of various amounts of probiotics, especially when mixed and/or food-based probiotics were explored.

This systematic review aimed to identify clinical trials on the use of probiotics alone or in combination with prebiotics for improving fatty liver, obesity, or dyslipidemia. The selected studies must quantify the number of probiotics and explicitly describe the outcome measures. This review did not restrict to any specific kind of probiotics or any country. Probiotics in functional foods or combined in a mixture with substances other than prebiotics were excluded.

## MATERIALS AND METHODS

### Protocol and registration

This systematic review has been registered in PROSPERO (CRD42019125511) and the protocol ID=CRD42019125511.

### Literature search

The conducting and reporting of this systematic review and meta-analysis followed the PRISMA statement guidelines<sup>[11]</sup> whereas the inclusion criteria reporting followed the PICOS scheme. A systematic literature search was performed by two independent authors (KJ and YD) using PubMed and Embase. The search was limited to human subjects and English language. Adult individuals of any gender who received probiotics were considered as the intervention group whereas those who received placebo were considered as the comparator group. Only controlled trials with and without randomization were included. The search strategy was based on various combinations of words for both database and focused on two main concepts: probiotics and fat metabolism. The last search was conducted on March 1, 2019.

For the PubMed database the following combination was applied: ((Overweight-[Mesh] OR overweight[tiab] OR obese[tiab] OR obesity[tiab] OR "body weight"[tiab] OR "body mass index"[tiab]) OR ("Fatty Liver"[Mesh] OR "fatty liver"[tiab] OR Fibroscan[tiab] OR Ultrasound[tiab] OR "liver function tests"[Mesh] OR "liver function tests"[tiab] OR "Aspartate Aminotransferases"[tiab] OR "Alanine Transaminase"[tiab] OR "Alkaline phosphatase"[tiab] OR "gamma-glutamyl transpeptidase"[tiab] OR albumin\*[tiab] OR bilirubin[tiab]) OR (Dyslipidemias[Mesh] OR dyslipidemia\*[tiab] OR Hyperlipidemia[tiab] OR Hyperlipoproteinemias[tiab] OR Hypertriglyceridemia[tiab] OR Hypercholesterolemia[tiab] OR Cholesterol[Mesh] OR cholesterol[tiab] OR plasma lipids[tiab] OR Triglycerides[Mesh] OR Triglyceride\*[tiab] HDL[tiab] OR LDL[tiab] OR VLDL[tiab])) AND ((Probiotics[Mesh] OR probiotics[tiab] OR probiotic[tiab] OR (Synbiotics[Mesh] OR synbiotics[tiab] OR synbiotic[tiab]) OR (Lactobacillales[Mesh] OR Lactobacillales[tiab] OR Lactobacillus[tiab] OR Pediococcus[tiab] OR Leuconostoc [tiab] OR Oenococcus[tiab] OR Weissella[tiab] OR Lactococcus[tiab] OR Streptococcus[tiab]) OR (Bifidobacteriales[Mesh] OR Bifidobacteriales[tiab] OR Bifidobacterium[tiab] OR Aeriscardovia[tiab] OR Allosiscardovia[tiab] OR Bifidobacterium[tiab] OR Bombiscardovia[tiab] OR Galliscardovia[tiab] OR Gardnerella[tiab] OR Neoscardovia[tiab] OR Parascardovia[tiab] OR Pseudoscardovia[tiab] OR Scardovia[tiab]) OR (Saccharomyces[Mesh] OR Saccharomyces[tiab])) AND (Humans[Mesh] AND English[lang])).

For the Embase database the following combination was applied: ('obesity'/exp OR 'adipositas':ti,ab OR 'adiposity':ti,ab OR 'alimentary obesity':ti,ab OR 'body weight, excess':ti,ab OR 'corpulency':ti,ab OR 'fat overload syndrome':ti,ab OR 'nutritional obesity':ti,ab OR 'obesitas':ti,ab OR 'obesity':ti,ab OR 'overweight':ti,ab OR 'body weight'/exp OR 'body weight':ti,ab OR 'total body weight':ti,ab OR 'weight, body':ti,ab OR 'body mass'/exp OR 'bmi (body mass index)':ti,ab OR 'quetelet

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### Study selection

A systematic review management software, Covidence, was used. The titles and abstracts of the primary studies identified in the electronic search were screened by the same two authors. The duplicate studies were excluded. The following inclusion criteria were set for inclusion in this meta-analysis: (1) Controlled clinical experiments with or without randomization on individuals of any gender at least 18 years of age who received probiotics with or without prebiotics for improving fatty liver, obesity, or dyslipidemia; and (2) Studies containing fibrosis score (kPa), body mass index (BMI; kg/m<sup>2</sup>), serum lipid profiles: Total cholesterol (TC; mg/dL), high density lipoprotein (HDL; mg/dL), low density lipoprotein (LDL; mg/dL), triglyceride (TG; mg/dL), liver enzymes: Alanine transaminase (ALT; IU/L), aspartate transaminase



(AST; IU/L), alkaline phosphatase (ALP; IU/L), gamma-glutamyl transpeptidase (GGT; IU/L), subcutaneous fat (%), subcutaneous fat area (cm<sup>2</sup>). The following exclusion criteria were set: (1) Review articles, letters, comments and case reports; (2) Studies on food-based probiotics (*e.g.*, yogurt, fermented/sour milk, soy product); (3) Studies where it was impossible to convert the probiotic dosage into colony-forming units (CFUs); and (4) Studies where it was impossible to calculate the outcomes of interest. The trial authors were requested if incomplete data were reported. If the trial authors did not respond within two weeks, only available data were used. Any disagreement was resolved through discussion and the final determination was made by the first author (KP).

### Data extraction

The same two authors extracted the data for the following variables: (1) Authors, year of publication, and study type; (2) Genus, species, and characteristics, including dosage of the probiotics; and (3) Clinical outcomes, including fibrosis score, BMI, serum lipid profile, liver enzymes, subcutaneous fat. All relevant text, tables, and figures were examined for data extraction. Discrepancies between the two reviewers were resolved by the first author (KP).

### Risk of bias

Two authors (KJ and YD) independently assessed the risk of bias in the included trials using the Cochrane Risk of Bias tool 2.0 in the following domains: bias arising from the randomization process; bias due to deviations from intended intervention; bias due to missing outcome data; bias in the measurement of the outcome; and bias in the selection of the reported result. The reviewers resolved any disagreement by discussion and consensus.

### Additional analysis

The analysis was performed by the following subgroups: Intake duration (< 12 weeks *vs* 12 weeks), dose per day (< 100x10<sup>8</sup> CFU *vs* ≥ 100x10<sup>8</sup> CFU), and the presence of prebiotics (with *vs* without).

### Statistical analysis

Mean differences (MD) between the intervention and control groups, along with 95% Confidence Interval (95%CI) were reported for continuous variables. Clinical and methodological heterogeneity was assessed by examining participant characteristics, probiotics type, duration of probiotics usage and dose, outcomes, as well as the design of the study. Statistical heterogeneity was assessed using the *I*<sup>2</sup> and *X*<sup>2</sup> statistics. The level of heterogeneity as defined in Chapter 9 of the Cochrane Handbook for Systematic Reviews of Interventions: 0% to 40% might not be important; 30% to 60% may represent moderate heterogeneity; 50% to 90% may represent substantial heterogeneity; 75% to 100% considerable heterogeneity. For the *X*<sup>2</sup> test, statistical heterogeneity of the included trials was assessed with a *p*-value of less than 0.05 (statistically significant). The random-effects meta-analysis by DerSimonian and Laird method was used as clinical, methodological, and statistical heterogeneity was encountered. The meta-analysis was performed using Stata/MP software version 15 (StataCorp 2017, College Station, TX, United States).

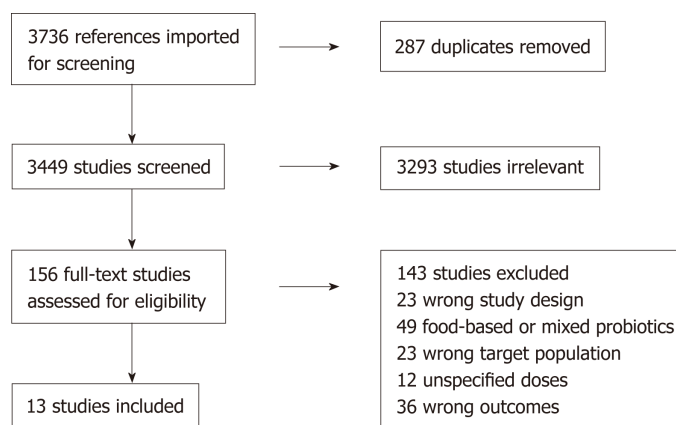
## RESULTS

### Study selection

The literature search yielded 3736 articles. After 287 duplicates were removed, 3449 titles and abstracts were screened, and 3293 irrelevant articles were removed. Of 156 articles selected for full-text screening, 143 were excluded for the following reasons: 23 were not controlled trials, 23 studies targeted irrelevant patient population, 49 studied focused on food-based probiotics and/or mixed probiotics, 12 did not specify the probiotic doses, 36 did not have quantifiable outcomes of interest. Finally, a total of 13 articles, dated between 1997 to 2018, fulfilled the selection criteria and were included in this meta-analysis<sup>[12-24]</sup> (Figure 1).

### Study characteristics

Included studies were published between 2006 to 2018 in various countries. All of the included studies are Randomized Controlled Trials (RCTs). Participants were randomly allocated to a control group or intervention group which reduce bias. Participants are 18 years of age or older which was related to inclusion criteria. Treatment periods were divided from 63 days to the longest period of 168 days (Table 1).



**Figure 1** Study selection.

### Probiotics

Ten studies used *Lactobacillus*, two studies used *Bifidobacterium*, and one study used *Pediococcus*. Due to the low number of studies accessing the effect of *Bifidobacterium* and *Pediococcus*, meta-regression for each genus was not performed. Especially, the study by Childs *et al.* intervened subjects by *Bifidobacterium* and *Bifidobacterium* plus a prebiotic. Meta-regression based on Childs's study suggested that *Bifidobacterium* had no significant impact on TC (SMD = 0.219; 95%CI: -0.213 to 0.651;  $P = 0.320$ ) and LDL (SMD = 0.00; 95%CI: -0.49 to 0.49;  $P = 1.000$ ). However, *Bifidobacterium*'s protective effect on HDL (SMD = 1.49; 95%CI: 0.51-2.47;  $P = 0.003$ ) and triglycerides (SMD = -0.40; 95%CI: -0.71 to -0.09;  $P = 0.011$ ) was significant.

### BMI

BMI was measured in seven trials before and after the administration of probiotic and placebo products (Figure 2). Overall, meta-analysis showed that probiotics significantly reduced BMI compared to placebo (SMD = -1.47; 95%CI: -2.63 to -0.13;  $P = 0.013$ ); however, between-study heterogeneity was high ( $I^2 = 95.5\%$ ;  $P = 0.000$ ). Subgroup analysis based on the type of probiotic genus revealed that *Lactobacillus* induced a great reduction in BMI (SMD = -1.56; 95%CI: -3.01 to -0.12;  $P = 0.034$ ) (Table 2). However, heterogeneity between studies in *Lactobacillus* was still large ( $I^2 = 96.1\%$ ;  $P = 0.000$ ).

### TC

A total of 10 studies examined the effects of probiotic on TC (Figure 3). The administration of probiotics was associated with significant decrease in TC levels (SMD = -0.72; 95%CI: -1.28 to -0.16;  $P = 0.011$ ), but with high heterogeneity ( $I^2 = 92.3\%$ ;  $P = 0.000$ ) between the studies. Subgroup analysis with regards to probiotic genus was performed. *Lactobacillus* significantly reduced TC levels (SMD = -0.72; 95%CI: -1.28 to -0.16;  $P = 0.011$ ). However, it is worth noting the high heterogeneity between studies ( $I^2 = 93.4\%$ ;  $P = 0.000$ ).

### LDL

The overall estimate of the ten studies showed a huge reduction in LDL in the treatment groups compared with the placebo groups (SMD = -0.85; 95%CI: -1.33 to -0.28;  $P = 0.006$ ), but the heterogeneity was large ( $I^2 = 91.3\%$ ;  $P = 0.000$ ) (Figure 4). The effect size was even larger in *Lactobacillus* group (SMD = -0.95; 95%CI: -1.62 to -0.28;  $P = 0.006$ ).

### HDL

An overall significant increase after intervention was reported for HDL levels in ten studies (SMD = 0.84; 95%CI: 0.09-1.59;  $P = 0.028$ ) (Figure 5). The effect of probiotic on HDL did not change much when it came to subgroup analysis for *Lactobacillus* (SMD = -0.95; 95%CI: -1.62 to -0.28).

### TG

The meta-analysis based on indicated a non-significant change in triglycerides post intervention (SMD = -0.06; 95%CI: -0.505 to 0.385;  $P = 0.792$ ) (Figure 6). However, an analysis for *Lactobacillus* showed a significant decrease in triglycerides (SMD = -0.32; 95%CI: -0.54 to -0.095;  $P = 0.005$ ) and with a low between-study heterogeneity ( $I^2 = 7.7\%$ ;  $P = 0.363$ ).

**Table 1** Characteristics of the included studies

First author	Year	Study period	Country	Study design	Participant, <i>n</i>	Age range (yr)	Treatment period (d)	Ref.
Simons	2006	2004-2005	Australia	Randomized, double blind, placebo-controlled	44	30-75	70	[24]
Ooi	2010	-	Malaysia	Randomized, double blind, placebo-controlled	32	18 years of age or older	84	[21]
Jones	2012	-	Canada	Randomized, double blind, placebo-controlled	127	20-75	63	[19]
Fuentes	2013	-	Spain	Randomized, double blind, placebo-controlled	60	18-65	84	[15]
Sanchez	2014	-	Canada	Randomized, double blind, placebo-controlled	93	18-55	168	[23]
Childs	2014	2008-2009	United Kingdom	Randomized, double blind, placebo-controlled, factorial, cross-over	42	25-65	21	[13]
Rajkumar	2015	-	India	Randomized, single blind, placebo-controlled	45	20-25	45	[22]
Ahn	2015	2012-2014	South Korea	Randomized, double blind, placebo-controlled	92	-	84	[12]
Higashikawa	2016	2013	Japan	Randomized, double blind, placebo-controlled	41	20-70	84	[17]
Fuentes	2016	2010	Spain	Randomized, double blind, placebo-controlled	60	18-25	84	[16]
Kim	2017	-	South Korea	Randomized, double blind, placebo-controlled	66	-	84	[20]
Costabile	2017	2015	United Kingdom	Randomized, double blind, placebo-controlled	46	18-50	84	[14]
Inoue	2018	-	Japan	Randomized, double blind, placebo-controlled	38	66-78	84	[18]

**Other outcomes**

No included studies reported fibrosis score, liver functions, subcutaneous fat outcomes.

**Risk of bias**

The analyses of the risk of bias of the included studies were summarized in **Figure 7**. Generally, all studies were classified as low risk of bias. Five articles clearly explained the methods used for randomization, while eight studies did not describe the process of randomizing. Twelve studies blinded the patients, researchers and outcome assessors whereas Rajkumar's study did not blind the patients and filed staff since the

**Table 2 Sensitivity analysis and subgroup analysis**

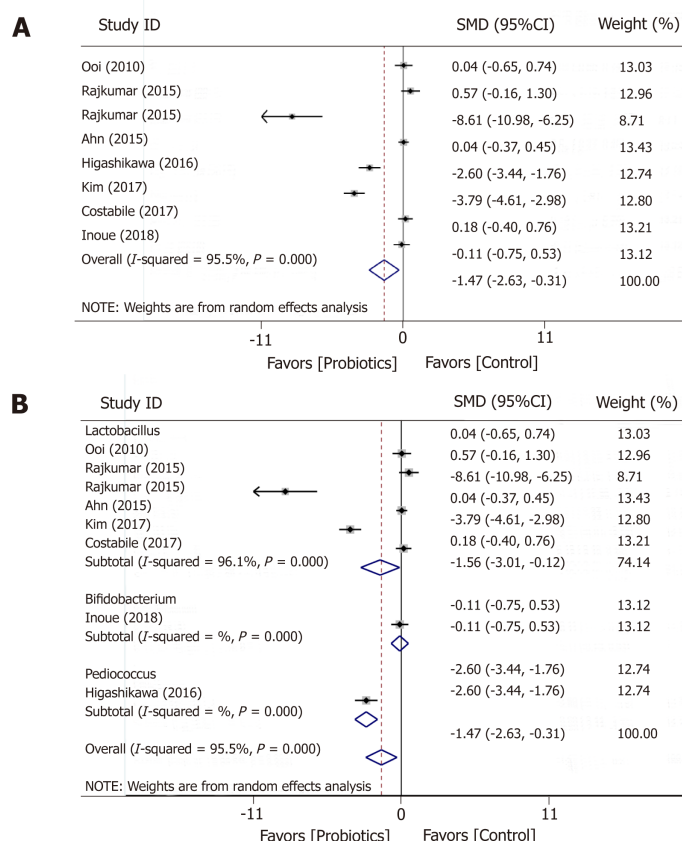
Subgroup/sensitivity analysis		No. of groups	SMD (95%CI)	P-value	Heterogeneity ( $I^2$ , P-value)
BMI					
Intake duration	< 12 wk	6	-0.535 (-0.782, -0.289)	0.000	95.1% (0.000)
	= 12 wk	2	-0.220 (-0.920, 0.479)	0.537	98.1% (0.000)
Dose per day	Low-dosage (< 100*10 <sup>8</sup> CFU)	4	0.024 (-0.351, 0.399)	0.900	94.4% (0.000)
	High-dosage (≥ 100*10 <sup>8</sup> CFU)	4	-0.829 (-1.125, -0.532)	0.00	96.7% (0.000)
Combined with or without prebiotics	Probiotic alone	6	-0.481 (-0.730, -0.233)	0.000	95.4% (0.000)
	Combined with prebiotics	2	-0.638 (-1.304, 0.027)	0.060	97.9% (0.000)
Total cholesterol					
Intake duration	< 12 wk	6	-0.225 (-0.443, -0.006)	0.044	86.9% (0.000)
	= 12 wk	4	-0.665 (-0.906, -0.424)	0.000	94.9% (0.000)
	> 12 wk	1	-0.200 (-0.607, 0.208)	0.337	
Dose per day	Low-dosage (< 100*10 <sup>8</sup> CFU)	9	-0.496 (-0.664, 0.328)	0.000	93.4% (0.000)
	High-dosage (≥ 100*10 <sup>8</sup> CFU)	2	0.031 (-0.310, 0.371)	0.860	0.00% (0.336)
Combined with or without prebiotics	Probiotic alone	9	-0.466 (-0.630, -0.303)	0.000	92.7% (0.000)
	Combined with prebiotics	2	0.004 (-0.377, 0.386)	0.983	93.6% (0.000)
LDL					
Intake duration	< 12 wk	4	-0.306 (-0.524, -0.088)	0.006	81.2% (0.000)
	= 12 wk	6	-0.871 (-1.113, -0.628)	0.000	94.1% (0.000)
	> 12 wk	1	-0.250 (-0.658, 0.159)	0.213	
Dose per day	Low-dosage (< 100*10 <sup>8</sup> CFU)	9	-0.558 (-0.725, -0.391)	0.000	91.9% (0.000)
	High-dosage (≥ 100*10 <sup>8</sup> CFU)	2	-0.333 (-0.685, 0.020)	0.064	92.7% (0.000)
Combined with or without prebiotics	Probiotic alone	9	-0.609 (-0.774, -0.445)	0.000	92.0% (0.000)
	Combined with prebiotics	2	-0.042 (-0.415, 0.331)	0.826	84.8% (0.010)
HDL					
Intake duration	< 12 wk	4	0.557 (0.329, 0.784)	0.000	94.0% (0.000)
	= 12 wk	6	0.273 (0.027, 0.520)	0.030	96.6% (0.000)
	> 12 wk	1	-0.501 (-0.914, -0.087)	0.018	
Dose per day	Low-dosage (< 100*10 <sup>8</sup> CFU)	9	0.445 (0.272, 0.617)	0.000	95.7% (0.000)
	High-dosage (≥ 100*10 <sup>8</sup> CFU)	2	-0.328 (-0.682, 0.025)	0.069	93.7% (0.000)
Combined with or without prebiotics	Probiotic alone	9	0.162 (-0.004, 0.329)	0.056	95.4% (0.000)
	Combined with prebiotics	2	1.158 (0.735, 1.581)	0.000	96.2% (0.000)
Triglycerides					
Intake duration	< 12 wk	4	-0.277 (-0.493, -0.061)	0.000	0% (0.614)
	= 12 wk	4	-0.135 (-0.411, 0.140)	0.336	93.0% (0.000)
Dose per day	Low-dosage (< 100*10 <sup>8</sup> CFU)	6	-0.298 (-0.489, -0.107)	0.770	97.4% (0.000)
	High-dosage (≥ 100*10 <sup>8</sup> CFU)	2	0.055 (-0.314, 0.424)	0.069	0.0% (0.567)
Combined with or without prebiotics	Probiotic alone	6	-0.185 (-0.376, 0.006)	0.058	88.3% (0.000)
	Combined with prebiotics	2	-0.368 (0.740, 0.004)	0.052	39.4% (0.199)

SMD: Standard mead difference; CI: Confidence interval; CFU: Colony forming unit; BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

capsules looked different. All studies explicitly explained the methods used for dealing with incomplete outcome data. Three studies worked well in allocation concealment whereas eight studies failed to make the process of allocation concealment clear. Two studies might have a high bias from the predictable allocation of intervention and placebo. At last, all studies had no problem with selective outcome reporting.

## DISCUSSION

This systematic review revealed that probiotics have a potentially beneficial effect on improving obesity and dyslipidemia. Probiotics were found to significantly decrease



**Figure 2** Meta-analysis forest plot concerning body mass index (A) and body mass index by genus (B).

BMI, levels of TC and LDL as well as increase HDL level. However, the effect of probiotics on TG was not statistically significant.

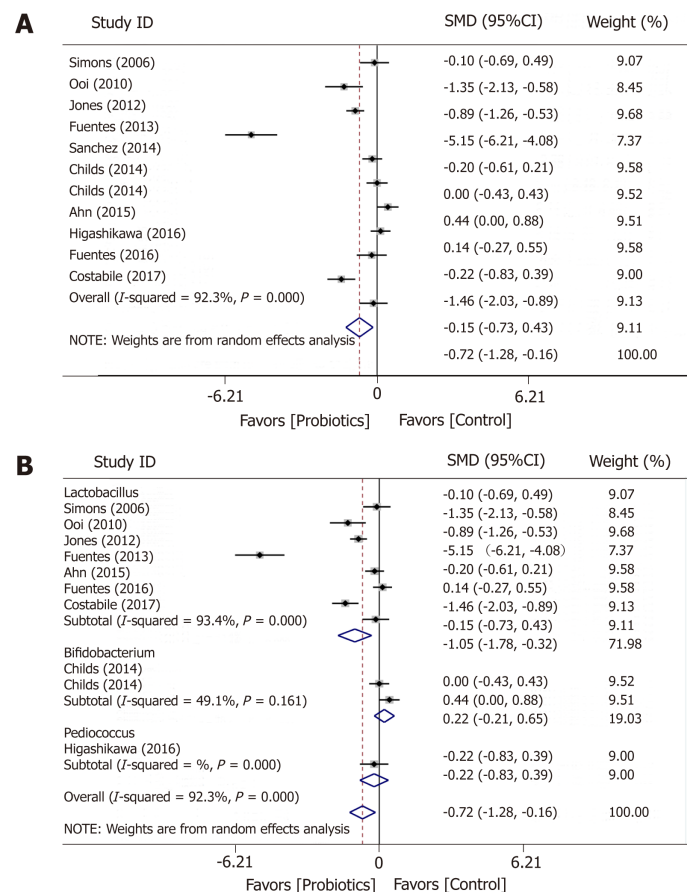
Our study filled the gap that previous studies assessing the effect of probiotics focused more on multiple probiotic strains by including trials using the single strain as treatment. Our results seem to be different from most of the previous studies. Sun's meta-analysis found that compared to multiple probiotic strains, a single strain did not have a significant effect on TC, HDL, and triglyceride<sup>[25]</sup>. However, due to the limited number of studies which intervened with a single strain included in their meta-analysis, caution is required while coming to the conclusion. In our study, 13 studies that used single strain as treatment are enrolled. The considerable number of trials enrolled guarantee more reliable results.

While existing studies showed that probiotics administered in different forms such as fermented milk, bread, tablet, powder or capsule had different effects on lipid profiles and BMI; in this meta-analysis, only studies not using food-based probiotic as interventions are included. Therefore, compared to previous studies, this meta-analysis could isolate the effects of probiotics from other supplements better. Multiple genera of probiotics could have different additive or synergistic effects in comparison with the single genus of probiotics. Hence, caution is needed to extrapolate multiple genera of probiotics' significant effects on lipid profiles and BMI to a single genus of probiotics. This meta-analysis fills the gap in this area. Thirteen studies are included in this meta-analysis. Compared to previous studies, a sufficient number of studies lead to more reliable conclusions.

Another impacted finding of this study is the daily consumption of probiotics more than  $100 \times 10^8$  CFU had a greater benefit on BMI reduction than daily dosage lower than  $100 \times 10^8$  CFU. Currently, there are no uniform standard regards to the among of daily intake of probiotics. This study suggests that to ensure an effect on reducing BMI, the number of probiotics may be more than  $100 \times 10^8$  CFU.

Another awaiting-to-answer question is the range of intake duration. Among 13 included studies, six trials treated subjects for less than 12 weeks, while the other six trials chose the exact 12 wk for intervention. Only one study intervened subjects for more than 12 wk. Considering studies' concentration around 12 weeks, we grouped studies into  $\leq 12$  wk and  $> 12$  wk. Omitting the study that was longer than 12 wk, sensitivity analysis showed that  $\leq 2$  wk' intake of probiotic has a significant effect on





**Figure 3** Meta-analysis forest plot concerning total cholesterol (A) and total cholesterol by genus (B).

TC, LDL, and HDL. Hence, although comparison among various lengths of administration terms should be done to further confirm the effect of administration term, we could come to a preliminary conclusion that intake duration of no more than 12 wk could ensure a significant effect of probiotics on TC, LDL, and HDL.

The meta-analysis revealed that probiotics did not significantly reduce the level of triglycerides. Subgroup analysis showed that when restricting studies to those whose duration of intake is less than 12 wk, the effect of probiotics on triglycerides became significant. This result was in agreement with other studies<sup>[25,26]</sup>.

A number of limitations of this study should be acknowledged. First, the findings were limited to fat metabolism but not metabolic syndrome as a whole. Second, the included studies did not report adverse effects, which indicates the safety and tolerance of probiotic capsules. Hence, when making a clinical recommendation of probiotic agents, adverse effects need to be taken into account and be carefully investigated. Third, limited studies reported effects of probiotics combined with other prebiotics. Two of the included studies reported synbiotics' effects on lipid profiles. Child's study found that compared with using Bifidobacterium alone, the combination with xylo-oligosaccharides resulted in a significant but modest change in HDL. In addition, Rajkumar's study showed a superior influence of synbiotics on lipid profiles in comparison to using probiotics alone. A further meta-analysis of more studies is required to confirm the augmentation of the impacts of probiotics alone on serum lipid profiles. Last but not least, crossover studies and parallel studies were included. Crossover studies have more methodological advantages and are easier to control individual-varying confounders compared to parallel RCTs. However, crossover studies could introduce additional bias when studies have insufficient washout periods. One of our included studies used crossover design with a washout period of 28 d. Whether the washout period is long enough to avoid additional bias needs further study.

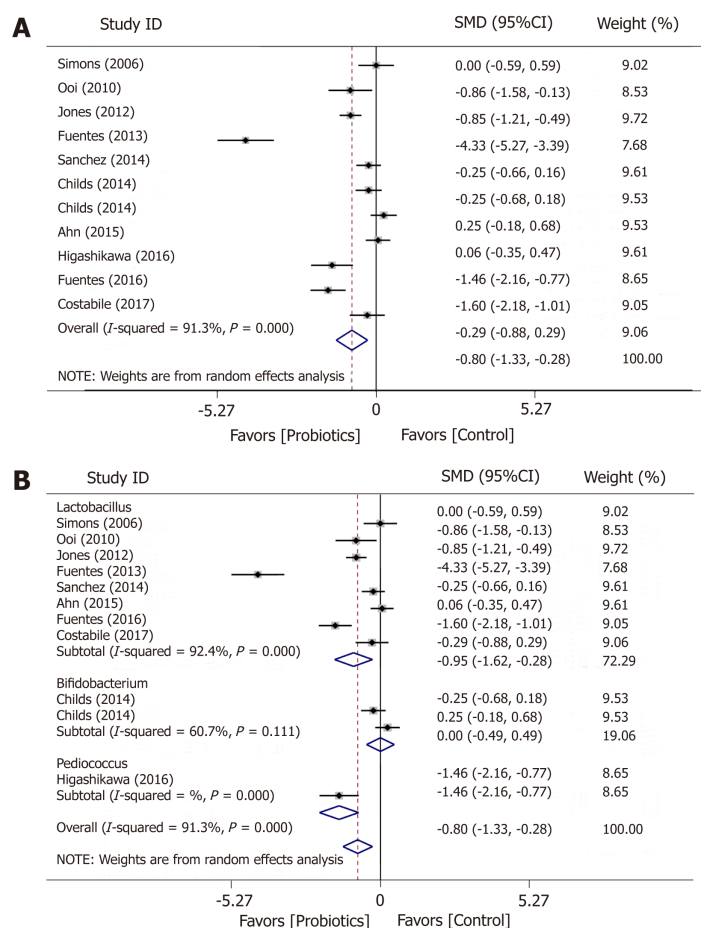


Figure 4 Meta-analysis forest plot concerning low density lipoprotein (A) and low density lipoprotein by genus (B).

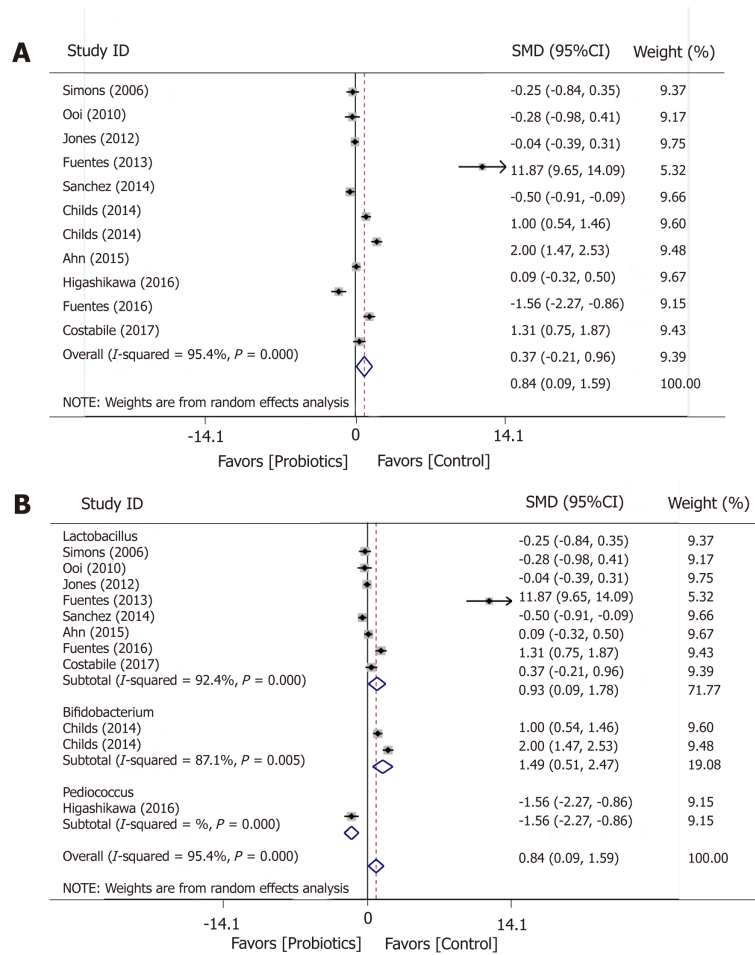


Figure 5 Meta-analysis forest plot concerning high density lipoprotein (A) and high density lipoprotein by genus (B).

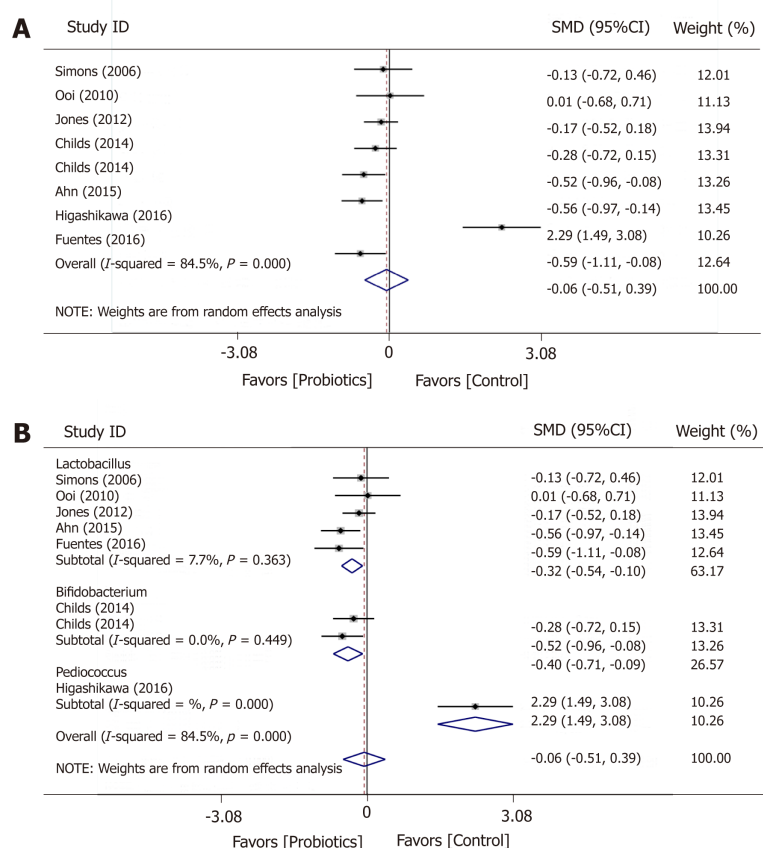


Figure 6 Meta-analysis forest plot concerning triglycerides (A) and triglycerides by genus (B).



Figure 7 Risk of bias of the included studies.

## ARTICLE HIGHLIGHTS

### Research background

An imbalance of the microorganisms could lead to many human diseases including dyslipidemia, fatty liver, and obesity. Probiotic supplementation has been considered an alternative treatment.

### Research motivation

Variety of probiotics has been available as 'healthy' products to consumers for many health purposes. These over-the-counter probiotics usually comprised of multiple probiotic strains with some health claims. Given limited evidence on the isolated effect of each probiotic strain, a systematic approach to synthesize current scientific evidence is essential.

### Research objectives

This study was aimed to identify clinical trials on the use of single probiotics alone or in combination with prebiotics for improving fatty liver, obesity, and dyslipidemia.

### Research methods

This systematic review and meta-analysis was conducted using a rigorous methodology and supported by the use of systematic review management software. Titles and abstracts of the primary studies listed in PubMed and Embase databases were screened by two assessors using standard sets of inclusion and exclusion criteria. Data from the included articles were extracted in order to synthesize the effect of single probiotics on specific outcome measures.

### Research results

A total of 13 randomized controlled trials were included. Three probiotics were included: *Lactobacillus* (10 studies), *Bifidobacterium* (2 studies), and *Pediococcus* (1 study). Probiotics significantly reduced BMI, reduced total cholesterol, reduced low-density lipoprotein, and increased high-density lipoprotein, compared to placebo; high study heterogeneities were observed. Only *Lactobacillus* could decrease triglyceride level with low heterogeneity. No included studies reported fibrosis score, liver functions, or subcutaneous fat outcomes.

### Research conclusions

This systematic review emphasizes the effects of single genus non-food-based probiotics on decreasing BMI, total cholesterol and low-density lipoprotein as well as increasing high-density lipoprotein levels.

### Research perspectives

Evidence on single genus probiotics is still limited. Additional clinical trials are needed for each of the single probiotics before combining two or more probiotics could be investigated.

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## Asymptomatic bacteriuria among hospitalized diabetic patients: Should they be treated?

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### Abstract

Diabetes Mellitus is a significant health care challenge in the United States. The Center for Disease Control and Prevention estimates approximately 9.4% of patients in the United States are afflicted by diabetes. The Infectious Disease Society of America asymptomatic bacteriuria in women as two consecutive clean-catch voided urine specimens with isolation of the same bacterial strain in counts  $\geq 10^5$  cfu/mL. It is understood that diabetic patients tend to be at higher risk for infections than non-diabetics. Urinary tract infections (UTIs) tend to be the most common infection contracted by this population. UTIs are not only a significant cause of morbidity and mortality, they are also a significant financial burden. The data are conflicting, in regard to treating asymptomatic bacteriuria in diabetic patients to avoid hospital complications and ultimately decrease healthcare costs associated with these complications. However, clinicians continue to prescribe antibiotics empirically. Further randomized controlled study looking into the specific population as immunocompromised diabetic patients, patient with diabetic ketoacidosis and patient in intensive care unit needs to be undertaken.

**Key words:** Asymptomatic bacteriuria; Diabetes mellitus; Hospitalized diabetics; Urinary tract infection

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**Core tip:** Urinary tract infections among diabetics can predispose patients to significant morbidity, mortality, and increased healthcare costs. Data remains controversial as it pertains to treatment of asymptomatic bacteriuria in hospitalized diabetics in reducing the risk of urinary tract infection, complications, and healthcare costs.

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## INTRODUCTION

It is known in the scientific community that diabetic patients are particularly susceptible to infections. Studies have suggested that diabetic patients are at four times higher risk of suffering from infections than non-diabetics<sup>[1]</sup>. Among infections in diabetics, urinary tract infections (UTIs) are the most common type of infection<sup>[2]</sup>. Prevalence of asymptomatic bacteriuria (ASB) is quite common among the diabetic population. The Infectious Disease Society of America (IDSA) ASB in women as two consecutive clean-catch voided urine specimens with isolation of the same bacterial strain in counts  $\geq 10^5$  cfu/mL in men IDSA recommends a single, clean-catch, voided urine specimen with one bacterial species isolated in a quantitative count of  $10^5$  cfu/mL to be defined as asymptomatic bacteriuria. For any asymptomatic patient, bacteriuria is defined as a single catheterized urine specimen with one bacterial species isolated in counts  $\geq 10^5$  cfu/mL<sup>[3]</sup>.

In a cross-sectional study, the prevalence of urinary tract infection (UTI) in diabetic patients was 16%<sup>[2]</sup>. What's more important is that the prevalence of ASB in diabetic patients has been shown to be at four times higher than the general population, however, whether ASB is a common precursor to UTIs and should ASB be treated or not is still inconclusive<sup>[4-8]</sup>.

## STUDY ANALYSIS

The diabetic population as whole are at higher risk for suffering from complications of UTIs which include renal and peri-renal abscess, emphysematous pyelonephritis, emphysematous cystitis, fungal infections, xanthogranulomatous pyelonephritis, and renal papillary necrosis<sup>[2]</sup>. Among diabetics, women, were found to have a higher incidence of UTIs than their male counterparts<sup>[3]</sup>. Consequently, diabetic women are also at higher risk of suffering from increased morbidity and mortality from UTI<sup>[4]</sup>. A study suggested that diabetic women are as much as 6 times to 24 times more likely than non-diabetic women to be admitted for acute pyelonephritis<sup>[5]</sup>. Whereas, diabetic men are 3.4-17 times more likely than their nondiabetic counterparts to be admitted for the same condition<sup>[6-9]</sup>.

UTIs are not only a significant cause of morbidity and mortality by elevating the risk of pyelonephritis, premature delivery, impaired renal function, and end-stage renal disease in patients, but also is a significant financial burden. The estimated annual cost of community-acquired UTI is significant, at approximately 1.6 billion USD/year and treatment of the same incurs in significant cost in the United States to about 1.6 billion dollars in 1995 with and about 25.5 billion USD over the course of 20 years<sup>[10]</sup>.

Given the clinical burden and economic cost of UTI it raises the question if the data that we currently have can be directly translated from a community over to a hospital setting and if ASB, a potentially treatable cause of UTI should be taken into consideration among the hospitalized diabetic patient?

In a study performed on hospitalized patients from 1996-2003 the rate of ASB was 12.76% (117 out of 917) and 11.4% (296 out of 2596) respectively in diabetic and nondiabetic males. The rate of ASB was 14.97% (229 out of 1529) and 13.1% (679 out of 5175) in diabetic and nondiabetic females, respectively<sup>[17]</sup>. Furthermore, prospective study done in two tertiary care university affiliated teaching hospitals demonstrated an overall prevalence of ASB to be 7.9% (85 cases per 1072 women) and a higher likelihood to have occult upper UTIs in certain aboriginal diabetic populations with lower level of education and socioeconomic status (53% of aboriginals *vs* 20% of non-aboriginals,  $P = 0.016$ )<sup>[6,9]</sup>. Studies among diabetic populations have found that the length of time with diabetes rather than diabetic control, as interpreted by hemoglobin A1c was shown to have an increased risk for both ASB and UTIs, however studies failed to mention how many, if any at all, of these patients were diagnosed in inpatient *vs* outpatient setting<sup>[11,11]</sup>. In another study, conducted in the year 2000, risk factors for ASB for Type II Diabetic women included: Age,

macroalbuminuria, a lower BMI, and a UTI during the previous year. ASB in Type II Diabetic women was noted to be an independent risk factor for UTIs<sup>[12]</sup>. The same study failed to demonstrate ASB as a risk factor for UTI in Type I Diabetics<sup>[12]</sup>. Sexual activity has also consequently been associated with ASB<sup>[13]</sup>. However, none of these studies manage to conclude any significance within the hospitalized population nor do they answer the question should treatment of ASB be beneficial to diabetic patients, particularly women. Some contest that rather than being condition or findings, we should consider this to be a complication of longstanding diabetes, along with albuminuria, and peripheral neuropathy<sup>[12,14]</sup>. In a large multicenter prospective study with an 18-month follow up did not demonstrate any significant association with ASB and renal function decline<sup>[15]</sup>. A long-term prospective study confirmed no significant association with ASB and renal function impairment in diabetic women at 6 years<sup>[16]</sup>.

Although, ASB does have some data that supports increased risk for symptomatic UTI, does this mean should we treat all ASB? Well, the question is more complicated than that. Should we treat depending on the pathogen? Several studies have demonstrated *E. Coli* to be the most commonly isolated bacteria in diabetic patients with ASB, this however is in keeping community acquired UTIs in non-diabetic patients as well<sup>[1]</sup>. Other studies in hospitalized patients demonstrate the contrary with more pathogenic organisms being isolated (*Klebsiella*, *Pseudomonas aeruginosa*) however no changes in antibiotic resistance were noted in comparison with non-diabetic patients<sup>[17,18]</sup>. Another emergency room based study demonstrated a correlation between diabetes and bacterial antibiotic resistance<sup>[19]</sup>.

## CONCLUSION

The question still stands; should ASB in diabetic patients be treated? Prospective randomized control trial done in 2002 comparing antimicrobial *vs* non antimicrobial therapy approach in diabetic women with ASB and followed for 36 mo, the study found no decrease in number of symptomatic episodes of hospitalizations during long term follow up and a high rate of recurrent bacteriuria after antibiotic treatment was given. The study went further to conclude no benefit to continued screening for and treatment of asymptomatic bacteriuria<sup>[20]</sup>. Current recommendations from the Infectious Disease Society of American Guidelines and United States Preventive Services Task Force recommend against routine screening of diabetic patients for asymptomatic bacteriuria<sup>[4,21]</sup>.

In conclusion, However, to our knowledge quality data and multiple high-quality studies are lacking. The conclusions are limited due to most of these studies focusing on the female population thus educated decisions for management of male diabetic patients is largely unclear. Further studies are required to determine the certain subpopulations of diabetic patients that would benefit, if any, especially for patients in ICU, patients with DKA or patients with significant immunosuppression from routine treatment of ASB. Some studies however have suggested the use of prophylactic measures such as probiotics may be beneficial to avoiding UTIs and its possible complications<sup>[22]</sup>. However, significant gaps in knowledge exists among the hospitalized patients. The jury is still out!

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## Artificial intelligence for endoscopy

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### Abstract

In recent times, there has been progressive development in artificial intelligence (AI) following the introduction of deep learning in the medical field including gastroenterology and endoscopy. Most of the reported studies were based on retrospective data. Several prospective studies of real-time diagnosis of moving images using the AI system are expected to match the real clinical situation and to aid the endoscopists in the detection and diagnosis of neoplasms without missing any lesion. AI can read a large number of endoscopic images in a few minutes and make a diagnosis; therefore, it is expected to cover the lack of support for the screening esophagogastroduodenoscopy in the health check-up and a large number of capsule images, thereby freeing the endoscopists from this burden. AI can help make the diagnosis during the endoscopic procedure and thereby prevent an unnecessary biopsy for patients taking antithrombotic drugs. AI can also be useful for education and training in endoscopy. Trainees can learn to perform endoscopy and the detection and diagnosis of lesions by the support of AI. In the near future, real-time endoscopic diagnosis using AI is expected to lessen the burden of endoscopists, to enhance the quality level of endoscopists, to overcome the miss of lesions and to make optimal diagnosis.

**Key words:** Artificial Intelligence; Endoscopy; Gastric cancer; Colonic neoplasm

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**Core tip:** Artificial intelligence (AI) has an increasing role in medical imaging in recent times. It has numerous benefits in the field of endoscopy. It aids in the accurate identification and diagnosis of lesions. AI also helps in reading and accurately interpreting large volumes of endoscopic images. It can play a role in the training of endoscopists as well.



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## ARTIFICIAL INTELLIGENCE FOR ENDOSCOPY

In recent times, there has been progressive development in artificial intelligence (AI) following the introduction of deep learning in various fields. As expected, AI has been introduced into the medical field, and many papers have reported on its use in different specialties in the medical field, including gastroenterology and endoscopy<sup>[1]</sup>.

In the field of endoscopy, conventional endoscopy, magnifying endoscopy and endocytoscopy using white light images and optical digital images for gastrointestinal neoplasia, gastritis related to *Helicobacter pylori* infection, and ulcerative colitis have been reported. Most of the reported studies were based on retrospective data. In addition, the results from these retrospective studies have been based on good-quality still images and not on either low-quality images or moving images<sup>[2,3]</sup>. Real-time imaging involves moving images from different levels in the endoscopic fields and not still images. Therefore, the results from retrospective studies might not match the real images. Several prospective studies of real-time diagnosis using the AI system have also been reported<sup>[4]</sup>. The data from these studies are expected to match the real clinical situation and to be more beneficial. For more robust clinical verification, well-designed multicenter prospective studies with adequate inclusion/ exclusion criteria that represent the target population are needed<sup>[1]</sup>. Moreover, the efficiency and accuracy of AI increases as the amount of data increases: For example, the use of moving images. Based on the prospective studies, real-time detection and diagnosis of lesions during endoscopic procedure are expected to become feasible. At present there is high sensitivity for the detection of gastric cancers; however, the specificity is not so good<sup>[5]</sup>, because most gastric cancers involve gastric inflammation as well, which makes the gastric mucosa red or white, irregular, and granular. Gastric inflammation causes erosion, ulcer, or polyp, and is relatively similar in appearance to the mucosa in gastric cancer. On the other hand, the diagnosis of colonic neoplasms using narrow band imaging (NBI), magnifying endoscopy with NBI, or endocytoscopy shows high sensitivity as well as specificity. Recently, EndoBRAIN software (Olympus Medical Systems, Japan) which is an AI system used in endocytoscopy for colonic neoplasms has become available in Japan. This allows the differential diagnosis of a colonic lesion and the confirmation of a colonic neoplasm to be made in a very short time. The endocytoscopy involves a special scope which magnifies the target lesion 520 times and enables observation at the cell level as with a microscope, whereas, EndoBRAIN software has not detected colonic polyps automatically yet.

Until now, it usually took huge time and effort for endoscopists to learn about the many gastrointestinal diseases and train in the endoscopic detection and diagnosis of gastric cancer or colonic neoplasm. Even when the endoscopists are experts, they might sometimes miss the detection and diagnosis of the neoplasms due to similar color of the lesions to the surrounding area, small size of the lesion, difficult location such as behind the folds or on the bending site, and lesions endoscopically observed in a moment among a lot of visual images. AI is expected to aid the endoscopists in the detection and diagnosis of neoplasms without missing any lesion.

AI also helps in the quick interpretation of endoscopic images taken in screening esophagogastroduodenoscopy (EGD) without waste of time. In Japan, screening EGD is conducted as part of the health check-up, and a double check of the screening EGD images is necessary. However, the number of endoscopists is not enough, especially in the local suburbs. Extensive time is required to interpret numerous endoscopic images, and the interpretation of endoscopic images is a burden for Japanese endoscopists. AI can read a large number of endoscopic images in a few minutes and make a diagnosis; therefore, it is expected to cover the lack of support for the screening EGD in the health check-up, thereby freeing the endoscopists from this burden. Moreover, there are numerous software that can easily detect lesions in capsule endoscopy. The interpretation of a large number of capsule images by the endoscopists takes a lot of time, and AI can free the endoscopists from the burden of capsule endoscopy image interpretation.

Lately, there has been a gradual increase in the number of patients taking antithrombotic drugs, and there is a hesitancy in performing a biopsy in these

patients. Although the guidelines for patients taking antithrombotic drugs is available not only in Western countries<sup>[6]</sup>, but also in Eastern countries<sup>[7,8]</sup>, and endoscopic biopsies are allowed in these patients, bleeding following a biopsy is sometimes observed. In addition, colonic polyps were sometimes resected and discard during colonoscopic procedure, therefore, it is important to decide the indication of resection and discard for colonic polyps. AI can help make the diagnosis in vivo during the endoscopic procedure and thereby prevent an unnecessary biopsy and resection, and careless discard.

AI can also be useful for education and training in endoscopy. Generally, instructors teach trainees on the various aspects of endoscopy. Even in the absence of an instructor in some hospitals, trainees can learn to perform endoscopy and the detection and diagnosis of lesions by the support of AI. However, if they always rely on AI for the diagnosis, the diagnostic acumen of the trainees does not improve. The trainees need to learn detection and diagnosis of lesions without the use of AI as well.

On the other hand, as deep learning algorithm is black-box, the machine-generated decision is sometimes hard to understand for endoscopists and it does not match the diagnosis by endoscopists. Therefore, the level of detection and diagnosis sometimes has to be checked periodically.

In the near future, real-time endoscopic diagnosis during endoscopic procedures with real moving images using AI is expected to become widespread in all endoscopic fields, to lessen the burden of endoscopists and to enhance the quality level of endoscopists. AI is desired to overcome the miss of lesions by endoscopy and to make endoscopic diagnosis comparable with optimal diagnostic accuracy by histopathological findings, and to reduce medical costs by avoiding pathological examination and unnecessary resection.

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# Phantom of the inflammasome in the gut: Cytomegalovirus

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## Abstract

Cytomegalovirus (CMV) is frequently detected in inflammatory bowel tissue, especially in corticosteroid-refractory patients, and it has been blamed for adverse outcomes. However, the first acquisition of CMV does not involve the colon. In particular in the colonic mucosa, which evolved due to the gut microbial relationship, CMV promotes inflammation *via* recruited monocytes and not through replication in resident macrophages. Whether CMV is the last straw in the process of mucosal inflammation, a doomed agent, or an innocent bystander is a difficult question that remains elusive. With this work, we will try to review the relationship between intestinal mucosa and CMV in the framework of basic virological principles.

**Key words:** Cytomegalovirus; Ulcerative colitis; Gancyclovir; Inflammatory bowel disease

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**Core tip:** We will here draw an analogy between the cytomegalovirus and the hero of the Gaston Leroux's "The Phantom of the Opera" novel, with the intestinal mucosa as the opera building. We aimed to emphasize the viral pathogenesis process to understand the elusive character of cytomegalovirus in the inflammatory bowel diseases.

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## INTRODUCTION

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Gaston Leroux's "The Phantom of the Opera" novel features a Paris opera building haunted by a phantom, an ugly genius of a man who had devoted his life to music and desperate love. The dungeons and tunnels that he created led to dynamic movements, enabling him to appear and disappear like a phantom all over the opera building. The protagonist may be interpreted as creative or destructive throughout the novel. He is a great composer and he makes clever devices, while strangling people with a Punjab lasso at the same time. What circumstances push the protagonist to lash out with violence and destruction? While that question is of interest to literary critics, we will here draw an analogy between the cytomegalovirus (CMV) and the hero of the aforementioned novel, with the intestinal mucosa as the opera building. For that, however, we must look at the basic viral pathogenesis before any deeper analysis.

## VIRUSES

Viruses are nonliving particles, and their replication is entirely dependent on the ability to infect the cells of their hosts<sup>[1]</sup>. This obligate dependence should suggest that every conditional change must be of some interest to the host as well as the virus. The first step in viral infection is entry into the target cell *via* specific receptors that provide tropism and fusion to the cells. Glycoprotein complexes act as an entry and fusion activator<sup>[2]</sup>. The second step in viral infection is replication and the production of new virions to spread<sup>[3]</sup>. After this step, there is another important component to complete infection: The immune response. A nonspecific role is played by the host cell's intrinsic defenses: Apoptosis, autophagy, RNA silencing, and antiviral proteins, while pathogen-specific responses harness the innate and adaptive immunity process<sup>[4]</sup>. Intracellular detection of viral infection occurs *via* receptors (Toll-like receptors, RIG, MDA) on cellular compartments (cytoplasm, plasma, and endosomal membranes)<sup>[5-7]</sup>. Following recognition, virus-infected cells and uninfected sentinel cells (dendritic cells, macrophages, natural killer cells) produce interferons in response to cellular products. Cytokines, both proinflammatory and anti-inflammatory, and chemokines are complementary elements in the ongoing inflammation<sup>[8]</sup>. Encoding cytokine homologs to block receptors and soluble cytokine receptors to neutralize cytokines, or altering the cytokine signaling pathway, are the preferred targets in herpesvirus survival strategies<sup>[9]</sup>. This diversity in the virus-host interaction causes different inflammatory stimulations that differ in clinical presentation. In particular, non-cytopathic viruses do not stimulate inflammation and may persist over a long duration. However, encoding at least one regulator of intrinsic/innate defenses is an essential component of viral pathogenesis.

## CYTOMEGALOVIRUS

CMV is a member of the  $\beta$ -herpesvirus subfamily. The virion consists of a 235-kb double-stranded linear DNA core in an icosahedral nucleocapsid, enveloped by a proteinaceous matrix. Nearly 200 of its genes encode proteins, but some express only noncoding RNAs, including approximately 14 microRNAs<sup>[10]</sup>. The genes with functions beyond transcription and proliferation necessitate a look at the human-CMV interaction from a co-evolutionary point of view. For instance, some virally encoded proteins show homology with the human chemokine receptor family<sup>[9]</sup>. Thus, this lifelong interaction may have a positive effect on our immunity, which can be revealed through animal studies<sup>[11]</sup>.

Worldwide CMV seroprevalence has increased from 40% to 99%, and population-based studies have shown that young children are an important source of CMV for childbearing women<sup>[12,13]</sup>. A survey has also demonstrated that primary school-age children continue to shed CMV in urine and live viremia at higher rates when compared with older children<sup>[14]</sup>. Moreover, 18-30 year-old college students shed CMV in saliva and urine without antibody response<sup>[15]</sup>. Notably, neither children nor college students experienced any clinical conditions. In contrast, mother-to-child transmission can occur even in the womb or during birth, as well as through breastfeeding. The association between fetal infection or frailty and human-CMV interactions from the beginning to the end of life is being investigated by researchers<sup>[16]</sup>.

In light of the latest data, platelet-derived growth factor receptor alpha (PDGFR- $\alpha$ ) has been identified as an entry receptor that forms a heterotrimeric complex with gH/gL/gO in fibroblasts<sup>[17]</sup>. Additionally, while cell line-based *in vitro* studies show some proteins, such as neuropilin 2, act as epithelial/endothelial receptors, in real life most of these receptors are found inside the immune cells<sup>[18,19]</sup>. Fibroblasts, a type of

stromal cells, endogenously express PDGFR. In a previous report, perivascular stromal cells were found to be susceptible to CMV infection in an ulcerative colitis murine model *via* PDGFR- $\beta$  and CXC chemokine ligand 12<sup>[20]</sup>. Additionally, in another investigation, more PDGFR $\alpha^+$  cells (smooth muscle cells) were found in the distal than in the proximal colon, which may be related to the frequency of CMV colitis rather than cell involvement<sup>[21]</sup>. If the inflammasome affects stromal and not epithelial cells, it may be inferred that CMV participates in the ongoing process at least *via* its immunomodulatory effect. In contrast, it is interesting to think that the lifelong persistence of the virus and the protective and dormant structure of the epithelial/endothelial cells may interact in terms of the infectious process.

CMV persists (latency) over the host's lifetime in specific progenitor cells that undergo reprogramming from hemopoietic stem cells<sup>[22,23]</sup>. This latency is broken intermittently through viral reactivation that is controlled by the adaptive immunity<sup>[24]</sup>. Moreover, monocyte recruitment to the relevant locations is the main mechanism in clinical manifestations of CMV<sup>[25]</sup>. In particular in the colonic mucosa, which evolved due to the gut microbial relationship, CMV promotes inflammation *via* recruited monocytes and not through replication in resident macrophages<sup>[26]</sup>. Although monocyte recruitment is essential in the effective control and elimination of viral, bacterial, fungal, and protozoal infections, it is worth questioning whether the intruder, here CMV, can alter the infection dynamics on its own. As mentioned before, we postulate that CMV plays a role akin to "The Phantom of the Opera" in the mucosa, with a balance between creative and destructive behaviors. Like the Phantom, CMV has gained a bad reputation, especially where inflammatory diseases are concerned, whereas a viral genome study revealed a higher ebstein barr virus (EBV) load in mucosal samples<sup>[27]</sup>. Both the CMV and EBV encode a viral ortholog of cellular interleukin-10 that impedes inflammatory responses and modulates host immunity<sup>[28]</sup>.

The rumors about the tortures inflicted by the Phantom show similarities with the existence of CMV and inflammations flaring in the mucosa. Be they a sign of direct or indirect pathogenicity, the first acquisition of CMV does not involve the colon. Whether CMV is the last straw in the process of mucosal inflammation, a doomed agent, or an innocent bystander is a difficult question that remains elusive. Thus, another important question follows on: To treat or not to treat?

CMV is frequently detected in inflammatory bowel tissue, especially in corticosteroid-refractory patients, and it has been blamed for adverse outcomes. Ganciclovir treatment is preferred by some clinicians, with or without other immunomodulatory drugs. Since clinical relevance and treatment efficacy have not been determined precisely, an accepted approach is not available. Many observational studies and a few meta-analyses have been carried out on the effect that ganciclovir treatment has on CMV reactivation in inflammatory bowel diseases<sup>[29]</sup>. Unfortunately, these uncontrolled and selection bias studies have not delivered adequate conclusions. Colectomy rates show high variability in both ganciclovir-treated and untreated groups.

## CONCLUSION

Researchers should focus the novel basic scientific data about the host and CMV interaction and re-review the clinical definitions, and treatment effectiveness of antivirals in the light of the evolutionary perspective.

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# Pediatric recurrent *Clostridium difficile* infections in immunocompetent children: Lessons learned from case reports of the first twelve consecutive patients

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## Abstract

### BACKGROUND

Recurrent *Clostridium difficile* infection (CDI) in children can be difficult to manage and may represent an unidentified underlying pathology. Recurrence can be frequently encountered in immunodeficiency disorders and inflammatory bowel disease (IBD).

### AIM

To report cases of a select population of children with recurrent CDI who are immunocompetent and do not have an identified IBD and examine the potential for any underlying risk factors, disease course and disease outcome.

### METHODS

Review of charts for children aged 1-21 years with recurrent CDI referred to see pediatric gastroenterology service was performed. All subjects with known immunosuppression or IBD were excluded. Subjects were followed for at least 24 mo.

### RESULTS

Twelve children seen consecutively were identified. All patients were treated with antibiotic courses for CDI prior to their referral. Five out of 12 patients had an underlying pathology that was not previously identified, including eosinophilic colitis and IBD. CDI symptoms resolved after treatment of underlying colitis without the need to target therapy for CDI. There were 9

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patients that failed antibiotic treatment of CDI and required fecal microbiome transplant, which was safe and highly effective in preventing recurrence (100% efficacy). The gut microbial changes after fecal transplant were characterized by a remarkable and durable increase in diversity and in abundance of *Bacteroides*.

### CONCLUSION

Pediatric patients with frequent recurrence of CDI may have an unidentified underlying gastrointestinal pathology that may warrant further investigation by a specialist who can identify these diseases and help optimize management. Many of these children may benefit from fecal microbial transplant which appears to be a safe, highly effective therapy that results in long term changes in the gut microbiome.

**Key words:** Recurrent *Clostridium difficile* infection; Eosinophilic colitis; Inflammatory bowel disease; Fecal microbiome transplant

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**Core tip:** Children with recurrent *Clostridium difficile* infection who do not have known immunodeficiency or inflammatory bowel disease deserve a thorough workup as many may have an underlying gastrointestinal disease.

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## INTRODUCTION

The incidence of *Clostridium difficile* infections (CDI) in both adults and pediatrics is increasing<sup>[1-5]</sup>. CDI can result in a spectrum of disorders that ranges from carrier and asymptomatic state to causing significant morbidity and even mortality<sup>[6]</sup>. Infants frequently test positive but are asymptomatic<sup>[7]</sup>. Part of the rise in CDI could be from increasing testing among infants, which needs to be done with caution given the high prevalence of asymptomatic colonization in young infants<sup>[7]</sup>. There is also a higher incidence of colonization and colitis with *C. difficile* in pediatric inflammatory bowel disease (IBD) compared to adult IBD as well as patients with celiac disease<sup>[8]</sup>.

There have been multiple studies showing correlation between certain risk factors predisposing to the development of CDI. Risk factors such as acid suppressing agents, especially H2 receptor antagonists, exposure to antibiotics and immunosuppressants, comorbidities such as cancer, cystic fibrosis and IBD, and hospitalization have been known to increase the incidence of CDI for some time<sup>[1,9]</sup>. These studies are charged with the task of understanding the risk for developing the infection in general, however, there is a paucity of studies that describe a select population of children that have *recurrence* of this infection. While community acquired CDI is more common in pediatrics than adults, recurrent CDI is not common in children<sup>[10]</sup>. A study by Kocielek in 2015<sup>[11]</sup> showed an association between recurrent CDI and malignancy and IBD. The study identified thirty children with recurrent infection and demonstrated that the majority of these subjects (19 subjects or 63%) have malignancy, underwent solid organ transplant or have IBD.

In this study, we aimed to understand CDI in a very unique population of children who are not immunocompromised and do not have any identified IBD. This study describes important discoveries of unidentified underlying gastrointestinal conditions which may not be recognized unless the child is adequately evaluated by a specialist in the field. The study also describes the success, and the durable gut microbial changes after fecal microbial transplant in this population. These discoveries contribute to the successful outcome in management of these subjects by identifying and addressing the underlying disease.

## MATERIALS AND METHODS

Institutional Review Board (IRB) approval was obtained to study pediatric patients with recurrent CDI, defined as two or more distinct episodes of CDI associated with diarrhea or bloody diarrhea who were referred for evaluation to pediatric gastroenterology service. Subjects younger than one year and older than twenty-one years of age were excluded. All subjects with known immunosuppression or IBD prior to referral were excluded. Subjects had been followed up for at least one year.

### Stool microbiome methods

The 16S bacterial DNA region from stool DNA and negative controls were amplified by PCR using a shared forward primer 806rB (CAAGCAGAAGACGGCATACGAGATAGTCAGCCAGCCGACTACNVGGGTWTCTAAT) for all samples, while each sample had its own unique identifying reverse primer, which were modified from the original 515F-806R primer pairs. All samples were pooled and sequenced using custom sequencing primers; R1 (TATGGTAATTGTGTGYCAGCMGCCGCGGTAA), R2 (AGTCAGCCAGCCGACTACNVGGGTWTCTAAT) and Index (AATGATACGGCGACCACCGAGATCTACACGCT). Paired-end sequencing (2 × 150bp) using Illumina MiSeq Reagent Kit v2 flowcell was performed on an Illumina MiSeq System.

Reads were de-multiplexed using QIIME v1.9.1. Statistical analyses were performed using the “phyloseq” (v1.20.0) package in the R statistical environment.

## RESULTS

Twelve consecutive children were identified that fit the criteria described above. Children averaged 7.5 years of age (range 1-17 years). All children were treated with at least one course of metronidazole and one course of enteral vancomycin prior to referral. Nine children were exposed to antibiotic therapy prior to their first CDI. Three children had multiple antibiotic courses including amoxicillin. The most common single antibiotic course prior to CDI was amoxicillin as well. Three children did not receive antimicrobials prior to their first CDI. Two of the three children who did not receive antibiotics prior to their first CDI, were found to have an underlying gastrointestinal disease. The identification of the underlying disease changed the management of these patients. Five of the 12 children were previously healthy. The remaining children had different co-morbidities as described in Table 1 without a known history of colitis or immunodeficiency prior to referral. There were 9 patients that failed antibiotic treatment of CDI and required fecal microbiome transplant (FMT), which ultimately relieved CDI symptoms. Of these nine patients, 4 had a gastrostomy or gastrojejunostomy tube (Table 1), seven had history of antibiotic use, and 3 had history of acid suppressants.

After a thorough gastrointestinal workup, two patients were found to have eosinophilic disease, one subject had eosinophilic colitis and another subject had eosinophilic esophagitis. The child with eosinophilic colitis was placed exclusively on crystalline amino acid formula which resulted in resolution and prevention of any further CDI even after future exposure to antimicrobial therapy. One patient was found to have IBD proctitis, and CDI resolved after treatment of IBD. There were three subjects diagnosed with lactase deficiency.

One of the children treated with FMT, experienced a change in disease phenotype from *C. difficile* colitis that required hospitalization for bloody diarrhea with endoscopic confirmation of *C. difficile* colitis, to an asymptomatic *C. difficile* colonizer for 12 mo, followed by loss of colonization. No further CDI treatment was required despite the use of antimicrobial therapy for respiratory infection after FMT. From the FMT safety perspective, one subject developed transient fever for one day but was otherwise asymptomatic. Another subject developed bloating on the day of FMT. No serious adverse events were seen related to FMT.

Gut microbial profiles were examined before and after fecal transplant and compared to the donor profile. Children with recurrent CDI had very low abundance of *Bacteroidaceae* (Figure 1) prior to fecal transplant as well as low diversity of microorganisms compared to healthy donor ( $1.3 \pm 0.2$  vs  $3.2 \pm 0.4$ , Shannon diversity index,  $P = 0.031$ ). After fecal transplant, the fecal microbial profile diversity improved. This phenomenon seemed to be durable for the twelve months following fecal transplant (Figure 2). Similarly, *Bacteroidaceae* became quite abundant after fecal transplant and this effect was seen over twelve months (Figure 1).

Table 1 Patient demographics and final diagnosis

Age of onset (yr)	Gender	# of CDIs	Medications	Co-morbidities	Devices	Prior hosp	Final diagnosis
8.6	F	> 5	PPI, EES, multiple antibiotics course including amoxicillin	DD, BPD	GT	Yes	Eosinophilic colitis
1.17	M	> 5	History of ranitidine, multiple antibiotic courses including amoxicillin	36 wk prematurity, SGA, GERD, cleft lip	GJT	Yes	
7	M	> 5	Erythromycin	CP, multiple orthopedic surgeries	GT	Yes	Lactase deficiency
10	F	3	Amoxicillin-clavulanate	None	None	No	
3	F	> 5	PPI, multiple antibiotics courses including amoxicillin	DD, renal disease, recurrent pneumonia	GT	Yes	
9	M	3	None	ASD	none	No	IBD proctitis
17	M	4	PPI, clindamycin	CP, DD	GT	Yes	
12	M	> 5	None	None	None	No	Eosinophilic esophagitis and lactase deficiency
4	F	4	Amoxicillin-clavulanate	History of UTI, hydronephrosis	None	Yes	
2	M	4	Amoxicillin	None	None	No	Lactase deficiency
2	M	3	None	None	None	No	
14	F	3	Cephalosporin	None	None	Yes	

Hosp: Hospitalization; PPI: Proton pump inhibitor; EES: Erythromycin ethylsuccinate; DD: Developmental delay; BPD: Bronchopulmonary dysplasia; SGA: Small for gestational age; GERD: Gastroesophageal reflux disease; CP: Cerebral palsy; ASD: Atrial septal defect; UTI: Urinary tract infection; GT: Gastrostomy tube; GJT: Gastrojejunostomy tube.

## DISCUSSION

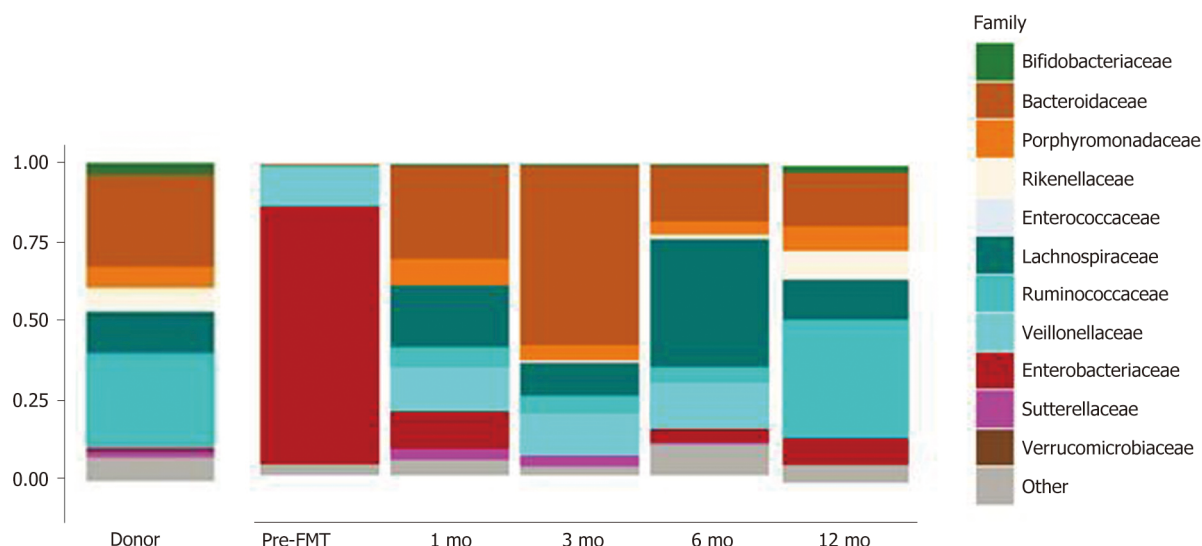
In adults, a recent meta-analysis showed age > 65 years, additional antibiotic use during follow-up, use of proton-pump inhibitors (PPIs), and renal insufficiency were most frequently associated with recurrent CDI<sup>[12]</sup>. There have been a few pediatric studies that describe risk factors for CDI in pediatric patients as well<sup>[1,9,11,13]</sup>. Underlying chronic medical condition, recent antibiotic use (specifically cephalosporins as described by Crews *et al*<sup>[13]</sup>), acid-suppressing agents, gastro-intestinal feeding device, and past or prolonged hospitalization increase the risk of developing CDI in pediatrics<sup>[1,9,13]</sup>. There is a paucity of data in the literature that focuses on recurrent infections. Kociolek *et al*<sup>[11]</sup> described a cohort of children who have recurrent CDI and found that the majority have malignancy or solid organ transplant, or IBD ( $n = 19$ , or 63%). Although there have been studies implicating IBD and immunosuppression increasing children's susceptibility to CDI<sup>[4,8]</sup>, to our knowledge, no studies have directly linked recurrent CDI to undiagnosed underlying gastrointestinal disease.

With regards to antibiotic use in children who developed recurrent CDI, most of the children in our study received amoxicillin therapy, in contrast to the study by Crews *et al*<sup>[13]</sup> that showed more exposure to cephalosporins. Most children (two thirds), who did not receive antimicrobials prior to their first CDI were found to have an underlying gastrointestinal disease which was only identified when a work up was performed after referral to specialist. The authors recognize the limitation of the findings due to the small overall number of subjects in this sub-population.

Perhaps over one third of infants younger than 12 mo are colonized with *C. difficile*<sup>[14]</sup>. The rate of colonization then drops to 15% between ages 1-8 years and then 5% after age 8 years, similar to the rate in adults<sup>[14]</sup>. Due to the high rate of colonization in infants, patients under 12 mo of age were excluded from this study.

Five of the twelve children in our cohort had a gastrostomy or a jejunostomy





**Figure 1** Bar plots depicting the percent abundance of gut microbial communities before and after fecal transplant compared to donor stools.

feeding tube, which are known to be associated with an increased risk of acquiring *C. difficile*, in adults and children<sup>[14-16]</sup>. This is likely due to spore contamination of equipment or formula, or use of formula that promotes *C. difficile* growth in the gut<sup>[14,15,17]</sup>. Most of the children in our study have been hospitalized in the past, which again would expose them to an environment that could harbor *C. difficile* spores and increasing their risk of acquiring *C. difficile*<sup>[14,18]</sup>.

While there are many medical conditions known to predispose pediatric patients to CDI, such as hematopoietic stem cell transplant, IBD, cancer, fungal infections, and human immunodeficiency virus infection<sup>[14]</sup>, those co-morbidities are diagnosed prior to the onset of CDI. In our study, 5 out of 12 patients had underlying pathology that was not previously identified. There have been many single study reports of other medical conditions that are associated with CDI<sup>[14]</sup>, such as cystic fibrosis<sup>[19]</sup>, Hirschsprung's<sup>[20]</sup>, and Henoch-Schonlein purpura<sup>[21]</sup>. In our study, two patients had eosinophilic disease, which has not been described in prior studies as an association or risk factor for CDI. The discovery and treatment of an underlying colitis, namely eosinophilic colitis and IBD proctitis, resulted in prompt resolution of the recurrence of CDI.

Three of the twelve subjects were diagnosed with lactase deficiency by disaccharidase assay. Since there is overlap in symptoms with CDI and lactase deficiency, namely diarrhea and abdominal pain, the discovery and treatment of lactase deficiency allowed optimizing management and more appropriate assignment of symptoms to the correct underlying disease. However, as expected, management of lactase deficiency did not result in resolution of CDI recurrence. FMT in both subjects resulted in prompt resolution of symptoms.

All the subjects receiving FMT had resolution of symptoms for at least one year. One subject became an asymptomatic colonizer of *C. difficile* after FMT. The colonization was seen for 12 mo followed by resolution of colonization.

FMT in this patient population appeared to be highly effective and safe. Fecal transplant resulted in improved gut microbial diversity and abundance of *Bacteroides*, which appeared to be durable and seen to persist for at least twelve months. The overall numbers are small and more research will be necessary to confirm these observations.

In this subset population, it is recommended that children with recurrent CDI who do not have immunodeficiency or identified IBD be evaluated by a provider who can investigate the presence of an underlying gastrointestinal disease. In about one third of these subjects, a gastrointestinal disorder may be discovered that can impact the management of recurrent infection.

In conclusion, there are likely risk factors that are still unknown that can predispose to CDI. Pediatric patients that have more than one episode of CDI recurrence have an increased likelihood of underlying gastrointestinal pathology especially if there has been no prior use of antimicrobials and should be investigated so that proper treatment can be offered. Fecal microbial transplant is a highly effective and safe therapy for these children and results in durable changes in the gut microbiome.

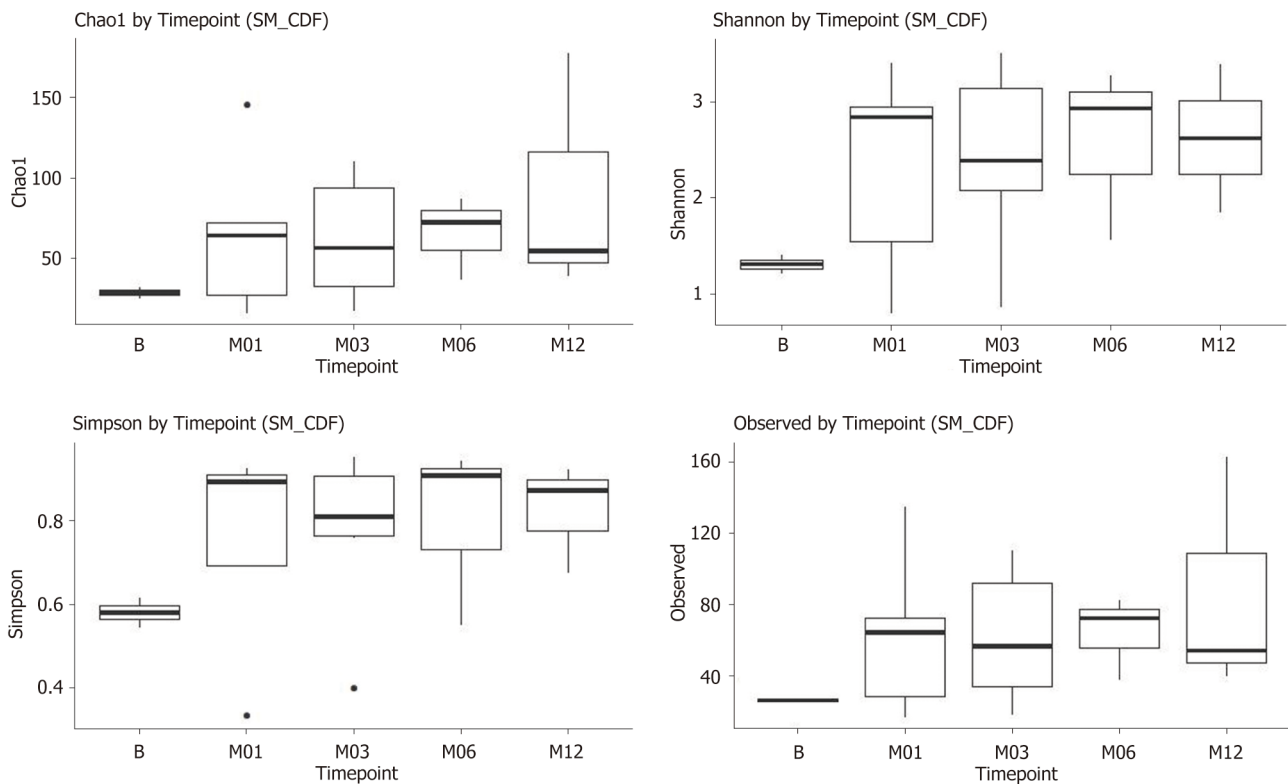


Figure 2 Diversity index at baseline and 1, 3, 6 and 12 mo after fecal transplant, showing consistent increase in diversity compared to baseline.

## ARTICLE HIGHLIGHTS

### Research background

Childhood recurrent *Clostridium difficile* infections (CDI) may be difficult to control and may represent an unknown underlying pathology. Recurrence often occurs in immunodeficiency disorders and inflammatory bowel disease (IBD).

### Research motivation

There have been multiple studies showing correlation between certain risk factors predisposing to the development of CDI. Risk factors such as acid suppressing agents, especially H2 receptor antagonists, exposure to antibiotics and immunosuppressants, comorbidities such as cancer, cystic fibrosis and IBD, and hospitalization have been known to increase the incidence of CDI for some time. These studies are charged with the task of understanding the risk for developing the infection in general, however, there is a paucity of studies that describe a select population of children that have *recurrence* of this infection. While community acquired CDI is more common in pediatrics than adults, recurrent CDI is not common in children.

### Research objectives

The main objectives of this report are understanding CDI in a very unique population of children who are not immunocompromised and do not have any identified IBD. This study describes important discoveries of unidentified underlying gastrointestinal conditions which may not be recognized unless the child is adequately evaluated by a specialist in the field. The study also describes the success, and the durable gut microbial changes after fecal microbial transplant in this population. These discoveries contribute to the successful outcome in management of these subjects by identifying and addressing the underlying disease.

### Research methods

Pediatric patients with recurrent CDI, defined as two or more distinct episodes of CDI associated with diarrhea or bloody diarrhea who were referred for evaluation to pediatric gastro-enterology service were identified. Subjects younger than one year and older than twenty-one years of age were excluded. All subjects with known immunosuppression or IBD prior to referral were excluded. Subjects had been followed up for at least one year.

### Research results

We have observed 12 children in succession. All patients received CDI antibiotics prior to referral. Five of the 12 patients had previously undiscovered potential pathologies, including eosinophilic colitis and IBD. After the treatment of basal colitis, the symptoms of CDI disappear and there is no need for CDI treatment. Nine patients required fecal microbial transplantation for antibiotic CDI failure, which is safe and effective (100% efficacy) for preventing recurrence.

Intestinal microbial changes following fecal transplantation are characterized by a significant and sustained increase in diversity and the abundance of *Bacteroides*.

### Research conclusions

Children with recurrent CDI deserve a thorough gastrointestinal workup as they may frequently have an underlying disease which can contribute to the management of the condition. When medical therapy fails in this population, fecal microbial transplant is a safe and durable therapy. Children with recurrent CDI may have unidentified gastrointestinal disease contributing to the recurrence of the infection. Children with recurrent *Clostridium difficile* frequently have an unidentified gastrointestinal disorder, which when identified and addressed, can help with management of *Clostridium difficile* recurrence.

### Research perspectives

Children with recurrent CDI need a thorough gastrointestinal workup to optimize their care and management. Future research should focus on individualized medicine and targeting underlying disease on a case by case basis.

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## Effect of dl-3-n-butylphthalide on infarction volume in animal models of ischemic stroke: A meta-analysis

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### Abstract

#### BACKGROUND

Ischemic stroke is a frequently-occurring disease in the elderly and characterized by high morbidity and mortality. DL-3-n-butylphthalide (NBP), a synthetic compound based on natural celery seeds, has potential therapeutic effects on cerebral ischemia, brain trauma, memory impairment, and epilepsy.

#### AIM

To evaluate the effect of NBP on infarct volume in experimental ischemic stroke.

#### METHODS

Twenty one relevant literatures were included from the PubMed, EMBASE, Web of Science, Chinese National Knowledge Infrastructure, VIP information database, and Wanfang database, and data on the effect of dl-3-n-butylphthalide on infarction volume in the middle cerebral artery occlusion model were extracted. Statistical analysis was performed using standard mean difference with random effects model of Revman 5.3.

#### RESULTS

The data of meta-analysis of the 21 studies had suggested that NBP reduced the cerebral infarction volume of middle cerebral artery occlusion model animals compared to the control group significantly [SMD: -3.97, 95%CI: -4.71 to -3.23,  $P < 0.01$ ; heterogeneity:  $\chi^2 = 59.09$ ,  $df = 20$  ( $P < 0.01$ );  $I^2 = 66\%$ ].

#### CONCLUSION

NBP was effective in experimental ischemic stroke.

**Key words:** Butylphthalide; Animal model; Ischemic stroke; Meta-analysis



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**Core tip:** The systematic review of animal research is of great significance in drug development. This study reports for the first time a systematic review and meta-analysis of the effects of butylphthalide on the volume of cerebral infarction in experimental ischemic stroke.

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## INTRODUCTION

Ischemic stroke is a frequently-occurring disease in the elderly and characterized by high morbidity and mortality<sup>[1,2]</sup>. The current treatment includes drug-based thrombolysis and interventional therapy in acute stage, however there were many inherent limitations of it<sup>[3,4]</sup>. To date, more than 1000 clinical trials of potential neuroprotective drugs have been verified to be failures<sup>[5]</sup>.

DL-3-n-butylphthalide (NBP), a synthetic compound based on natural celery seeds, has potential therapeutic effects on cerebral ischemia, brain trauma, memory impairment, and epilepsy, of which the injectable formulations have been approved for the treatment of acute ischemic stroke in China<sup>[6]</sup>. NBP protects the integrity of cerebrovascular structures<sup>[7]</sup>, promotes the formation of collateral circulation, accelerates the proliferation of neonatal capillary<sup>[8,9]</sup>, and increases the cerebral blood perfusion<sup>[10]</sup>; by targeting mitochondria, it improves neuronal energy metabolism<sup>[11]</sup>, reduces oxidative stress damage and neuronal apoptosis<sup>[12]</sup>. The systematic review of animal research is of great significance in drug development<sup>[13]</sup>. To this end, we conducted a meta-analysis of preclinical studies to evaluate the efficacy and the mechanisms of NBP for experimental ischemic stroke.

## MATERIALS AND METHODS

### Literature search strategies

This meta-analysis was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement<sup>[14]</sup>. All Chinese and English literatures before August 2018 on the effects of NBP for experimental ischemic stroke were searched in the six databases, which included PubMed, EMBASE, Web of Science, Wanfang database, VIP Chinese Journal Service Platform database and China National Knowledge Infrastructure database. Furthermore, to further confirm the relevant literature, we searched the list of references for potential publications. In the retrieval of the Web of Science, PubMed and EMBASE databases, only one keyword of "butylphthalide" was retrieved. In the searching of other databases, the following search strategy: "butylphthalide" AND "cerebral ischemia OR brain ischemia OR cerebral infarction OR brain infarction OR stroke OR cerebral ischemia/reperfusion OR cerebral I/R" were performed.

### Inclusion and exclusion criteria

The inclusion criteria must be met the follows: (1) The experimental ischemic stroke model was established by the adoption of middle cerebral artery occlusion (MCAO); (2) The intervention group used NBP and the control group applied blank or non-functional solvent; and (3) The cerebral infarct volume was included in the study results and the unit of infarct volume was "%", and the calculation formula was (infarction volume / whole brain volume) × 100%. The exclusion criteria were followed: (1) The intervention group was not administered NBP or the intervention group was taken NBP with other medicines concomitantly; (2) The animal models was not adopt for proceeding to MCAO; (3) Without control group; (4) Repeating literature; and (5) The data were not available.

### Literature screening and data extraction

By reading the title, abstract and full text according to the inclusion and exclusion criteria, the literature and extracted data were screened independently and cross-checked by the first author and the second author. When there was a disagreement, the point must be reached through the discussion panel which consisted of all authors, and the final conclusion was determined by the corresponding author.

Extracting the following data from the included literature: (1) The year of publication and the name of the first author; (2) The species, age, weight, gender, anesthesia methods, and model types of experimental animals (transient MCAO or permanent MCAO); (3) Therapeutic dose, route of administration, time of onset of treatment, and duration of treatment of the interventions; (4) The mean value and standard deviation of the cerebral infarction volume; and (5) The potential therapeutic mechanism of NBP for ischemic stroke.

Moreover, to study the multiple doses and multiple time points on effects of NBP, the final experimental data using the highest dose was extracted; If the volume of cerebral infarction cannot be obtained directly from the original text, the author of the literature is contacted by e-mail to get complete data, and if not, calculation was performed by using digital scale software.

### Quality assessment

The risk of bias tool of the Systematic Review Centre for Laboratory animal Experimentation's was applied to assess the methodological quality of the included studies<sup>[15]</sup>: (1) Baseline characteristics: The strain, gender, age, weight, anesthesia methods, name and anesthetic dose of the experimental animals were involved; (2) Allocation concealment: The experimental animals were grouped randomly; (3) Sequence generation: The generation of allocation sequence was random; (4) Random housing: The living environment and feeding conditions of each group of animals were in the conformity with those of each group; (5) blindly feeding: The blind method was adopt for the breeder; (6) Random outcome assessment: Evaluate the outcomes stochastically on the premise of random selection of animals; (7) Result evaluator blindly: The blind method adopted by the outcome evaluator; (8) Outcome data completely: All data of animals were included in the final analysis; (9) No selective outcome reporting: No report bias; and (10) No other sources of bias: There were no other factors that contributed to the risk of high bias.

### Statistical analysis

The Revman 5.3 software was used to analyze all data, the cerebral infarction volume was considered as continuous data, and the standard mean difference with random effects model were used to assess the combined effect sizes.

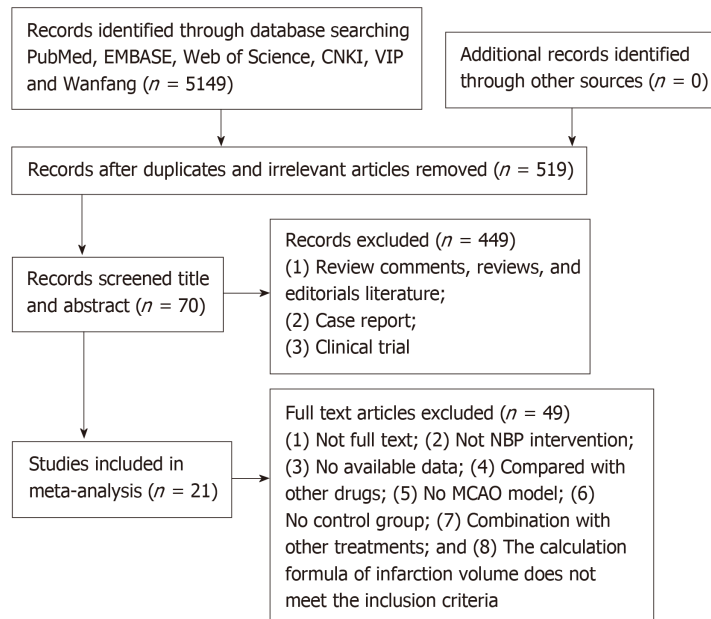
## RESULTS

### Study inclusion

A total of 5149 relevant literatures were retrieved from six databases, in them, there were 4630 duplicates and irrelevant were excluded, resulting in 519 literatures. After reading the titles and abstracts of the enrollment articles, 449 were rejected due to review comments, reviews, case reports, clinical trials and editorials literatures. By reading the full text of the remaining 70 articles, 49 articles were eliminated as the animal model was not established by the MCAO method and the intervention drug was not NBP monotherapy; there was no control group and the infarct volume calculation formula did not meet the inclusion criteria. Ultimately, 21 eligible articles were identified<sup>[8,16-35]</sup> (Figure 1).

### Study characteristics

A total of 21 articles were collected, and among which, 5<sup>[24,25,32,33,35]</sup> were published in Chinese and the remaining were in English. A total of 314 animals were included in 21 studies to investigate the effect of NBP on the volume of cerebral infarction in the experimental ischemic stroke model. A total of 314 animals, including 159 in the experimental group and 155 in the control group were involved in 21 studies to investigate the effect of NBP on the volume of cerebral infarction in the experimental ischemic stroke model. Moreover, in 21 articles, there were 15 studies were performed on SD rats (15/21, 71.4%), 3<sup>[25,29,35]</sup> on Wistar rats (3/21, 14.3%), one<sup>[22]</sup> on C57BL6/J mice (1/21, 4.8%), one<sup>[16]</sup> on CD1 mice (1/21, 4.8%), one<sup>[31]</sup> on 129S2/Sv mice (1/21, 4.7%); meanwhile, twenty studies of them were applied males (20/21, 95.2%), and both males and females (1/21, 4.8%) one were adopted in one study<sup>[18]</sup>. As for anesthesia method, intraperitoneal injection of chloral hydrate in animals was used in the study of fourteen(14/21, 66.7%), injection of sodium pentobarbital in the abdominal cavity was adopted in one<sup>[19]</sup> study (1/21, 4.8%), ketamine and xylazine



**Figure 1 Literature inclusion flow chart.** NBP: DI-3-n-butylphthalide; MCAO: Middle cerebral artery occlusion.

injection into the peritonealcavity in anesthesia of experimental animals were employed in one<sup>[29]</sup> study (1/21, 4.7%), isoflurane inhalation anesthesia was accepted in the study of one<sup>[22]</sup> (1/21, 4.8%), and only one study<sup>[34]</sup> was adopted both atropine sulfate to reduce airway secretions, and inhalation anesthesia with isoflurane (1/21, 4.7%), in addition, three studies<sup>[17,18,33]</sup> that were not mentioned the anesthesia methods (3/21, 14.3%). Meanwhile, the animal models used in the fifteen studies were transient MCAO (tMCAO) (15/21, 71.4%), and a permanent MCAO (pMCAO) were proceeded in six studies (6/21, 28.6%)<sup>[16,25,29,33,35]</sup>. To detect infarction volume, there were 19 and 2<sup>[8,22]</sup> studies adopting the 2,3,5-triphenyltetrazolium chloride and cresyl violet as the staining agents respectively (Table 1).

### Study quality

Of the 21 studies, five studies got 7 points, four studies got 6 points, eight studies got 5 points, two studies got 4 points, and two studies got 3 points. None of the studies described blind feeding and random outcome assessment; the result evaluator blindness was described only in two studies<sup>[8,32]</sup>; all studies described the data of baseline characteristics; two studies<sup>[18,29]</sup> have found other sources of bias; no incomplete outcome data, and no selective outcome reporting were described in 11 and 17 studies, respectively (Table 2).

### Effectiveness

The data of Meta-analysis of the 21 studies had suggested that NBP reduced the cerebral infarction volume of MCAO model animals compared to the control group significantly [SMD: -3.97, 95%CI: -4.71 to -3.23,  $P < 0.01$ ; heterogeneity:  $\chi^2 = 59.09$ ,  $df = 20$  ( $P < 0.01$ );  $I^2 = 66\%$ ] (Figure 2). Moreover, the data of meta-analysis of fifteen studies adopting the tMCAO model also had verified that NBP reduced infarct volume significantly [SMD: -3.67, 95%CI: -4.52 to -2.82,  $P < 0.01$ ; heterogeneity:  $\chi^2 = 42.34$ ,  $df = 14$  ( $P < 0.01$ );  $I^2 = 67\%$ ] (Figure 3A). The same is true of studies using the pMCAO model [SMD: -4.70, 95%CI: -5.92 to -3.47,  $P < 0.01$ ; heterogeneity:  $\chi^2 = 9.26$ ,  $df = 5$  ( $P = 0.10$ );  $I^2 = 46\%$ ] (Figure 3B). To analyzed the effects of the NBP on the volume of cerebral infarction with pre- or post-administrated NBP in proceeding the MCAO model, the data had showed that both the pre-administration [SMD: -3.93, 95%CI: -5.51 to -2.36,  $P < 0.01$ ; heterogeneity:  $\chi^2 = 25.58$ ,  $df = 6$  ( $P < 0.01$ );  $I^2 = 77\%$ ] (Figure 4A) and the post-administration [SMD: -3.62, 95%CI: -4.32 to -2.92,  $P < 0.01$ ; heterogeneity:  $\chi^2 = 17.92$ ,  $df = 11$  ( $P = 0.08$ );  $I^2 = 39\%$ ] (Figure 4B) all reduced the infarct volume of the model animals. A funnel plot was adopted to evaluate publication bias and a slight bias was found (Figure 5A).

## DISCUSSION

Table 1 Characteristics of the included studies

Study (yr)	Species / Age, Sex / Weight, Number (Control group / Experimental group)	Anesthetic	Model	Intervention dose administration method time point / duration	Measurement of infarction volume	Outcome index	P-values
Qin <i>et al</i> <sup>[8]</sup> , 2018	SD rats/8 weeks	5 % chloral hydrate	tMCAO 2 h	90 mg/kg, daily	Cresyl violet	1 Infarction volume	$P < 0.05$
	Male/250-300 g	400 mg/kg	Reperfusion 7 d	Gavage	Image J	2 Ameliorate body weight loss	$P < 0.05$
	6/6	Intraperitoneally		Postoperative / 7 d		3 Improve neurological behavior scores	$P < 0.05$
						4 Reduce brain atrophy volume	$P < 0.01$
						5 Upregulate <i>PTGIS</i> , <i>PTGES</i> ; downregulate <i>TBXAS 1</i>	$P < 0.05$
						6 Prevent <i>REN</i> , <i>AGT</i> , <i>ACE 1</i> , <i>AGTR 1</i> ; upregulate <i>RoA</i>	$P < 0.05$
						7 Increase the diameter of middle cerebral artery	$P < 0.05$
Zhao <i>et al</i> <sup>[16]</sup> , 2018	CD 1 mice / 10-12 wk	10 % chloral hydrate	pMCAO 24 h	120 mg/kg	2% TTC	1 Infarction volume	$P < 0.05$
	Male/27-30 g	35 mg/g				2 Improve neurological behavior scores	$P < 0.05$
	10/10	Intraperitoneally				3 Decrease the water content of brain	$P < 0.05$
						4 Decrease the permeability of blood-brain barrier	$P < 0.05$
						5 Decrease pinocytotic vesicles of capillary endothelial cells	
						6 Downregulate <i>MMP 9</i>	$P < 0.05$
						7 Upregulate <i>Claudin 5</i> , <i>VEGF</i> , <i>GFAP</i> , <i>Nrf 2</i> and <i>HO 1</i>	$P < 0.05$
Wang <i>et al</i> <sup>[18]</sup> , 2018	SD rats/unknown	Unknown	tMCAO 2 h	1 mg/kg	2% TTC	1 Infarction volume	$P > 0.05$
	Male and female/250-280 g		Reperfusion 48 h	Intravenously	Image Pro Plus	2 Improve neurological behavior scores	$P > 0.05$
	8/12			postoperative/4 h and 24 h			
Yan <i>et al</i> <sup>[17]</sup> , 2017	SD rats/adult	Unknown	tMCAO 2 h	75 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.01$
	Male/180-220 g		Reperfusion 24 h	Gavage		2 Decrease the water content of brain	$P < 0.05$
	8/8			Preoperative/7 d		3 Decrease the permeability of blood-brain barrier	$P < 0.01$

						4 Decrease cell apoptosis	
						5 Decrease ROS,cleaved caspase-3, p-p38; increase SOD	$P < 0.01$
						6 Decrease MDA, p-JNK	$P < 0.05$
Zhang <i>et al</i> [19], 2016	SD rats/unknown	Sodium pentobarbital	tMCAO 1 h	4.5 mg/kg	TTC	1 Infarction volume	$P < 0.01$
	Male/250-320 g	50 mg/kg	Reperfusion 24 h	Intraperitoneally		2 Improve neurological behavior scores	$P < 0.001$
	8/8			Postoperative/-		3 Decrease the water content of brain	$P < 0.05$
						4 Upregulate HGF; downregulate TLR4	$P < 0.001$
Yin <i>et al</i> [20], 2016	SD rats/unknown	Chloral hydrate	tMCAO 2 h	80 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.001$
	Male/280-320 g	300 mg/kg	Reperfusion 24 h	Gavage	Image Pro Plus	2 Improve neurological behavior scores	$P < 0.05$
	6/6	Intraperitoneally		preoperative/7 d		3 Decrease the water content of brain	$P < 0.01$
						4 Decrease MDA	$P < 0.01$
						5 Increase SOD	$P < 0.05$
						6 Increase GSH-Px	$P < 0.001$
Hua <i>et al</i> [21], 2015	SD rats/57-61 days	Chloral hydrate	tMCAO 2 h	60 mg/kg	2% TTC	1 Infarction volume	$P < 0.05$
	Male/250-280 g	300 mg/kg	Reperfusion 24 h	Gavage	Image Pro Plus	2 Improve neurological behavior scores	$P > 0.05$
	6/6	Intraperitoneally		Postoperative/-		3 Decrease the water content of brain	$P < 0.01$
						4 Decrease MDA; increase GSH, SOD, Nrf 2, Trx, Bcl-2	$P < 0.01$
						5 Decrease NF- $\kappa$ B p65; increase Txnip	$P < 0.05$
Lu <i>et al</i> [22], 2014	C57BL6/J/ dult	Isoflurane	tMCAO 0.75 h	10 mg/kg	Cresyl violet	1 Infarction volume	$P < 0.05$
	Male/ nknown	Initiated 3 %	Reperfusion 23 h	Intravenous	Image J	2 Improve neurological behavior scores	$P < 0.05$
	10/10	Maintained 1.5 %		Preoperative/-		3 Decrease MMP 9; increase TIMP 1	$P < 0.01$
						4 Increase SBP, p-ERK	$P < 0.01$
Wang <i>et al</i> [23], 2013	SD rats/unknown	Chloral hydrate	tMCAO 2 h	80 mg/kg, daily	TTC	1 Infarction volume	$P < 0.05$
	Male/280-320 g	300 mg/kg	Reperfusion 24 h	Gavage		2 Improve neurological behavior scores	$P > 0.05$
	6/6	Intraperitoneally		preoperative/7 d		3 Decrease the water content of brain	$P > 0.05$
Wang <i>et al</i> [24], 2013	SD rats/unknown	10 % chloral hydrate	tMCAO 2 h	80 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.05$
	Male/250 $\pm$ 20 g (n = 30)	3 mL/kg	Reperfusion 24 h	Gavage		2 Downregulate GRP78, CHOP	$P < 0.05$



	5/5	Intraperitoneally		Preoperative / 7 d			
Pan <i>et al</i> <sup>[25]</sup> , 2013	Wistar rats/unknown	10 % chloral hydrate	pMCAO 24 h	0.8 g/kg, daily	2% TTC	1 Infarction volume	$P < 0.05$
	Male/200-250 g	3 mL/kg		Gavage	Biosens Digitan Image	2 Decrease the water content of brain	$P < 0.05$
Zhang <i>et al</i> <sup>[26]</sup> , 2013	8/8	Intraperitoneally		Preoperative/14 d		3 Decrease Smac, S100B	$P < 0.05$
	SD rats/unknown	6 % chloral hydrate	tMCAO 2 h	200 mg/kg	2% TTC	1 Infarction volume	$P < 0.01$
	Male/250 ± 20 g		Reperfusion 24 h	Intraperitoneally	Image Pro Plus	2 Improve neurological behavior scores	$P < 0.01$
						3 Increase VEGF	$P < 0.01$
Wang <i>et al</i> <sup>[27]</sup> , 2012	8/8	Intraperitoneally		Postoperative/-		1 Infarction volume	$P < 0.05$
	SD rats/unknown	chloral hydrate	tMCAO 2 h	90 mg/kg, daily	2% TTC	2 Improve neurological behavior scores	$P < 0.05$
	Male/250-300 g	300 mg/kg	Reperfusion 3 d	Gavage		3 Decrease the water content of brain	$P < 0.05$
						4 Decrease the ratio of TXB <sub>2</sub> : 6-keto-PGF <sub>1α</sub>	$P < 0.05$
Wu <i>et al</i> <sup>[28]</sup> , 2012	10/10	Intraperitoneally		Postoperative/3 d		1 Infarction volume	$P < 0.001$
	SD rats/unknown	Chloral Hydrate	tMCAO 2 h	80 mg/kg, daily	2% TTC	2 Improve neurological behavior scores	$P < 0.001$
	Male/250-280 g	350 mg/kg	Reperfusion 24 h	Gavage		3 Decrease the water content of brain	$P > 0.05$
	6/6	Intraperitoneally		Preoperative/7 d		4 Decrease MDA; increase SOD	$P < 0.001$
Zhang <i>et al</i> <sup>[29]</sup> , 2012	Wistar Kyoto rats/3 mo	Ketamine, xylazine	pMCAO 7 d	80 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.01$
	Male/unknown	75 mg/kg, 10 mg/kg		Gavage	Image Pro Plus		
Zhao <i>et al</i> <sup>[30]</sup> , 2012	6/6	Intraperitoneally		Postoperative/7 d			
	SD rats/unknown	Chloral hydrate	tMCAO 2 h	50 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.05$
	Male/250-300 g	300 mg/kg	Reperfusion 3 d	Gavage		2 Improve neurological behavior scores	$P < 0.05$
Li <i>et al</i> <sup>[31]</sup> , 2010	6/6	Intraperitoneally		Postoperative / 3 d			
	129S2/Sv/adult	4 % chloral hydrate	pMCAO 24 h	100 mg/kg	2% TTC	1 Infarction volume	$P < 0.05$
	Male/20-25 g			Intraperitoneally		2 Decrease cleaved-caspase 3; caspase 9, p-JNK; p-p38	$P < 0.05$
Cao <i>et al</i> <sup>[32]</sup> , 2009	10/10	Intraperitoneally		Postoperative 1 h/-		3 Reduce mitochondrial release of cytochrome c and AIF	$P < 0.05$
	SD rats/3-4 mo	10 % chloral hydrate	tMCAO 2 h	25 mg/kg, twice a day	TTC	1 Infarction volume	$P < 0.01$
	Male/280-350 g		Reperfusion 3 d	Gavage	Image Pro Plus	2 Improve neurological behavior scores	$P < 0.05$
Li <i>et al</i> <sup>[33]</sup> , 2008	5/5	Intraperitoneally		Postoperative/3 d		3 Upregulate VEGF, bFGF	$P < 0.05$
	SD rats/3-4 mo	Unknown	pMCAO 3 d	25 mg/kg, twice a day	TTC	1 Infarction volume	$P < 0.05$

Zhang <i>et al.</i> <sup>[34]</sup> , 2006	Male/280-350 g			Gavage	Image Pro Plus	2 Improve neurological behavior scores	$P < 0.05$
	5/5			Postoperative/3 d		3 Upregulate VEGF, bFGF	$P < 0.05$
	SD rats/unknown	3 % isoflurane	tMCAO 2 h	10 mg/kg	4% TTC	1 Infarction volume	$P < 0.001$
	Male/270-330 g	Endotracheal intubation	Reperfusion 24 h	Intravenously	SPOT Biometrics	2 Improve neurological behavior scores	$P < 0.01$
Lin <i>et al.</i> <sup>[35]</sup> , 1996	10/10			intraoperative / -			
	Wistar rats/unknown	Chloral hydrate	pMCAO 24 h	240 mg/kg	4% TTC	1 Infarction volume	$P < 0.001$
	Male/250-350 g			Gavage		2 Improve neurological behavior scores	$P < 0.001$
	8/8			Postoperative/-			

PTGIS: Prostacyclin synthase; PTGES: Prostaglandin E synthase; TBXAS 1: Thromboxane A2 synthase 1; REN: Renin; AGT: Angiotensinogen; ACE 1: Angiotensin converting enzyme 1; AGTR 1: Angiotensin II receptor type 1; tMCAO: Transient middle cerebral artery occlusion; pMCAO: Permanent middle cerebral artery occlusion; TTC: 2,3,5-triphenyltetrazolium chloride; MMP 9: Matrix metalloproteinase 9; VEGF: Vascular endothelial growth factor; GFAP: Glial fibrillary acidic protein; Nrf 2: NF-E2-related factor 2; HO 1: Heme oxygenase 1; ROS: Reactive oxygen species; SOD: Superoxide dismutase; MDA: Malonaldehyde; HGF: Hepatocyte growth factor; TLR4: Toll like receptor 4; GSH-Px: Glutathione peroxidase; GSH: Glutathione; Trx: Thioredoxin; Txnip: Thioredoxin-interacting protein; TIMP 1: Tissue inhibitor of metalloproteinase 1; SBP: Spectrin breakdown product; GRP 78: Glucose-regulated protein 78; CHOP: C/EBP-homologous protein; Smac: Second mitochondria-derived activator of caspases; S100B: S100 calcium binding protein B; TXB2: Thromboxane B2; 6-keto-PGF1 $\alpha$ : 6-keto-prostaglandin F1 $\alpha$ ; AIF: Apoptosis-inducing factor; bFGF: Basic fibroblast growth factor.

### Summary of evidence

The preclinical meta-analysis study evaluated the effects of NBP on infarct volume in experimental ischemic stroke, which was based on experimental data from 314 animals in five Chinese literatures and sixteen English literatures. The evidence obtained from present study suggest that NBP might play potential neuroprotective roles for ischemic stroke by increasing cerebral blood flow, enhancing mitochondrial function, protecting integrity of the structure and function of blood-brain barrier, and developing anti-inflammatory and antioxidant stress.

### Limitations

First, the absence of relevant literatures in other languages other than Chinese and English, may lead to selective bias. Second, none of study provides sample size calculations, blind feeding, and random outcome assessment. Third, the lack of negative research may result in an overestimation of the efficacy of NBP. Fourth, cerebral infarction is usually accompanied by other conditions, such as old age, hypertension, hyperlipidemia, diabetes, heart disease and so on<sup>[36-39]</sup>. We did not analyze the effects of NBP on cerebral infarction when the accompanying situation occurred. Fifth, one study<sup>[18]</sup> using female animals does not rule out estrogen neuroprotection, that has been reported<sup>[40]</sup>. One study<sup>[29]</sup> of anesthetic drugs containing ketamine did not eliminate its neuroprotection, that has been reported in preclinical and clinical studies<sup>[41,42]</sup>.

### Potential neuroprotective mechanisms

Summarizing the included literatures, we have found that NBP plays a neuroprotective role in experimental ischemic stroke by acting on multiple targets (Figure 5B). We have drawn a conclusion of the underlying mechanisms as follows: (1) Increase blood supply to brain tissue in the ischemic area: Dilate the middle cerebral artery<sup>[8]</sup>; regulate the expression of REN, AGT, ACE 1, AGTR 1, RoA, PTGIS, PTGES and TBXAS 1 in ischemic brain tissue<sup>[8]</sup>, decrease TXB2 and 6-keto-PGF1 $\alpha$  ratio<sup>[27]</sup>, and reduce thrombosis; (2) Promote angiogenesis: Increase VEGF and bFGF in ischemic brain tissue<sup>[16,26,32,33]</sup>; (3) Anti-inflammatory: Inhibition of TLR4/NF- $\kappa$ B signaling pathway<sup>[19,21]</sup>; decrease the expression of S100B in ischemic brain tissue<sup>[25]</sup>. (4) Protect the structure and function of the blood-brain barrier: Modulate the expression of MMP-9 and claudin-5 in ischemic brain tissue<sup>[16,22]</sup>; up-regulate the expression of GFAP in ischemic brain tissue, stabilize astrocytes<sup>[16]</sup>; increase the expression of TIMP1 and decrease the expression of SBP in ischemic brain tissue<sup>[22]</sup>; (5) Antioxidative stress: Enhance Nrf-2/HO-1 signaling pathway<sup>[16]</sup>; reduce the expression of ROS and MDA in ischemic brain tissue; increase the expression of SOD, GSH-px, GSH, Trx and Txnip in ischemic brain tissue<sup>[17,20,21,28]</sup>; (6) Protect the structure and function of mitochondria: Increase the expression of Bcl-2 in ischemic brain tissue<sup>[21]</sup>; reduce the expression of

**Table 2 Risk of bias of the included studies**

Study (yr)	A	B	C	D	E	F	G	H	I	J	Score
Qin <i>et al</i> <sup>[8]</sup> , 2018	√	√	√				√	√	√	√	7
Zhao <i>et al</i> <sup>[16]</sup> , 2018	√	√	√						√	√	5
Wang <i>et al</i> <sup>[18]</sup> , 2018	√		√	√				√	√		5
Yan <i>et al</i> <sup>[17]</sup> , 2017	√			√					√	√	4
Zhang <i>et al</i> <sup>[19]</sup> , 2016	√			√					√	√	4
Zhao <i>et al</i> <sup>[16]</sup> , 2018	√	√	√						√	√	5
Yin <i>et al</i> <sup>[20]</sup> , 2016	√	√	√					√	√	√	6
Hua <i>et al</i> <sup>[21]</sup> , 2015	√	√	√	√				√	√	√	7
Lu <i>et al</i> <sup>[22]</sup> , 2014	√	√	√	√				√	√	√	7
Wang <i>et al</i> <sup>[23]</sup> , 2013	√	√	√						√	√	5
Wang <i>et al</i> <sup>[24]</sup> , 2013	√	√	√					√		√	5
Pan <i>et al</i> <sup>[25]</sup> , 2013	√	√	√					√		√	5
Zhang <i>et al</i> <sup>[26]</sup> , 2013	√	√	√						√	√	5
Wang <i>et al</i> <sup>[27]</sup> , 2012	√	√	√						√	√	5
Wu <i>et al</i> <sup>[28]</sup> , 2012	√	√	√						√	√	5
Zhang <i>et al</i> <sup>[29]</sup> , 2012	√	√	√	√				√	√		6
Zhao <i>et al</i> <sup>[30]</sup> , 2012	√	√	√	√				√	√	√	7
Li <i>et al</i> <sup>[31]</sup> , 2010	√								√	√	3
Cao <i>et al</i> <sup>[32]</sup> , 2009	√	√	√	√			√	√		√	7
Li <i>et al</i> <sup>[33]</sup> , 2008	√	√	√	√				√		√	6
Zhang <i>et al</i> <sup>[34]</sup> , 2006	√	√	√	√					√	√	6
Lin <i>et al</i> <sup>[35]</sup> , 1996	√								√	√	3

A: Baseline characteristics; B: Allocation concealment; C: Sequence generation; D: Random housing; E: Blind feeding; F: Random outcome assessment; G: Result evaluator blind; H: No incomplete outcome data; I: No selective outcome reporting; J: No other sources of bias.

Smac in ischemic brain tissue<sup>[25]</sup>; reduce mitochondrial release of cytochrome C and AIF<sup>[31]</sup>; and (7) Anti-apoptosis: Reduce the expression of cleaved caspase-3, p-p38 and p-JNK in ischemic brain tissue<sup>[17,31]</sup>; increase the expression of HGF and p-ERK in ischemic brain tissue<sup>[19,22]</sup>; decrease the expression of GRP78 and CHOP in ischemic brain tissue, and inhibit endoplasmic reticulum stress-induced apoptosis<sup>[24]</sup>.

### Implications

Animal experiments are an important link between basic research and clinical experiments. The results have reference value for the next step in designing and implementing clinical research. Compared with clinical research, the principles of randomization and blindness are theoretically easier to be implemented in animal experiments. Animal research is important for comprehending disease mechanisms, and high-quality preclinical research is also critical for translational medicine<sup>[43,44]</sup>. Therefore, to obtain more accurate and less biased experimental data, designing animal programs should follow the guidelines all the time<sup>[15,45]</sup>, calculate sample size in the beginning, apply applicable animals, use appropriate anesthetic drugs, adopt random feeding, and blind models during the experiment, and employ random outcome measurements at the time of evaluation.

Similar to artemisinin, NBP is also a plant-derived drug approved for the treatment of acute ischemic stroke in China. We envision that NBP promote to treat more patients in the world, like artemisinin, and it requires a large number of randomized, double-blind, and multi-center clinical trials in terms of safety and efficacy.

In conclusion, we conducted the first preclinical systematic review and meta-analysis of the effects of NBP on experimental ischemic stroke, and found that NBP was effective in experimental ischemic stroke.

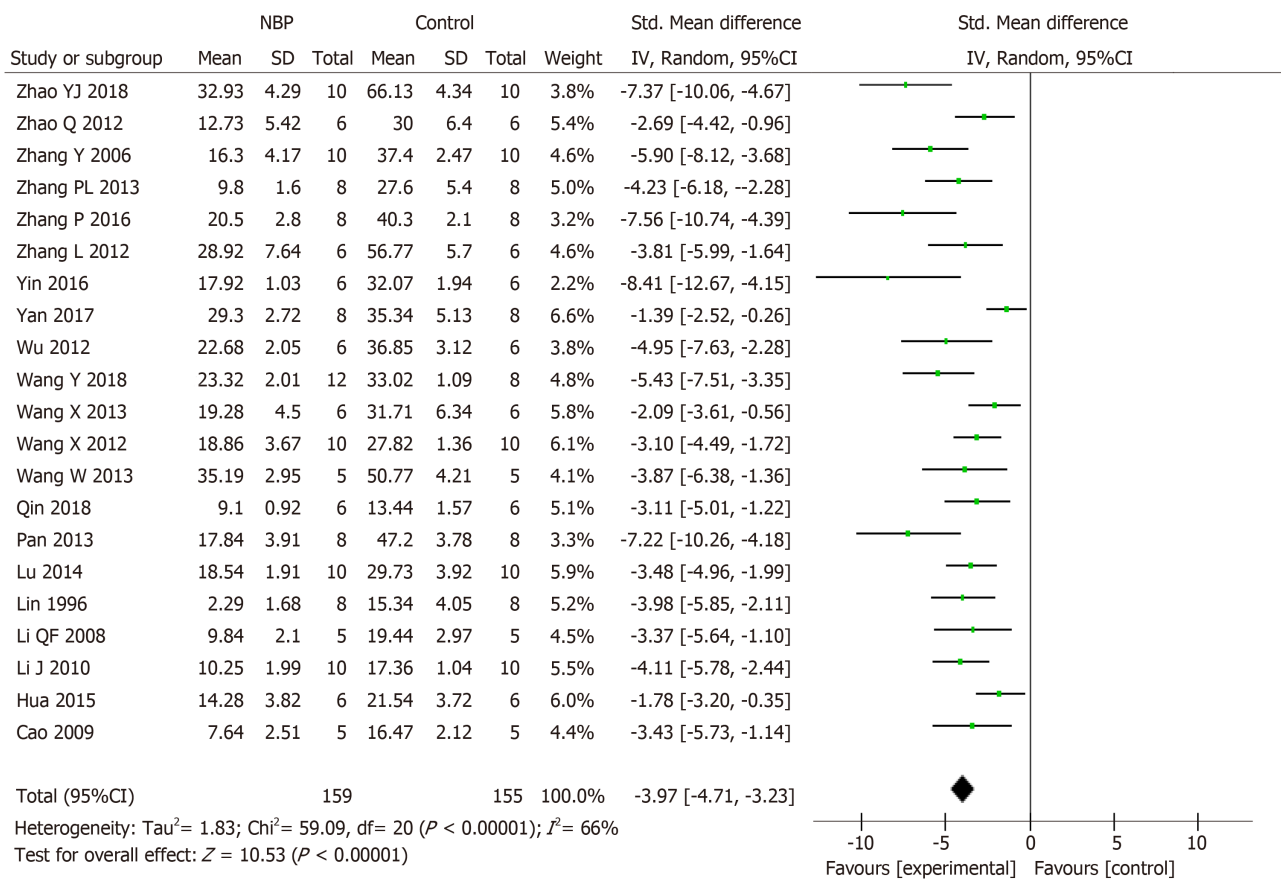
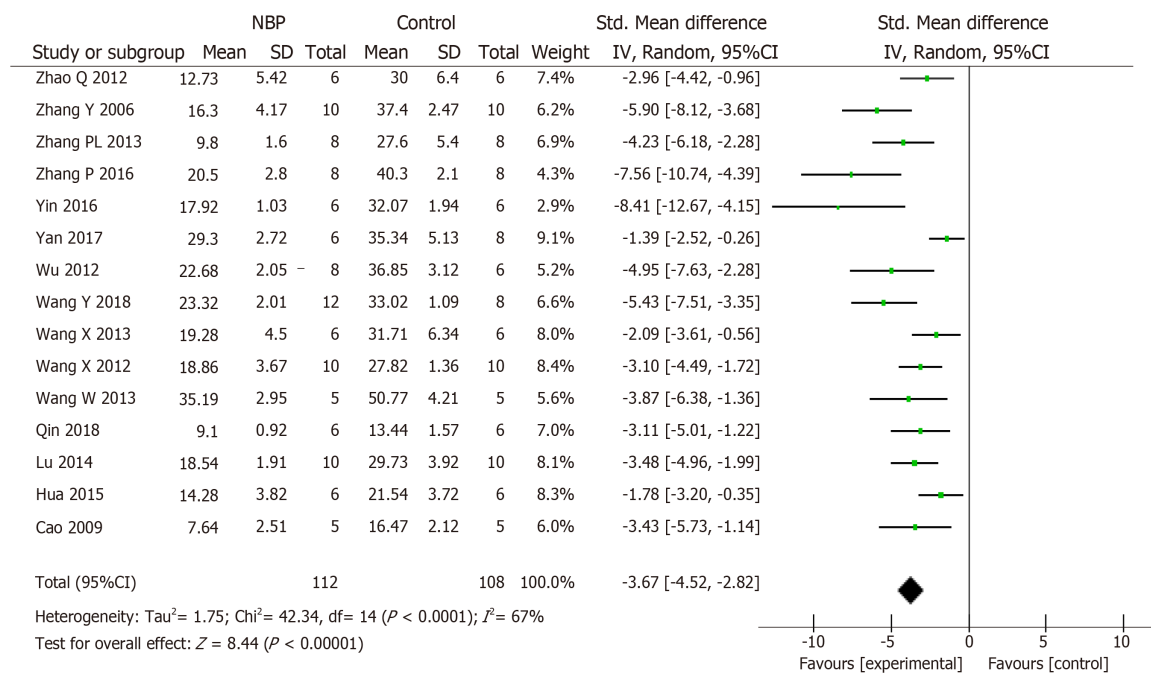
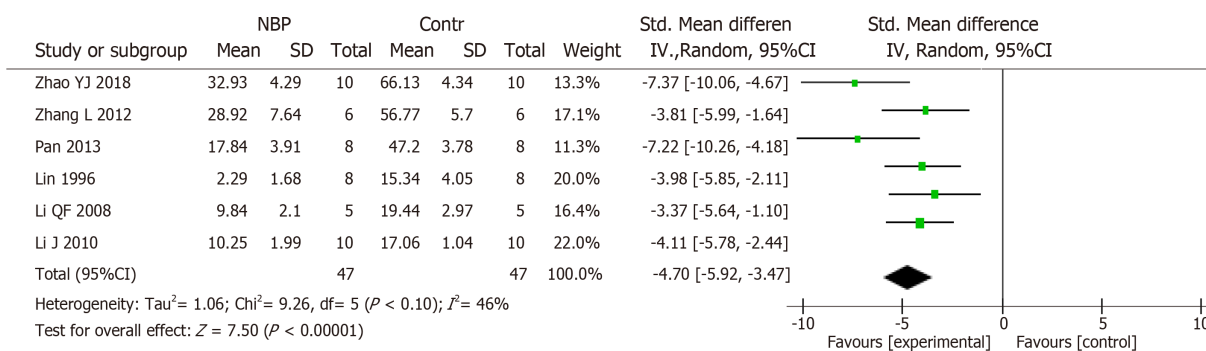
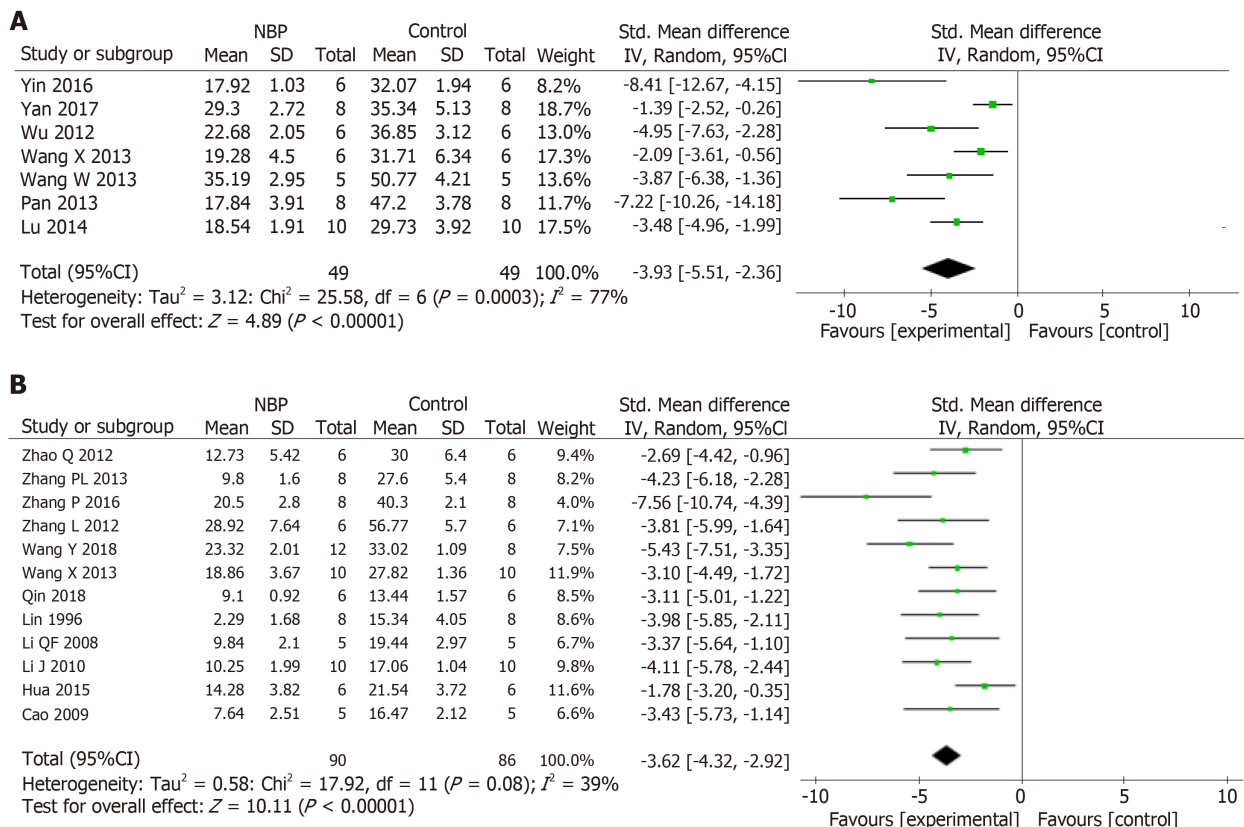


Figure 2 The forest plot: Effects of dl-3-n-butylphthalide for decreasing the cerebral infarction volume compared with control group.

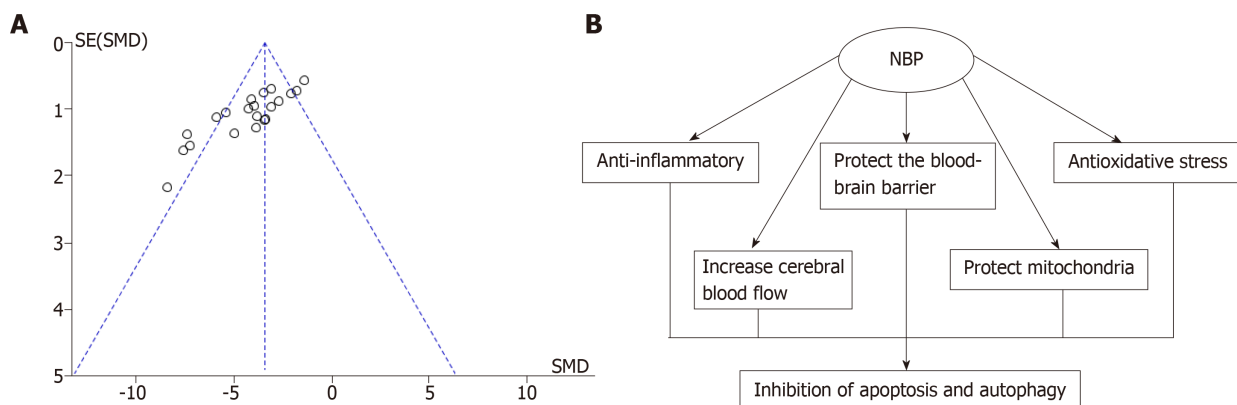
**A****B**

**Figure 3** The forest plot: Effects of dl-3-n-butylphthalide for decreasing the cerebral infarction volume compared with control group in transient (A) and permanent (B) middle cerebral artery occlusion model, respectively.





**Figure 4** The forest plot: Effects of dl-3-n-butylphthalide for decreasing the cerebral infarction volume compared with control group in pre (A) and post-administration (B) model, respectively.



**Figure 5** The funnel plot evaluation publication bias and underlying mechanism of dl-3-n-butylphthalide in neuroprotection. A: The funnel plot evaluation publication bias for the dl-3-n-butylphthalide on infarction volume; B: The underlying mechanism of dl-3-n-butylphthalide in neuroprotection.

## ARTICLE HIGHLIGHTS

### Research background

Ischemic stroke is a frequently-occurring disease in the elderly and characterized by high morbidity and mortality. DI-3-n-butylphthalide (NBP), a synthetic compound based on natural celery seeds, has potential therapeutic effects on cerebral ischemia, brain trauma, memory impairment, and epilepsy. The systematic review of animal research is of great significance in drug development.

### Research motivation

There are many studies on the therapeutic effects of NBP in the middle cerebral artery occlusion model, and there is controversy about whether NBP reduces the volume of cerebral infarction.

### Research objectives

To evaluated effect of NBP on infarct volume in experimental ischemic stroke.

### Research methods

We searched Chinese and English databases to screen NBP-related literature. Data such as cerebral infarction volume and potential therapeutic mechanisms were extracted. The risk of bias tool of the Systematic Review Centre for Laboratory animal Experimentation's was applied to assess the methodological quality of the included studies. Data analysis was performed by Revman 5.3 software.

### Research results

The data of meta-analysis of the 21 studies had suggested that NBP reduced the cerebral infarction volume of middle cerebral artery occlusion (MCAO) model animals compared to the control group significantly. Moreover, the data of meta-analysis of fifteen studies adopting the tMCAO model also had verified that NBP reduced infarct volume significantly. The same is true of studies using the pMCAO model. To analyze the effects of the NBP on the volume of cerebral infarction with pre- or post-administrated NBP in proceeding the MCAO model, the data had showed that both the pre-administration and the post-administration all reduced the infarct volume of the model animals.

### Research conclusions

NBP was effective in experimental ischemic stroke.

### Research perspectives

Animal experiments are an important link between basic research and clinical experiments. The results have reference value for the next step in designing and implementing clinical research. Compared with clinical research, the principles of randomization and blindness are theoretically easier to be implemented in animal experiments. Animal research is important for comprehending disease mechanisms, and high-quality preclinical research is also critical for translational medicine. Therefore, to obtain more accurate and less biased experimental data, designing animal programs should follow the guidelines all the time, calculate sample size in the beginning, apply applicable animals, use appropriate anesthetic drugs, adopt random feeding, and blind models during the experiment, and employ random outcome measurements at the time of evaluation.

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## Tofacitinib for the treatment of ulcerative colitis: A review of the literature

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### Abstract

Ulcerative colitis (UC) is a chronic immune-mediated inflammatory condition affecting the colon. Recently, tofacitinib, an oral small molecule that is an inhibitor of the Janus kinase signal transduction pathway, was proven efficacious for inducing and maintaining remission in adult patients with moderate to severe UC in three global Phase III studies. The purpose of this review is to summarize existing data on the efficacy, safety, and quality of life issues related to use of tofacitinib as well as highlight recent real-world experience with this drug among patients with UC.

**Key words:** Ulcerative colitis; Tofacitinib; Review; Inflammatory bowel disease; Treatment

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**Core tip:** Tofacitinib is a small molecule that is an inhibitor of the Janus kinase signal transduction pathway, and it is the first oral medication approved for chronic use among adults with moderately to severely active ulcerative colitis (UC). Three large phase III trials have shown overall efficacy and safety; however, long-term results and real-world data are lacking in the literature. Our objective is to consolidate the current literature to better understand what is currently known about efficacy, safety, quality of life, and real-world experience with this medication among patients with UC.

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## INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory condition that primarily affects the colon, due to an abnormal dysregulation of the immune system. The pattern of disease activity is most often described as relapsing and remitting, with some patients experiencing persistent disease activity despite diagnosis and medical therapy. Therapeutic decisions are subcategorized into induction and maintenance modalities, with a primary treatment endpoint of obtaining and maintaining both endoscopic healing and symptomatic remission. The current therapeutic armamentarium for UC treatment includes corticosteroids, immunosuppressants, aminosalicylates, immunomodulators, anti-tumor necrosis factor (TNF) agents, as well as anti-integrins. Recently, tofacitinib, an oral small molecule that is an inhibitor of the Janus kinase (JAK) signal transduction pathway, was found to be effective in both inducing and maintaining remission in adult patients with moderate to severe UC in three global Phase III studies<sup>[1,2]</sup>. Tofacitinib has been used for the treatment of adults with moderate-to-severe rheumatoid arthritis (RA) since its initial Food and Drug Administration (FDA) approval in 2012, and in 2018 the FDA expanded this approval to include treatment of adults with moderate to severe UC. It should be noted that this medication has not been FDA approved for the use in pediatric populations. It is unique in that it is the first of its kind oral medication with FDA approval for treatment of moderate to severe UC.

Given its status as a relative newcomer in the treatment of UC, there is limited evidence of the long-term safety and efficacy of tofacitinib in this patient population. The purpose of this review is to summarize existing data on the safety, efficacy, and quality of life issues related to the use of tofacitinib as well as highlight recent real-world experience with this drug among patients with UC.

## REVIEW OF THE LITERATURE

### Data on efficacy

In a phase 2 double-blind, randomized, placebo-controlled trial by Sandborn *et al*<sup>[1]</sup> involving patients with moderate-to-severe UC, a significantly higher rate of response at 8 wk was found among those who received tofacitinib at a dose of 15 mg twice daily than among those who received placebo and also a significantly higher rate of remission with tofacitinib at doses of 3 mg, 10 mg, and 15 mg twice daily than with placebo.

Subsequently, Sandborn *et al*<sup>[2]</sup> reported the results of phase 3 trials of tofacitinib as induction therapy (OCTAVE Induction 1 and 2) and maintenance therapy (OCTAVE Sustain) in patients with moderate-to-severe UC. Enrolled patients had moderate-to-severe UC and had experienced previous treatment failure with or unacceptable side effects from glucocorticoid, thiopurine, or anti-TNF therapy. For all three trials, the primary end point was remission, which was based on Mayo scores. The rate of remission at 8 wk was significantly higher in the 10-mg tofacitinib group than in the placebo group in the OCTAVE Induction 1 trial (18.5% *vs* 8.2%,  $P = 0.007$ ) and in the OCTAVE Induction 2 trial (16.6% *vs* 3.6%, ( $P < 0.001$ )). The rate of remission at 52 wk was significantly higher in the 5-mg and 10-mg tofacitinib groups (34.3% and 40.6%, respectively) than in the placebo group (11.1%) in the OCTAVE Sustain trial.

Although there have been no head-to-head clinical trials comparing tofacitinib to biologics, meta-analyses have been conducted to address this important question. A recent systematic review and network meta-analysis by Bonovas *et al*<sup>[3]</sup> aimed to comparatively assess efficacy of tofacitinib and biologics (infliximab, adalimumab, golimumab and vedolizumab) in adult patients not previously exposed to anti-TNF agents. In terms of clinical response, clinical remission, and mucosal healing, each drug demonstrated superiority over placebo. However, no indirect comparisons between tofacitinib and biologics reached statistical significance.

A recent network meta-analysis found that tofacitinib has the highest rank for induction of clinical remission among patients with prior anti-TNF exposure. In an effort to analyze the comparative safety and efficacy of differing therapies as first line (biologic-naïve) and second line (previous exposure to anti-TNF agents) therapies for moderate-severe UC, Singh *et al*<sup>[4]</sup> conducted a systematic review and network meta-



analysis. They found that while infliximab and vedolizumab were ranked highest for induction of clinical remission amongst biologic-naïve patients, among patients with prior anti-TNF exposure, tofacitinib was ranked highest for induction of clinical remission [OR: 11.88 (2.32-60.89)] and mucosal healing.

### Safety and adverse events

Tofacitinib has been associated with an increased risk of infections among patients with RA<sup>[5]</sup> and psoriasis<sup>[6]</sup>. In the OCTAVE trials<sup>[2]</sup>, there were higher rates of infections with tofacitinib as compared to placebo, and the rate of serious infection was found to be increased with tofacitinib in the induction trials, but similar across treatment groups in the maintenance trial. Overall, 2.9% of subjects suffered at least one serious infection compared with 1.0% of the placebo controls, including anal abscess, pneumonia, herpes zoster (HZ) infection, *Clostridium difficile* infection, and cytomegalovirus colitis.

In OCTAVE Sustain, HZ infections occurred in 14 patients total, 3 (1.5%) in the 5 mg group, 10 (5.1%) in the 10 mg group and 1 (0.5%) in the placebo group. An analysis of the safety of tofacitinib for the treatment of moderate to severe UC based on more than four years of data from global clinical trials by Sandborn *et al*<sup>[7]</sup> again suggest what appears to be a dose-dependent relationship with HZ infection, with those taking 10 mg BID at highest risk. For the overall cohort, the incident rate of HZ infection was 4.1 (95%CI: 3.1-5.2). Winthrop *et al*<sup>[8]</sup> conducted an analysis specifically examining the risk of HZ in patients with UC using tofacitinib. They found that among HZ incidence was 4.07 per 100 person-years among all patients with UC treated with tofacitinib, and again found a dose-dependent risk. It should be noted that the majority of HZ events were uncomplicated and mild to moderate in severity. Independent risk factors for HZ in these patients with UC included advanced age and prior anti-TNF failure<sup>[9]</sup>. In addition, patients with Asian race (IR: 6.49; 95%CI: 3.55-10.89), oral corticosteroid use at baseline (IR: 5.14; 95%CI: 3.56-7.18), history of diabetes mellitus (IR: 8.06; 95%CI: 2.96-17.55), and those who received the 10 mg twice daily dosing (IR: 4.25; 95%CI: 3.18-5.65) were at higher risk for HZ infection.

The new recombinant HZ subunit vaccine (RZV) could decrease the risk of HZ from tofacitinib; it is currently only recommended for immunocompetent adults aged  $\geq 50$  years. However, given the known risk of this infection, it remains to be seen whether it may be warranted to administer the RZV vaccine to all inflammatory bowel disease (IBD) patients of all ages treated with tofacitinib, including those younger than 50. A recent study by Caldera *et al*<sup>[10]</sup> attempts to further clarify this question by calculating the number needed to harm (NNH) in order to quantify the risk of HZ in patients treated with tofacitinib as compared to those with alternative treatments for UC, including infliximab and vedolizumab. They found that the higher 10 mg twice a day dosing of tofacitinib had the highest risk for HZ infection when compared to placebo with an NNH of 22 patients; the combined NNH for both treatment groups (5 mg and 10 mg) combined was 36 patients. The information gathered from these studies can collectively inform our clinical approach towards addressing the potential risk of HZ. Currently suggested approaches for lowering the risk of HZ include potentially vaccinating younger patients including those less than 50 years old on tofacitinib, who demonstrate risk factors for HZ including steroid use, Asian race, or diabetes mellitus. Moreover, educating patients to recognize early symptoms of HZ, and closely monitoring patients with UC during induction therapy in order to maintain the lowest effective dose – or, to withdraw the drug entirely in non-responders are other approaches. Of note, it is recommended to avoid the use of live vaccines concurrently with this medication<sup>[11]</sup>. Further research is needed both on understanding risk factors for HZ as well as regarding the safety and efficacy of the RZV series in patients receiving tofacitinib for treatment of UC.

Among RA patients, gastrointestinal perforations have been observed with the use of tofacitinib<sup>[5]</sup>. Across the OCTAVE trials, one intestinal perforation occurred with tofacitinib; in the OCTAVE Induction 1 trial, 1 patient in the 10-mg tofacitinib group had a serious adverse event of intestinal perforation. In the OCTAVE Induction 2 trial, a single patient in the placebo group had a serious adverse event of intestinal perforation. No patients in the OCTAVE Sustain trial experienced intestinal perforation<sup>[2]</sup>.

There is some data to suggest an increase in malignancy risk among RA patients treated with tofacitinib. In a worldwide, 3-year, post-marketing surveillance study on tofacitinib in patients with RA<sup>[12]</sup>, the relative risk per 100 patient-years for neoplasms was 0.45, with the most common neoplasms being nonmelanoma skin cancers (NMSCs). Fifteen cases of lymphoma were documented over approximately 34000 patient-years of exposure, and the risk of lymphoma was not found to increase over time. The data on malignancy risk among UC patients using tofacitinib is much more limited. In an integrated analysis of tofacitinib UC clinical trials, eleven patients had

malignancies (excluding NMSC), all during OCTAVE Open<sup>[7]</sup>. There 1 case reported for each of the following cancers: Cervical cancer, hepatic angiosarcoma, cholangiocarcinoma, cutaneous leiomyosarcoma, Epstein-Barr-virus-associated lymphoma, renal cell carcinoma, essential thrombocythemia, acute myeloid leukemia, adenocarcinoma of colon, lung cancer, and breast cancer. In the overall cohort, IR of malignancy (excluding NMSC) including all 11 patients with events was 0.7 (95%CI: 0.3-1.2).

Additional studies have analyzed other important safety-related questions regarding tofacitinib. Cases of maternal and paternal exposure to tofacitinib (defined as parental exposure to tofacitinib before or at the time of conception and/or during the course of pregnancy) were identified in the Pfizer safety databases in a study by Mahadevan *et al*<sup>[13]</sup>. Of 1157 patients enrolled in the UC interventional studies, 11 cases of maternal exposure and 14 cases of paternal exposure to tofacitinib (doses of 5 mg or 10 mg twice daily) before or at the time of conception or during pregnancy were identified. Outcomes included 15 healthy newborns, no fetal deaths, no neonatal deaths, no congenital malformations, 2 spontaneous abortions, and 2 medical terminations. Overall, they found that outcomes across other tofacitinib studies and post-marketing cases were consistent, with a healthy newborn being the most common outcome and no fetal deaths. However, it is important to note that tofacitinib has been found to be teratogenic in animal models and is contraindicated in patients who are attempting to become pregnant<sup>[11]</sup>.

There has been interest in understanding the association between tofacitinib and lipid profiles since an early pooled analysis demonstrated dose-dependent increases in total cholesterol, LDL-C, and HDL-C among patients with RA<sup>[14]</sup>. In the OCTAVE trials, as compared with placebo, tofacitinib was associated with increased lipid levels as well<sup>[2]</sup>. More recently, Sands *et al*<sup>[15]</sup> analyzed lipid concentrations and incidence rates of major adverse cardiovascular (CV) events (MACEs) in patients with UC who received and found that after 8 weeks of therapy, there were greater increases from baseline in total cholesterol, HDL-C, and LDL-C in patients on tofacitinib compared with placebo. Four MACEs were reported; the incidence rate was 0.24 (95%CI: 0.07-0.62), and 3 of these patients had 4 or more CV risk factors. Overall, they did not find clinically meaningful changes in lipid ratios or CV risk scores, and MACEs were found to be infrequent and not dose-related.

Importantly, an association between thromboembolic events and higher doses of tofacitinib was recently noted. Early results from the RA Study, an ongoing open-label clinical trial of patients over the age of 50 with at least one cardiac risk factor, show an increased risk of pulmonary embolism and overall mortality among study participants receiving tofacitinib at 10 mg twice daily as compared to 5 mg<sup>[16]</sup>. Currently, the European Medicines Agency's safety committee is recommending against the use of 10 mg twice daily dose of tofacitinib in patients who are at high risk of thromboembolic disease including pulmonary embolism, as well as those with heart failure, cancer, history of blood clots, or taking combined hormonal contraceptives<sup>[17]</sup>. Given that the recommended induction dosage for UC is 10mg twice daily, more data is needed to evaluate this potentially serious association.

### Quality of life

Paschos *et al*<sup>[18]</sup> conducted a systematic review with network meta-analysis aiming to compare the impact of interventions for moderate-to-severe UC on health-related quality of life (HRQL); they found that induction therapy with tofacitinib improves quality of life of patients with moderate-to-severe UC, the beneficial effect of which is maintained during maintenance therapy. This was supported by Panés *et al*<sup>[19]</sup> who found that tofacitinib 10 mg twice daily induction therapy significantly improved HRQL versus placebo at week 8. These improvements were persistent through 52 wk' maintenance therapy with tofacitinib 5 mg and 10 mg twice a day.

### Real-world experience

Recently, Weissshof *et al*<sup>[20]</sup> published their real-world experience with tofacitinib used for treatment of patients with moderate-to-severe IBD. In this retrospective, observational study, 58 patients (including 53 with UC) completed at least 8 wk of treatment with tofacitinib. Clinical response and adverse events were assessed at 8 wk (induction), at 26 wk (maintenance), at 52 wk, and at the last available follow-up. They found that at 8 wk of treatment, 21 patients (36%) achieved symptomatic improvement, and 19 (33%) achieved clinical remission. Steroid-free remission at 8 wk was achieved in 15 patients (26%). Of the 48 patients followed for 26 wk, 21% had clinical, steroid-free remission. Of the 26 patients followed for 12 mo, 27% were in clinical remission and remained steroid-free.

Rapid clinical response has been suggested in several studies. Hanauer *et al*<sup>[21]</sup> assessed the timing of symptom improvement in post-hoc analyses of data from 2

phase 3 trials of induction therapy with tofacitinib in patients with UC (OCTAVE Induction 1 and 2); they found significant improvements in symptoms among patients given tofacitinib compared with placebo within 3 d, indicating a rapid onset of effect of this drug in patients with UC. In a case study by Griller *et al*<sup>[22]</sup>, tofacitinib and infliximab were used as combination rescue therapy to avoid colectomy in a hospitalized patient with severe UC. The patient received intravenous steroids and 2 loading doses of infliximab with minimal improvement and then started on 10 mg tofacitinib twice daily as rescue therapy; the patient improved dramatically within 48 hours and subsequently achieved clinical remission.

In an off-label use of tofacitinib, Berinstein *et al*<sup>[23]</sup> presented the first reported use of tofacitinib in 4 patients with acute severe UC (ASUC) predicted to fail medical management, based on severe Truelove and Witt's criteria, C-reactive protein (CRP) > 100 mg/L at presentation, endoscopic features during admission, and prior failure of IV corticosteroids or infliximab therapy. After receiving tofacitinib, all 4 patients had a rapid improvement in clinical symptoms and decline in CRP. Two patients achieved clinical remission with a combination of tofacitinib and IV corticosteroids, whereas one patient achieved clinical remission with tofacitinib and budesonide only. One patient was unable to achieve clinical remission, although they did experience an initial rapid improvement in symptoms and CRP until tofacitinib was reduced. No major adverse effects directly attributable to the use of tofacitinib were reported during the induction phase of drug administration or up to 18 mo of reported follow-up.

## DISCUSSION

IBD is a chronic condition affecting millions of people of all ages worldwide, with prevalence highest in Europe and North America, and rising incidence in newly industrialized countries in Africa, Asia and South America<sup>[24]</sup>. With ever-increasing targeted research on novel therapeutics, the treatment of IBD continues to evolve. Tofacitinib is currently the only JAK kinase inhibitor with FDA approval for the treatment of patients with moderate-to-severe UC.

Overall, clinical data shows that tofacitinib is effective in inducing and maintaining clinical remission, clinical response, and mucosal healing. Additionally, analysis of the OCTAVE 1 and 2 trials suggests a rapid onset of action with response as early as day 3<sup>[21]</sup>. Studies also indicate that tofacitinib has a favorable effect on quality of life<sup>[18,19]</sup>.

In the OCTAVE trial, HZ reactivation was more frequent among patients under tofacitinib 10mg twice a day (5.1%) compared with other treatment groups (1.5% and 0.5% across tofacitinib 5mg twice a day and placebo, respectively). Vaccination can help lower the risk of infection, and an inactivated recombinant varicella zoster vaccine is now available, which in clinical trials has demonstrated 97% efficacy among adults ≥ 50 years of age<sup>[25]</sup>. Further research is needed both on understanding risk factors for HZ as well as regarding the safety and efficacy of the RZV series in patients on tofacitinib.

Recent safety data suggests that pulmonary embolism may potentially be a class-wide issue for JAK inhibitors; however, these data need to be confirmed by future adverse events reporting trends and clinical trials. Currently, the European Medicines Agency's safety committee is recommending against the use of 10 mg twice daily dose of tofacitinib in patients who demonstrate risk factors for thromboembolic disease.

Real-world experiences with the use of tofacitinib are lacking in the literature. Weisshof *et al*<sup>[20]</sup> published their real-world experience with the use of tofacitinib for treatment of patients with moderate-to-severe IBD; they found that at 8 wk of treatment, 21 patients (36%) achieved symptomatic improvement, 19 (33%) achieved clinical remission, and 15 (26%) achieved steroid-free remission. Overall, tofacitinib induced clinical response in 69% of the patients and 27% were in clinical, steroid-free remission by 1 year of treatment, suggesting that tofacitinib can be an effective treatment alternative for patients with anti-TNF resistant IBD. Tofacitinib has also been used as a combination rescue therapy with infliximab to avoid colectomy in a hospitalized patient with severe UC<sup>[22]</sup>, as well as in inpatients with ASUC predicted to fail medical management<sup>[23]</sup> with good success.

Currently, there is an ongoing Phase III long-term extension study known as OCTAVE Open that aims to assess the safety, tolerability, and efficacy of long-term tofacitinib therapy; it includes non-responders in OCTAVE Induction 1 or 2, treatment failures in OCTAVE Sustain, and those who completed OCTAVE Sustain. OCTAVE Open will assess safety through an analysis of adverse events, clinical laboratory parameters, and physical examination, as well as efficacy as determined by clinical response and endoscopy at predetermined intervals.

Other future research directions to be pursued include head-to-head trials to determine the most optimal therapies in UC. In addition, there is currently limited data on the efficacy of combining tofacitinib therapy with biologics among patients with UC. Within the RA population, there is some data to support safety with combination therapy; a case series of 6 patients with RA treated with tofacitinib–biologic combination therapy did not find any adverse events after a mean of 14 months of treatment<sup>[26]</sup>. Le Berre *et al*<sup>[27]</sup> report a case of successful combination of vedolizumab and tofacitinib in a patient with UC and spondyloarthropathy for whom anti-TNF therapy was contraindicated; after 3 mo of treatment with this combination therapy, the patient achieved clinical remission for both gastrointestinal and rheumatologic symptoms. No adverse events were observed, including no infections. Additionally, rapid remission was achieved recently in an inpatient as described by Griller *et al*<sup>[22]</sup>, when tofacitinib and infliximab were used as combination rescue therapy to avoid colectomy in a hospitalized patient with severe UC. Interestingly, as a stand-alone medication, it should also be highlighted that the economic burden to the patient for the cost of tofacitinib is likely less than compared to alternative therapies such as anti-TNFs and vedolizumab<sup>[28]</sup>. Overall, the available evidence remains limited regarding UC patients, and larger studies are needed to confirm the efficacy and safety profile of combination therapy in this patient population.

At this time, further novel subtype-selective JAK kinase inhibitors are currently being developed. Additional studies are required to better understand long-term efficacy, safety profiles, and the optimal positioning of agents like tofacitinib in management algorithms for UC.

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# Antidiabetic agents in patients with hepatic impairment

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## Abstract

Chronic liver disease (CLD) often coexists with type 2 diabetes mellitus, making diabetes management a challenge to the clinician. It is well known that liver is the major site of drug metabolism, and, therefore, its impairment affects hepatic metabolism of many antidiabetic agents. Furthermore, patients with CLD have serious comorbidities such as impaired renal function, hypoalbuminemia, lactic acidosis, hypoglycemia and malnutrition, making their treatment even more difficult. On the other hand, most of the antidiabetic agents, with the exception of insulin, need dosage titration due to alterations to their pharmacokinetics in patients with CLD. For well-established antidiabetic treatments, like metformin and sulfonylureas there are studies regarding their dosage chance in these patients. However, despite the growing problem of management of diabetes in patients with CLD the existing literature data, especially on newer antidiabetic agents, are limited and, furthermore, no direct guidelines exist. Therefore, in the present review article we try to summarize the existing literature data regarding management of diabetes in patients with CLD.

**Key words:** Hepatic impairment; Type 2 diabetes mellitus; Pharmacokinetics; Antidiabetic drugs

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**Core tip:** Most of the antidiabetic agents, with the exception of insulin, need dosage titration due to alterations to their pharmacokinetics in patients with chronic liver disease (CLD). For well-established antidiabetic treatments, like metformin and sulfonylureas there are studies regarding their dosage chance in these patients. However, despite the growing problem of management of diabetes in patients with CLD the existing literature data, especially on newer antidiabetic agents, are limited and, furthermore, no direct guidelines exist. Therefore, in the present review article we try to summarize the existing literature data regarding management of diabetes in patients with CLD.

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## INTRODUCTION

Liver is one of the principal organs in carbohydrate metabolism due to its important role in neoglucogenesis and glycogenolysis<sup>[1]</sup>. A link between type 2 diabetes mellitus (T2DM) and chronic liver disease (CLD) was observed for the first time before almost 100 years<sup>[1,2]</sup>. Since then it is well-known that diabetes and CLD often coexist. Even more, presence of CLD increases not only T2DM complications but it is recognized as a cause of premature mortality in patients with T2DM<sup>[3]</sup>. On the contrary, diabetes *per se* has been recognized as a risk factor for CLD and hepatocellular carcinoma (HCC). It is estimated that about 30%-60% of patients with cirrhosis have T2DM<sup>[4]</sup>. In another study, the prevalence of T2DM in patients with CLD was varied between 18%-71%<sup>[5]</sup>. On the other hand, glucose intolerance is present in the majority of patients with CLD<sup>[6]</sup>. It is obvious, that there is a two-side relationship between T2DM and CLD making the management of these patients a challenge to the clinicians.

Since liver is the major site of metabolism for most of the antidiabetic agents, management of T2DM in patients with CLD is still challenging for the reasons that are listed below. First of all, patients with CLD have serious comorbidities such as impaired renal function, hypoalbuminemia, lactic acidosis, hypoglycemia and malnutrition<sup>[7,8]</sup>. Secondly, patients with CLD are more prone to acute kidney injury leading to accumulation of either drugs or their metabolites resulting in various adverse events<sup>[9]</sup>. Finally, patients with CLD develop malnutrition as the liver plays a key role in carbohydrate, protein, lipid, vitamin, and mineral metabolism and energy balance<sup>[10,11]</sup>.

Liver is the major site of drug metabolism, and its impairment affects hepatic metabolism of drugs<sup>[12]</sup>. On the other hand, hypoalbuminemia, a result of protein deficiency<sup>[13]</sup>, can cause serious toxicity by highly protein bound drugs since their free plasma concentrations are increased in CLD. Furthermore, the potential hepatotoxicity of some oral antidiabetic agents (OADs) associated adverse events favored by CLD makes management of T2DM in patients with CLD even more complex<sup>[4]</sup>.

Until now, only limited literature data are available yet regarding the management of T2DM in patients with CLD<sup>[3,8]</sup>. Therefore, the aim of the present review is to summarize the existing literature data on the use of OADs and injectable agents in T2DM patients with CLD.

## CLASSIFICATION OF LIVER IMPAIRMENT

The Child-Pugh score is currently used to assess the overall prognosis of CLD, mainly cirrhosis<sup>[14]</sup>. The Child-Pugh score is consisted of 5 clinical characteristics of liver disease: total bilirubin level, serum albumin concentration, prothrombin or international normalized ratio value, presence of ascitis and hepatic encephalopathy. Each measure is scored from 1 to 3, with 3 indicating most severe derangement. Patients are classified into 3 Child-Pugh classes (A-C): Child-Pugh A = 5-6 points, Child-Pugh B = 7-9 points, and Child-Pugh C = 10 or more points.

## ANTIDIABETIC TREATMENT

### *Biguanides (metformin)*

Metformin, a biguanide compound, is the first-line therapy for T2DM patients for almost half a century<sup>[15]</sup>. Its action is mediated by the inhibition of gluconeogenesis and glycogenolysis in hepatocytes<sup>[15]</sup>. Metformin undergoes renal excretion and is excreted unchanged by the kidneys<sup>[16]</sup>.

One of the most life threatening adverse events of metformin is lactic acidosis. However, it must be noticed that metformin might cause lactic acidosis in predisposed patients (with heart, renal and liver failure), a rather rare, however, adverse event of metformin therapy. In patients with CLD, there is an increased risk

of low oxygen tension due to concurrent pulmonary or heart disease making lactic acidosis easy to happen. Even more, patients with CLD are at increased risk for sepsis or hemorrhage<sup>[17]</sup> making them vulnerable to lactic acidosis since metformin inhibits mitochondrial respiration in the liver<sup>[18]</sup>. It must be mentioned that lactic acidosis is rather a rare side effect of metformin since the incidence of lactic acidosis is 0.03-0.5 cases/1000 patient-years in metformin-treated population<sup>[19]</sup>.

According to the existing studies, metformin therapy is safe in T2DM patients with cirrhosis, and further prolong patient's survival time. A study in 22 T2DM cirrhotic patients showed that metformin therapy was related to overt hepatic encephalopathy. A possible pathogenetic mechanism proposed by authors was the inhibition of glutaminase activity<sup>[20]</sup>. Another study showed that metformin was related with reduced incidence of HCC and liver-related death/transplantation in T2DM patients with cirrhosis due to hepatitis C virus<sup>[21]</sup>. It is noteworthy that metformin therapy reduced the risk of death by 57% in T2DM patients with cirrhosis<sup>[22]</sup>.

The only risk of metformin therapy in patients with CLD, as it is mentioned above, is lactic acidosis. Therefore, according to the ADA guidelines, it is recommend to avoid metformin therapy in patients with severe hepatic impairment (HI) or in binge drinkers due to high risk for lactic acidosis<sup>[15]</sup> (Table 1).

### **Sulfonylureas**

Liver is the major site of biotransformation for sulfonylureas. Sulfonylureas are metabolized into active and inactive metabolites in the liver through hepatic oxidative enzymes (CYP P450s). Then, they are extensively bound to serum proteins and excreted through renal pathway. Therefore, protein binding of sulfonylureas may be reduced in patients with T2DM and CLD due to hypoalbuminemia resulting to increased drug plasma concentrations<sup>[23-25]</sup>. Therefore, sulfonylurea therapy in patients with CLD and renal failure increases the risk for hypoglycemia<sup>[26]</sup> that is more pronounced in the presence of malnutrition, a common comorbidity in CLD patients<sup>[7]</sup>, and diminished gluconeogenic capacity<sup>[27]</sup>. Furthermore, in patients with alcoholic liver disease alcohol-induced enzyme degradation of sulfonylureas decreases drug's effectiveness and further increases the risk of hypoglycemia<sup>[26]</sup>.

There are only a few studies examined the effect of CLD on sulfonylurea metabolism. A study examined the effect of glipizide on hepatic uptake of insulin, showed that glipizide caused an increase in the estimated uptake of insulin in T2DM patients with cirrhosis, whereas a small decrease was observed in the control group<sup>[28]</sup>.

Sulfonylureas therapy in patients with HI may be challenging since they are metabolized by the liver and excreted by the kidneys not only the parent drug but it's active metabolites as well. Glimepiride and gliclazide are contraindicated in severe HI<sup>[23-25]</sup>. According to the position statement of the ADA and EASD insulin secretagogues should be avoided in severe HI due to the risk of hypoglycemia<sup>[15]</sup> (Table 1).

### **Meglitinides (glinides)**

Glinides (nateglinide and repaglinide) have shorter half-lives than sulfonylureas and they do not have significant renal excretion<sup>[29,30]</sup>. They are extensively bound to serum albumin protein and are metabolized by oxidative biotransformation (CYP 450) and conjugation with glucuronic acid in the liver<sup>[31,32]</sup>. Repaglinide's metabolism is mainly affected by the presence of CLD while this is not the case for nateglinide. One possible explanation for this discrepancy is that repaglinide is metabolized by CYP isoform 2C8<sup>[33]</sup> and nateglinide by CYP isoform 2C9<sup>[30]</sup>.

Repaglinide clearance is significantly reduced in patients with HI and should be used with caution while in T2DM patients with severe HI the drug is contraindicated<sup>[34]</sup>. On the other hand, nateglinide pharmacokinetics (PK) is not affected in patients with HI and, therefore, no adjustment of nateglinide dosage is needed in patients with mild to moderate HI<sup>[35]</sup>. There are no data available in patients with severe HI (Table 1).

### **Alpha-glucosidase inhibitors (Acarbose)**

Acarbose acts locally within the gastrointestinal tract by inhibiting enzymes (glycoside hydrolases) needed to digest carbohydrates<sup>[36]</sup>. The lack of intestinal absorption and hepatic metabolism, makes acarbose a safe choice in CLD patients with a good tolerability and absence of toxic effects<sup>[38]</sup>, well-compensated non-alcoholic cirrhosis<sup>[39]</sup>, and low-grade hepatic encephalopathy<sup>[40]</sup>. However, there may be a possibility of hyperammonemia when acarbose is prescribed to T2DM patients with advanced HI<sup>[37]</sup>. The effect of acarbose in hepatic encephalopathy was studied in 107 cirrhotic patients with T2DM. Acarbose therapy was related with decreased ammonia blood levels. However, no change in biochemical parameters of liver function was observed at the end of the study<sup>[40]</sup>. The findings of another study with

**Table 1 Use of antidiabetic agent according to the degree of hepatic impairment**

Antidiabetic agent	Degree of hepatic impairment (HI)
Metformin	Avoid in severe HI
Sulfonylureas	
Glimepiride	Avoid in severe HI
Gliclazide	Avoid in severe HI
Glinides	
Repaglinide	Avoid in severe HI
Nateglinide	No adjustment of dosage in mild to moderate HI
Alpha-glucosidase inhibitors	
Acarbose	Well tolerated
Thiazolidinediones	
Pioglitazone	Safe in Child-Pugh Class A patients. Should be avoided in Class B and C patients
DPP-4 inhibitors	
Sitagliptin	Well tolerated
Vildagliptin	Well tolerated
Saxagliptin	Well tolerated
Alogliptin	Well tolerated
Linagliptin	Well tolerated
GLP-1 receptor agonists	
Exenatide	Well tolerated
Liraglutide	Well tolerated
Lixisenatide	Well tolerated
SGLT-2 inhibitors	
Canagliflozin	Safe in Child-Pugh Class A patients. Caution is needed in Class B patients. Should better be avoided in Class C patients
Dapagliflozin	Safe in Child-Pugh Class A patients. Caution is needed in Class B patients. Should better be avoided in Class C patients
Empagliflozin	Safe in Child-Pugh Class A patients. Caution is needed in Class B patients. Should better be avoided in Class C patients
Insulin	Safe in use

the use of acarbose showed that T2DM associated with HI might be safely and effectively treated with acarbose except for a small increase in ammonia blood levels. Therefore, acarbose treatment in T2DM patients with cirrhosis might increase the risk of hyperammonemia<sup>[37]</sup>. According to the position statement of the ADA acarbose is safe, useful, and well tolerated in CLD patients<sup>[15,16]</sup> (Table 1).

### Thiazolidinediones

**Pioglitazone:** Pioglitazone is the only drug available in the market of this class; it is extensively metabolized by hydroxylation and oxidation and it is metabolized mainly by CYP2C8<sup>[41]</sup>. It is excreted primarily as metabolites and their conjugates in bile and feces<sup>[41]</sup>. Hepatic safety of pioglitazone was evaluated in a large observational study in T2DM patients in Japan where no case of HI was reported and no alanine aminotransferase (ALT) abnormalities with pioglitazone therapy in different dosages<sup>[42]</sup>.

In a study, where the hepatic safety profile of pioglitazone (compared to glibenclamide) was examined in pioglitazone-treated patients, there was no case of hepatocellular injury in the pioglitazone group while and four cases were observed in the glibenclamide group. No case of hepatic dysfunction or HI was reported in the pioglitazone group<sup>[43]</sup>. However, in another study, the case-fatality rate of liver failure associated with rosiglitazone or pioglitazone was 81%, while only 14% of the patients recovered<sup>[44]</sup>. On the contrary, a large-scale study in Japan, in 24993 patients (28008 patient-years), no case of HI was found<sup>[42]</sup>. The above finding was confirmed in a retrospective data analysis of 1.12 patients with T2DM, where pioglitazone therapy was not associated with increased risk of HI or hepatitis compared to other OADs<sup>[45]</sup>.

According to the position statement of the ADA in case of cirrhosis or serum ALT level exceeding 2.5 times of upper normal limit (ULN), pioglitazone should be avoided<sup>[15]</sup>. Pioglitazone should be used with caution in CLD patients. It should be avoided in patients whose liver enzymes are > 3 times ULN range. Pioglitazone may be used in Child-Pugh Class A patients. However, it should be avoided in Class B and C patients<sup>[15]</sup> (Table 1).

### DPP-4 inhibitors

Dipeptidyl peptidase-4 (DPP-4) inhibitors (sitagliptin, vildagliptin, saxagliptin, alogliptin and linagliptin) belong to the incretin-based glucose-lowering agents<sup>[46]</sup>. Sitagliptin is primarily excreted by the kidney and only a small percentage of the drug undergoes hepatic metabolism (mainly through the CYP3A4 isoenzyme and less through CYP2C8 isoenzyme)<sup>[47]</sup>. Vildagliptin is metabolized *via* hydrolysis and its inactive metabolites show renal excretion<sup>[47]</sup>. Saxagliptin is metabolized *in vivo* to form an active metabolite, and both parent drug and metabolite are excreted primarily via the kidneys<sup>[48]</sup>. Saxagliptin is primarily metabolized by CYP3A4 and CYP3A5 isoforms and eliminated through renal and hepatic routes. Alogliptin is metabolized into M-I, an N-demethylated active metabolite *via* CYP2D6, and M-II, an N-acetylated inactive metabolite and it is excreted primarily *via* the kidneys<sup>[48,49]</sup>. In contrast to other DPP-4 inhibitors, approximately 80% of administered dose of linagliptin<sup>[50]</sup> is eliminated through enterohepatic recycling<sup>[48]</sup>.

The safety of DPP-4 inhibitors in T2DM patients was examined in a systematic review and meta-analysis, whereas no adverse events of hepatotoxicity were reported<sup>[51]</sup>. Regarding sitagliptin, a few cases of drug-induced hepatic injury<sup>[52]</sup> and of elevated hepatic enzymes<sup>[53]</sup> have been reported. However, the causal pathogenetic relationship is still unclear<sup>[54]</sup>. Despite the initial concern about a possible hepatotoxicity of vildagliptin a pooled analysis of 38 controlled trials showed that there is not any significant increase of liver enzymes with vildagliptin therapy<sup>[55]</sup>. The safety of vildagliptin was confirmed in another pooled analysis in clinical trials with duration more than two years<sup>[56]</sup>. Sitagliptin PK is not affected by moderate HI<sup>[57]</sup>. Similarly, vildagliptin PK is not affected in patients with mild, moderate or even severe HI<sup>[58]</sup>.

According to the already conducted studies, there is no liver safety issues for saxagliptin<sup>[59]</sup>. In the placebo-controlled SAVOR-TIMI 53 cardiovascular outcome trial, no signal of liver toxicity was found in the saxagliptin group<sup>[60]</sup>. Saxagliptin PK is affected only in a small degree in patients with HI<sup>[61,62]</sup>.

A meta-analysis of 8 placebo-controlled trials confirmed the hepatic safety of linagliptin<sup>[63]</sup>. In a study in patients with mild and moderate HI, linagliptin was well tolerated without any adverse events<sup>[64]</sup>. There is only one case report described a probable linagliptin-induced liver toxicity<sup>[65]</sup>. One study<sup>[64]</sup> reported that mild, moderate or severe HI did not affect linagliptin PK compared to normal hepatic function.

According to the already conducted studies, there is no concern for hepatotoxicity for alogliptin<sup>[66]</sup>. The large cardiovascular outcome study EXAMINE showed no signal of hepatotoxicity in the alogliptin group<sup>[67]</sup>. There is only one observational study coming from Japan where hypoglycemic symptoms under alogliptin therapy were reported and associated with liver disease and alcohol consumption<sup>[68]</sup>. Finally, in patients with moderate HI alogliptin PK is not affected<sup>[69]</sup>.

Summary of product characteristic of sitagliptin, saxagliptin, and linagliptin recommends no dosage adjustments in patients with CLD<sup>[70-72]</sup>, while vildagliptin should not be used in patients with CLD, including patients with

ALT or aspartate aminotransferase (AST) > 3x the ULN<sup>[73]</sup>. Therefore, DPP-4 inhibitors may be used in Child-Pugh Class A patients while their use requires caution in Class B patients. On the contrary, DPP-4 inhibitors are not preferred in Class C patients (Table 1).

### GLP-1 receptor agonists

Glucagon-like peptide-1 receptor agonists (GLP-1RA) (exenatide, liraglutide, lixisenatide and dulaglutide) belong to the incretin-based glucose-lowering agents and offer new opportunities for the management of T2DM<sup>[45]</sup>. Renal excretion is the main pathway for the elimination of exenatide. Liraglutide and dulaglutide are metabolized into their component amino acids by general protein catabolism pathways<sup>[74-76]</sup>.

The existing literature data regarding the effect of GLP-1RAs therapy in patients with CLD is limited. Therefore, until nowadays, clinical experience with liraglutide, exenatide and lixisenatide in CLD patients is limited. However, since exenatide is primarily excreted by the kidney, blood concentrations of the drug are not affected in patients with HI<sup>[77]</sup>. Regarding liraglutide it seems that drug concentrations are not affected by HI<sup>[78]</sup>.

According to the SPC of exenatide and lixisenatide no dosage adjustment is required regarding their administration to patients with HI, whereas for liraglutide the therapeutic experience in patients with CLD is limited. On the basis of available evidence, GLP-1RAs should be used with caution without dose modification in CLD patients. Drugs of this class can be administered to Child-Pugh Class A patients. However, GLP-1RAs should be avoided in Class B and C patients (Table 1).



### SGLT-2 inhibitors

Sodium-glucose co-transporter-2 (SGLT-2) inhibitors (canagliflozin, dapagliflozin, and empagliflozin) is a new class of antidiabetic agents acting through the inhibition of glucose reuptake in the kidney<sup>[79]</sup>. They undergo hepatic metabolism through glucuronidation, and small proportions of the parent drug are eliminated through renal route<sup>[79]</sup>.

The safety of empagliflozin in patients with HI has been confirmed in a study investigating the effect of various degrees of HI on the PK of empagliflozin. In patients with HI empagliflozin PK was affected in a very small degree and, therefore, no dose adjustment of the drug is required in patients with HI<sup>[80]</sup>. The same pattern was observed in a canagliflozin trial, where the canagliflozin PK was not affected by the presence of mild or moderate HI. Therefore, no dose adjustment of canagliflozin is required for these patients<sup>[81]</sup>. Finally, a study on the PK and safety profile of dapagliflozin in patients with HI showed that systemic exposure to dapagliflozin was correlated with the degree of HI<sup>[82]</sup>. Therefore, dapagliflozin should be used with caution in these patients.

On the basis of available evidence, SGLT-2 inhibitors can be used with caution and lower doses should be considered during initiation of therapy in CLD patients. These agents are contraindicated in severe HI. The risk of dehydration and hypotension is associated with the use SGLT-2 inhibitors; hence, caution is required. Precisely, SGLT-2 inhibitors are safe in Child-Pugh Class A patients; however, they should be used with caution in Class B patients. Agents of this class should better be avoided in Class C patients (Table 1).

### Insulin therapy

Liver is the major site of insulin metabolism. Almost half of the insulin produced by the pancreas is metabolized by the liver<sup>[83]</sup>. Hyperinsulinemia is a common finding in T2DM patients with cirrhosis, due to higher insulin secretion rate and reduced hepatic clearance. However, insulin requirement may vary in patients with CLD as a result of the reduced capacity for gluconeogenesis and hepatic breakdown of insulin. Therefore, daily dose requirements of exogenous administered insulin can vary in a high degree and, therefore, is difficult to control blood glucose levels in these patients<sup>[7,16]</sup>.

Insulin therapy is the safest and most effective therapy in patients with CLD. However, there is still the limitation of the increased risk of hypoglycemia<sup>[84]</sup>. Newer insulin analogs are preferred in CLD patients as their PK is unaltered and possesses low risk of hypoglycemia. However, it is suggested that frequent glucose monitoring and dose adjustments are required to minimize the risk of hypoglycemia or hyperglycemia in these patients<sup>[85-88]</sup>. The ADA guidelines highlight the importance of insulin therapy and suggest frequent dose adjustment and careful glucose monitoring in T2DM patients with CLD<sup>[15]</sup> (Table 1).

## CONCLUSION

Management of T2DM in patients with CLD is still a challenge for the clinician. Most of the antidiabetic agents are either contradicted or need dosage titration due to alterations to their pharmacokinetics in patients with CLD. Insulin therapy seems to be the safest choice in patients with CLD. The existing literature data regarding the management of T2DM in patients with CLD are limited<sup>[89]</sup> and only small studies and meta-analyses exist showing the effect of CLD on PK of the OADs. However, the need for the development of guidelines for the management of T2DM in patients with CLD is growing following the high prevalence of HI that characterizes T2DM.

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## Preventive strategies for anastomotic leakage after colorectal resections: A review

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### Abstract

Anastomosis is a crucial step in radical cancer surgery. Despite being a daily practice in gastrointestinal surgery, anastomotic leakage (AL) stands as a frequent postoperative complication. Because of increased morbidity, mortality, combined with longer hospital stay, the rate of re-intervention, and poor oncological outcomes, AL is considered the most feared and life-threatening complication after colorectal resections. Furthermore, poor functional outcomes with a higher rate of a permeant stoma in 56% of patients this could negatively affect the patient's quality of life. This a narrative review which will cover intraoperative anastomotic integrity assessment and preventive measures in order to reduce AL. Although the most important prerequisites for the creation of anastomosis is well-perfused and tension-free anastomosis, surgeons have proposed several preventive measures, which were assumed to reduce the incidence of AL, including antibiotic prophylaxis, intraoperative air leak test, omental pedicle flap, defunctioning stoma, pelvic drain insertion, stapled anastomosis, and general surgical technique. However, lack of clear evidence of which preventive measures is superior over the other combined with the fact that the decision remains based on the surgeon's choice. Despite the advances in surgical techniques, AL remains a serious health problem associated with increased morbidity, mortality with additional cost. Many preventative measures were employed with no clear evidence supporting the superiority of stapled anastomosis over hand-Sewn anastomosis, coating of the anastomosis, or pelvic drain. Defunctioning stoma, when justified it could decrease the leakage-related complications and the incidence of reoperation. MBP combined with oral antibiotics still recommended.

**Key words:** Anastomotic leakage; Colorectal; Resection; Anastomosis; Cancer; Anastomotic disruption



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**Core tip:** Although the most important prerequisites for the creation of anastomosis is well-perfused and tension-free anastomosis, surgeons have proposed several preventive measures, which were assumed to reduce the incidence of anastomotic leakage, including antibiotic prophylaxis, intraoperative air leak test, omental pedicle flap, defunctioning stoma, pelvic drain insertion, stapled anastomosis, and general surgical technique. However, the decision remains based on the surgeon's choice. This review found that many preventative measures were employed with no clear evidence supporting the superiority of stapled anastomosis over hand-Sewn anastomosis, coating of the anastomosis, or pelvic drain. Defunctioning stoma, when justified it could decrease the leakage-related complications and the incidence of reoperation. Mechanical bowel preparation combined with oral antibiotics still recommended.

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## INTRODUCTION

Anastomosis is a crucial step in radical cancer surgery. Despite being a daily practice in gastrointestinal (GI) surgery, anastomotic leakage (AL) stands as a frequent postoperative complication<sup>[1]</sup>. A recent analysis of the National Surgical Quality Improvement Program (NSQIP) database reported that rectal anastomoses were associated with the greatest incidence of AL attributing this to lacking serosa, the under tension anastomoses, technical difficulties in working in the deep pelvis, and easily compromised blood supply<sup>[2,3]</sup>.

Because of increased morbidity, mortality, combined with longer hospital stay, the rate of re-intervention, and poor oncological outcomes, AL is considered the most feared and life-threatening complication after colorectal resections. Furthermore, poor functional outcomes with a higher rate of a permeant stoma in 56% of patients this could negatively affect the patient's quality of life<sup>[4-6]</sup>.

Rojas-Machado *et al*<sup>[7]</sup> in a trial to develop a prognostic index for colorectal AL, they found that 54 potential risk factors were present in the literature. The two most common factors associated with a significantly higher risk of AL were anastomotic height, followed by male sex<sup>[8,9]</sup>. So, the incidence of AL following colorectal resections varies according to the anastomotic level, being 1% to 19% in colorectal or coloanal anastomoses; 0% to 2% in colocolic anastomoses; 0.02% to 4.0% in ileocolic anastomoses; and around 1% in ileoileal anastomoses<sup>[10-14]</sup>.

Surgeons advocated several surgical measures in order to reduce the incidence of AL, including antibiotic prophylaxis, intraoperative leak test, omental pedicle flap, defunctioning stoma, pelvic drain insertion, stapled anastomosis, and general surgical technique. Controversy still exists, which preventive measure is superior over the other combined with the fact that the decision remains based on the surgeon's choice<sup>[15,16]</sup>.

This review will cover intraoperative anastomotic integrity assessment and preventive measures in order to reduce AL.

## INTRAOPERATIVE ANASTOMOTIC INTEGRITY

Nachiappan *et al*<sup>[17]</sup> in a systematic review of intraoperative tests for the assessment of colorectal anastomotic integrity, they testified a reduction in the AL rate when these tests were applied and they divided these tests into: (1) Mechanical patency assessment including air or dye leak testing competence of the doughnuts, it tests the anastomosis by occluding proximal to the anastomosis followed by transanal filling with air or dye to assess any leaking point into the peritoneal cavity without permitting direct anastomotic inspection; (2) Endoscopic visualization which permits direct inspection with the possibility of therapeutic intervention; and (3)

Microperfusion methods permitting blood flow analysis or tissue perfusion showing oxygenated and deoxygenated hemoglobin and the properties of feeding vessels which in turn may modify the planned anastomotic site or reinforce it if needed<sup>[4]</sup>.

### **Intraoperative anastomotic air leak testing**

Wu *et al*<sup>[18]</sup> in a systematic review of the value of intraoperative leak test in prevention of colorectal AL they testified variable methods for performing air leak test (ALT) with variable volume of inflated gas/dye, while ALT group had a lower AL rate compared to the non-ALT group, however, this was non-significant. Patients with positive-ALT had a significantly higher clinical AL rate compared to those with negative-ALT. Additional sutures or diversion were applied to positive-ALT patients. Despite it does not reduce AL, they recommended the routine performance of ALT as it at least predicts high-risk anastomosis and allows additional repairs.

### **Evaluation of anastomotic perfusion “Microperfusion”**

Traditionally, surgeons rely on active mucosal bleeding, the bright coloration, and palpable mesenteric pulses as indicators of adequate perfusion. The search for a reliable objective method to determine tissue perfusion intraoperatively was warranted in order to reduce the incidence of AL, different modalities were applied, however, none has been used routinely in clinical practice<sup>[19]</sup>.

Recently, Near-Infrared (NIR) Fluorescence Angiography using Indocyanine Green (ICG) which is a tricarbo-cyanine molecule when it is injected intravenously, it remains confined to the intravascular space due to its hydrophobic properties allowing it to bind strongly to the plasma proteins. It also fluoresces when excited by light of a particular frequency due to its fluorophoric properties, so it can be used intraoperatively for LN mapping with higher sensitivity and specificity<sup>[20]</sup> as well as in intraoperative perfusion assessment using NIR light technology<sup>[21]</sup>.

Mizrahi and Wexner<sup>[22]</sup> in a review about the role of NIR of the colorectal anastomosis using ICG they reported 3.7%-19% change in the intraoperative decision with further proximal resection for the hypo-perfused anastomoses. They found 6 series with more than 100 patients showed a lower incidence of AL by 4%-12% compared to 75% published case-control series. Jafari *et al*<sup>[23]</sup> in the PILLAR II trial using NIR ICG in distal colorectal resections, they concluded its safety and feasibility. Degett *et al*<sup>[24]</sup> in a systematic review of the role of ICG Angiography for intraoperative perfusion assessment of GI anastomoses they testified regarding the colorectal anastomoses after colorectal cancer, that ICG Fluorescence Angiography had a significant lower AL rate compared to those without assessment. Similar results were reported by studies<sup>[25,26]</sup>.

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## **PREVENTIVE MEASURES**

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Although the most important prerequisites for the creation of anastomosis is well-perfused and tension-free anastomosis<sup>[27]</sup>, surgeons have proposed several preventive measures, which were assumed to reduce the incidence of AL, including antibiotic prophylaxis, intraoperative ALT, omental pedicle flap, defunctioning stoma, pelvic drain insertion, stapled anastomosis, and general surgical technique. However, the decision remains based on the surgeon's choice<sup>[1,28]</sup>.

### **Mechanical bowel preparation**

Traditionally, mechanical bowel preparation (MBP) through the last century was believed to be an important factor within the control of surgeons in order to reduce AL rate and infectious complications in elective colorectal surgery<sup>[29]</sup>. MBP was proposed to have a few theoretical advantages; decreasing the fecal bacterial count, which in turn decrease infectious complications, easier bowel manipulation, decrease the risk of unwanted spillage into the abdomen, decrease the chance of mechanical disruption of the anastomosis<sup>[30]</sup>.

Slim *et al*<sup>[31]</sup> in a meta-analysis of RCTs comparing colorectal surgery with or without prophylactic bowel preparation, they reported a significant AL rate in bowel preparation group. Furthermore, they recommended what was mentioned 40 years ago by Hughes<sup>[32]</sup>, “Omission of enemas and bowel washes from the preoperative procedures will be welcomed by both patients and nursing staff”.

Güenaga *et al*<sup>[33]</sup> in a Cochrane systematic review including a total of 5805 patients, there was not a significant evidence support the use of both MBP or rectal enemas. Additionally, bowel preparation can be omitted safely from colonic surgery, while few studies suggested its selective application in rectal surgery without known significant value. Anastomosis below the peritoneal reflection and laparoscopic rectal surgery still warranted further research<sup>[33]</sup>.

### Oral antibiotics

The concept of the use of oral antibiotics in order to reduce the AL was shown by Cohn and Rives<sup>[34]</sup> in 1955 in the animal model with a complete devascularization of the anastomotic site, the dogs which received oral antibiotics completely recovered with both serosa and mucosa were normal grossly and microscopically, while the control dogs died rapidly from perforated devascularized segment and fecal peritonitis.

Roos *et al*<sup>[35]</sup> in a systematic review and meta-analysis of RCTs about the selective decontamination of the digestive tract (SDD) using a combination of oral antibiotics in addition to intravenous antibiotics compared to intravenous antibiotics alone in elective GI surgery. They testified a significantly lower rate of AL in SDD, a further subgroup analysis for both upper and lower GI surgeries with SDD associated with reduced the incidence of AL in both subgroups.

Recently, data from the NSQIP by Scarborough *et al*<sup>[36]</sup> in a study aimed to determine the association between preoperative bowel preparation status and 30-d outcomes in including AL after elective colorectal resection, with a total of 4999 patients; 1494 patients received combined MBP and oral antibiotic preparation (OAP), 2322 MBP only, 91 OAP only, and 1092 no preoperative preparation. Patients in the combined MBP and OAP group had significantly the lowest incidence of postoperative AL (2.8%) compared to 5.7% of no preparation group, this significance was maintained after adjustment. Patients receiving MBP only or OAP only did not differ significantly from those did not receive preparation<sup>[36]</sup>. Similar results from NSQIP testified by Kiran *et al*<sup>[37]</sup> a total of 8442 patients, 3822 received MBP only, 2324 combined MBP and antibiotic, 2296 no preparation. On multivariate analyses, MBP with antibiotics compared to no preparation was independently associated with lower AL.

A recent pan-European study contacted by the European Society of Coloproctology collaborative group on 3676 patients from 343 centers across 47 countries who underwent left-sided colorectal resections. In this study 29.9% of the patients received no MBP, 52.9% received MBP only, and 16.8% received MBP plus oral antibiotics (Abx). In the multivariate analysis, MBP plus Abx was the only group with a lower risk of AL (OR 0.52, 0.30-0.92,  $P = 0.02$ )<sup>[38]</sup>.

### Creation of the anastomosis

Creation of an anastomosis is a hallmark of surgical practice, decades of practice and research brought a large variety of techniques which made it difficult when trying to conclude about the safest method<sup>[39]</sup>.

**Stapled vs hand-sewn anastomosis:** Stapled anastomoses were believed to have a better healing and less operative complications in comparison to hand-sewn anastomoses, this was explained by less tissue manipulation and better blood supply<sup>[40]</sup>. MacRae *et al*<sup>[41]</sup> in a meta-analysis found no significant difference in total, clinical, and/or radiological AL between stapled and hand-sewn colorectal anastomoses. Lustosa *et al*<sup>[42]</sup> in a systematic review and meta-analysis of RCTs comparing stapled and hand-sewn anastomoses, irrespective the level of colorectal anastomosis they were not able to address any superiority of stapled over hand-sewn anastomosis. The same conclusion was reported by Neutzing *et al*<sup>[43]</sup> in a Cochrane Systematic Review.

Slieker *et al*<sup>[39]</sup> in a systematic review of evaluating the technique of colorectal anastomosis with the clinical AL as the outcome measure, they found a level 1A evidence that there was no superiority between stapled and hand-sewn anastomoses. They also concluded that the hand-sewn anastomoses were constructed following an undefined technique, while the stapled anastomoses were much more uniform.

**Compression anastomoses:** Stapled or hand-sewn anastomoses both are characterized by the use of foreign material; the persistent existence of these foreign materials can be avoided by the use of compression anastomosis with a resultantly reduced inflammation which in turn decrease the duration of the lag phase of anastomotic healing<sup>[44]</sup>. A revolution took place starting from a silver ring by Denans in 1826, then in the Murphy button in 1892 by Murphy. In the 1980s, the Valtrac<sup>TM</sup> in colorectal anastomoses with the use of biofragmentable anastomotic ring by Hardy *et al*<sup>[45]</sup> in 1984, AKA-2 and subsequently the AKA-4 modification for transanal application in the lower rectal anastomoses using non-absorbable metal pins by Kanshin and colleagues in Russia. Recently in colorectal anastomose using nickel-titanium either a clip alloy (Compression Anastomosis Clip-CAC) or a ring compression device (Compression Anastomosis Ring-ColonRing)<sup>[44,45]</sup>. Slieker *et al*<sup>[39]</sup> testified a level 1B evidence similarity between hand-sewn and compression anastomoses.

**The colonic J-pouch:** A lower incidence of AL was testified between colonic J-pouch anastomosis and straight anastomosis<sup>[46-48]</sup>. Justifications of this difference in AL came from the idea that creation of the J-pouch necessitates the full mobilization of the splenic flexure and the obliteration of the pelvic dead space by the colon<sup>[49]</sup>. Later, Hallböök *et al*<sup>[46]</sup> considered the microcirculation difference at the anastomotic site between straight coloanal anastomosis and colonic J-pouch anal anastomosis. They settled a favorable healing anastomosis in the colonic J-pouch compared to colonic end in the straight coloanal anastomosis, due to unaffected blood flow at the anastomotic site of the pouch, whereas became relatively ischemic at the colonic end in the straight coloanal anastomosis.

Brown *et al*<sup>[50]</sup> in a Cochrane systematic review of the reconstructive techniques after rectal resection for rectal cancer they testified that colonic J-pouch leads to better bowel function and similar rates of postoperative complications when compared to the straight coloanal anastomosis. While there is limited literature comparing the transverse colectomy procedure to the colonic J-pouch, three small RCTs suggested that bowel function was similar in patients reconstructed with either procedure. However, there is some evidence that the transverse colectomy procedure results in more AL. Liao *et al*<sup>[51]</sup> in a meta-analysis comparing colonic J-Pouch vs transverse colectomy pouch after AR for rectal cancer, they found no significant difference in the incidence of AL. Hüttner *et al*<sup>[52]</sup> in a meta-analysis of the reconstruction techniques after LAR for rectal cancer they reported that there is no significant difference between straight or side-to-end coloanal anastomosis, colonic J pouch, and transverse colectomy.

### Coating of the anastomosis

It was proposed that external coating of the anastomosis with various materials may reduce clinical AL, especially for high-risk anastomoses as the coating material will seal off the defect. Pommergaard *et al*<sup>[1]</sup> in a systematic review to evaluate the external coating of colonic anastomoses, they reported variable materials had been used with contradictory results, this may be due to the fact that most of these series were studied in experimental animals of different species and of different designs, so their role remains unclear. Only fibrin sealant, omental pedicle graft, and hyaluronic acid/carboxymethylcellulose have been testified in humans.

**Fibrin sealant:** Vakalopoulos *et al*<sup>[53]</sup> in a systematic review of the use of tissue adhesive in GI anastomoses they found it difficult to draw a conclusion on the effects of the tested tissue adhesives on each level of GI anastomosis due to too much heterogeneity in the animal model, absence of details of the amount or the method of applied sealant, and the anastomotic technique was not standardized. They reported 9 studies in rats on fibrin sealant showed to decrease the incidence of AL. The only report on human by Huh *et al*<sup>[54]</sup> in a non-randomized trial of patients who underwent laparoscopic LAR for rectal cancer without diversion, they compared 104 patients in whom fibrin sealant was applied to intracorporeal stapled anastomosis to 119 patients without the use of fibrin sealant was not found to decrease the incidence of AL. They did not describe the amount of the sealant. Nordentoft *et al*<sup>[55]</sup> in a systematic review to assess the potential effect of fibrin sealant on the healing of GI anastomoses, they indicated that it is a physical and mechanical effect neither due to improving the healing power of the anastomosis.

**Omental pedicle graft (Omentoplasty):** A controversy still exists over the use of omentoplasty to decrease the AL rate after colorectal resection<sup>[56]</sup>. Wrapping the anastomosis with intact or pedicled omentum has been designated since 1977 in order to reduce the rate or the severity of AL after colorectal resections, however, insufficient randomized controlled trials exist with conflictive results such as necrosis of the wrap and anastomotic stricture<sup>[56,57]</sup>. Theoretically, when resections are performed for cancer, omentoplasty patients are exposed to further risks of radiation necrosis and local recurrence which was described recently in the animal model<sup>[57]</sup>.

Hao *et al*<sup>[58]</sup> in a meta-analysis of the role of omentoplasty in the prevention of AL after colorectal resection found that there is no supportive evidence to use or not to use omentoplasty as a measure to reduce AL after colorectal resection. Wiggins *et al*<sup>[59]</sup> in a systematic review and meta-analysis in GI anastomoses, they testified on three RCTs of colorectal anastomoses, there was no significant difference in the incidence of AL nor the in-hospital mortality.

### The defunctioning stoma

The value of defunctioning stoma is still controversial, the debate is still present, whether AL rates are lower in diverted anastomoses in comparison to non-diverted anastomoses or both are similar<sup>[60-62]</sup>. Many surgeons delineated the routine use of



proximal diversion for poor patient general condition, narrow male pelvis, neoadjuvant chemoradiotherapy, intraoperative complications related to the anastomosis, low-lying rectal cancer with total mesorectal excision (TME), the goal was to divert the fecal stream from the anastomotic site, which in turn could reduce the incidence of AL and its related morbidity<sup>[63,64]</sup>.

Tan *et al*<sup>[65]</sup> in a meta-analysis about the role of the defunctioning stoma in LAR for rectal cancer testified that value conferred by defunctioning stoma in decreasing the rate and in mitigating the severity of AL. Hüser *et al*<sup>[60]</sup> in a systematic review and meta-analysis of the role of the defunctioning stoma in low rectal cancer surgery, they reached the same conclusion with a significantly lower AL and reoperation rates, whereas mortality rates remained comparable between the groups. These results also were verified by Montedori *et al*<sup>[61]</sup> in a Cochrane systematic review about the use of covering stoma in anterior resection for rectal cancer. Matthiessen *et al*<sup>[9]</sup> in a study of risk factors of AL after rectal resection concluded that in the presence of intraoperative adverse events, defunctioning stoma did not decrease the risk of symptomatic AL. Despite many surgeons delineates a concept of diverting colorectal anastomosis, a controversy still stands whether the best defunctioning could be achieved by loop ileostomy or loop colostomy to address this controversy Güenaga *et al*<sup>[62]</sup> in a Cochrane systematic review found it is not possible to express a preference for use of either loop ileostomy or loop colostomy<sup>[62]</sup>.

However, these benefits must be justified by the fact that routine stoma creation will reduce the quality of life in patients in whom leakage will not occur, the stoma itself is a source of high morbidity reach up to 30%<sup>[66]</sup>. Moreover, the stoma reversal is associated with a mortality of up to 2.3%, requires a second reintervention and hospital readmission<sup>[60,67-69]</sup>. Chow *et al*<sup>[70]</sup> in a systematic review about the morbidity of the reversal of defunctioning ileostomy, they testified that an underestimation of the consequence of stoma reversal. They recommended a selective use of defunctioning ileostomy with patient counseling about the possible complications of reversal at the time of the initial operation. Lindgren *et al*<sup>[5]</sup> in a multicenter RCT about the risk of permeant stoma after LAR for rectal cancer, 234 patients randomly assigned to defunctioning stoma ( $n = 116$ ) or a group without defunctioning stoma ( $n = 118$ ), they testified that 19% of patients their stoma became permanent and this risk was significant for those who developed AL 56% compared to 11% for those without AL.

### **Pelvic drainage**

The purpose of pelvic drainage is to obliterate the pelvic dead space preventing the accumulation of fluid or blood which in turn may form a pelvic abscess or infected pelvic hematoma, both may erode through the anastomosis. Pelvic drainage also may permit the early detection of AL. Some surgeons adopted the use of routine pelvic drainage, other surgeons place drain only in case of doubt about the quality of the anastomosis<sup>[71]</sup>. Pelvic drainage was believed not to prevent AL, nevertheless, the drain serves as “an eye” into the pelvis, allowing for early detection of silent leakage of feculent, pus, or air. It also may contribute to the conservative management of AL<sup>[3]</sup>.

Tsujinaka and Konishi<sup>[72]</sup> in a review article about the usage of drainage in colorectal surgery, they testified that the use of drain should be justified against its own related complications like drain-site infection (up to 2.5%), pain, bleeding, bowel evisceration or injury (0.1%-0.5%), and omental herniation (up to 1.0%). Placing the drain may even disrupt the anastomosis itself. Smith *et al*<sup>[73]</sup> in the animal model showed the danger of placing latex drains near to a colonic anastomosis, as this was associated with a significantly higher incidence of AL, they assumed that latex seems to have a local inhibitory effect on anastomosis healing process. Urbach *et al*<sup>[74]</sup> in meta-analysis and systematic review testified that the use of prophylactic drain has no benefit in prevention of AL or even controlling it if occurs. Jesus *et al*<sup>[71]</sup> in a Cochrane systematic review of RCTs about the role prophylactic anastomotic drainage for colorectal anastomoses they testified this practice devoid evidence. Petrowsky *et al*<sup>[75]</sup> in a systematic review and meta-analysis testified that AL was not significantly different between drained and no drained anastomoses. Rolph *et al*<sup>[76]</sup> reported the same results in another Cochrane review.

On the other hand, Zhang *et al*<sup>[77]</sup> in a systematic review of the use of prophylactic pelvic drainage in colorectal anastomosis to reduce postoperative complications. They testified that no statistically significant difference between the drain and the no drain groups in term of clinical or radiological AL. An unclear value of draining extraperitoneal anastomosis was testified by Rondelli *et al*<sup>[78]</sup> in a meta-analysis, they revealed a lower incidence of AL in drained anastomosis than in the non-drained anastomosis, furthermore, a significantly lower rate of reintervention was found in the drained group than in the non-drained. Karliczek *et al*<sup>[79]</sup> in a systematic review and a meta-analysis on RCTs generally testified that there is no significant difference in the occurrence of clinical or radiological AL. According to the anastomotic level,



they reported no benefit of extraperitoneal anastomosis drainage, but this was based on 2 RCTs.

### Transanal tube drainage

The transanal tube drainage may potentially lower the incidence of AL and its clinical consequences this may be attributed to direct drainage, decreasing the intraluminal pressure and promotion of motility<sup>[80]</sup>. Lee *et al*<sup>[81]</sup> investigated the impact of using a transanal tube drainage after LAR without defunctioning stoma on the incidence of AL, when a propensity score matching was applied the incidence of AL in patients with transanal tube drain had a lower incidence of AL with a reduced number of patients with peritonitis, however, all these difference did not reach significant level.

Shigeta *et al*<sup>[82]</sup> in a meta-analysis tested that transanal tube drainage was associated with a significantly lower rate of AL and reoperation compared with those without. Wang *et al*<sup>[83]</sup> recently in a systematic review and meta-analysis based on three observational studies and one RC, they testified that transanal tube drainage associated with a significantly lower incidence of AL and reoperation with unknown mechanism may be attributed to the reduced intraluminal pressure. Ha *et al*<sup>[84]</sup> in a systematic review and meta-analysis about the role of transanal tube placement after LAR for rectal cancer in RCTs of 475 patients they testified no difference between both groups, while in non-randomized studies of 643 patients the placement of transanal tube was associated with a lower incidence of AL.

## CONCLUSION

Despite the advances in surgical techniques, AL remains a serious health problem associated with increased morbidity, mortality with additional cost. Many preventative measures were employed with no clear evidence supporting the superiority of stapled anastomosis over hand-Sewn anastomosis, coating of the anastomosis, or pelvic drain. Defunctioning stoma, when justified it could decrease the leakage-related complications and the incidence of reoperation. MBP combined with oral antibiotics still recommended.

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## Blood glucose control in the intensive care unit: Where is the data?

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### Abstract

Blood glucose control, including hyperglycemia correction, maintaining glucose at optimal level and avoiding hypoglycemia, is a challenge clinicians face every day in intensive care units (ICUs). If managed inadequately, its related mortality can increase. Prior to 2001, no relevant data from randomized, controlled studies assessing glucose control in the ICU were available. In the past 18 years, however, many clinical trials have defined criteria for managing abnormal blood glucose levels, as well as provided suggestions for glycemic monitoring. Point-of-care blood glucose monitors have become the preferred bedside technology to aid in glycemic management. In addition, in some institutions, continuous glucose monitoring is now available. Cost-effectiveness of adequate glycemic control in the ICU must be taken into consideration when addressing this complex issue. Newer types of glycemic monitoring may reduce nursing staff fatigue and shorten times for the treatment of hyperglycemia or hypoglycemia. There are a variety of glycemic care protocols available. However, not all ICU clinicians are aware of them. The following minireview describes some of these concepts.

**Key words:** Blood glucose control; Critical illness; Intensive care unit; Insulin therapy; Critical care

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**Core tip:** Blood glucose control in the intensive care unit has remained a controversial topic since 2001, with many clinical trials attempting to elucidate which method provides the best option in terms of cost-effectiveness and in providing good clinical outcomes. As technology plays an important role in this matter, this minireview



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compiles the many features of state-of-the-art glycemic monitoring in the intensive care unit and treatment strategies for blood glucose control.

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## INTRODUCTION

Critically ill patients present a special challenge when dealing with glycemic control, as they require correcting hyperglycemia while avoiding hypoglycemia and keeping blood glucose (BG) at optimal levels. This can have significant repercussions on the prognosis of these patients<sup>[1]</sup>. In the last 2 decades there have been a series of studies and added recommendations for glycemic control in the intensive care unit (ICU) setting<sup>[2-5]</sup>. For example, Van den Berghe *et al*<sup>[2,3]</sup> conducted a study among patients in the surgical ICU, who were managed with a rigorous glucose control protocol (maintenance of BG between 80-110 mg/dL) versus conventional treatment (infusion of insulin if BG > 215 mg/dL). They showed an increased survival rate and better prognosis, overall decrease in the mortality rate by 34%, as well as by sepsis (46%), polyneuropathy (44%) acute kidney injury (41%), and a significant decrease in blood transfusion requirements (50%)<sup>[2]</sup>. That particular study elicited some controversies, and additional randomized controlled trials were conducted. In 2009, the Normoglycemia in Intensive Care Evaluation-Survival Using Glucose Algorithm Regulation study (known by its acronym, NICE-SUGAR) revealed an increased mortality rate in those patients that underwent the tight glucose control (TGC) of 81-108 mg/dL, while moderate glucose control target of 140-180 mg/dL was associated with a higher survival rate<sup>[6]</sup>. This multicenter study emphasized the significant risk of hypoglycemic episodes with TGC due its proximity to the lower limit of the BG levels and other similar studies followed<sup>[6-8]</sup>.

Independent of diabetes mellitus, there are many other clinical scenarios that may cause alterations in BG level among critically ill patients, although diabetics are most susceptible to these alterations<sup>[9-11]</sup>. Indeed, critically ill patients are usually admitted to the ICU with stress-induced hyperglycemia (50%-85%)<sup>[5,12]</sup>. For that reason, it is important to identify adequate BG monitoring methods. Continuous BG monitoring would be ideal but can be complex to interpret and treat. Current glucose monitoring devices are rudimentary, and laboratory results may take longer periods of time<sup>[13]</sup>. In this review, we present some aspects regarding the diagnosis, monitoring and management of glycemia in the ICU and discuss some of the newer technological advances that are at the forefront of continuous care of BG.

### Complications

Hyperglycemia has been an important issue when dealing with glucose control in critically ill patients. Krinsley *et al*<sup>[9]</sup> conducted a retrospective study evaluating 1826 patients admitted to the ICU and reported a significant increase in mortality related to glycemic levels, reaching 42.5% in patients with higher mean glucose levels (> 300 mg/dL). These results are consistent with those from other studies, which also have shown that hyperglycemia is a marker of mortality in the ICU<sup>[1,14]</sup>.

Hypoglycemia, on the other hand, is also an important contributing factor for mortality in critically ill patients. Many trials have tested the effectiveness of TGC and have shown it to be a risk factor for developing hypoglycemia (BG < 40 mg/dL) as well as a powerful marker for mortality; it was also found to be superior to hyperglycemia<sup>[6-8,15]</sup>. For example, hypoglycemia in intensive insulin therapy (IIT) was found to be 6-fold more common in patients with more liberal glycemic control<sup>[2,16]</sup>.

## MATERIALS AND METHODS

The authors independently searched an electronic database (PubMed™) using MeSH identifiers with the terms “blood glucose” and “intensive care unit” to identify articles published up to December 2018 with relevancy to glycemic care in the ICU. This search yielded 309 articles. Of those articles, after independent manual review, 160

potential articles were identified and reviewed. As the topic of this search was narrowed to the care of the critically ill patients, only 49 articles were included in this review. Abstract-only, posters, duplicate information, comments and conference papers were excluded. All data acquired were discussed later between the authors, and any disagreements were resolved (Figure 1).

## GUIDELINE RECOMMENDATIONS ON GLUCOSE CONTROL

Several different guidelines recommend certain parameters for glycemic control, with slight differences between the reference values, but a common denominator is the minimization of TGC. In 2011, the American College of Physicians recommended the use of the moderate range of 140-200 mg/dL and did not recommended TGC of 80-110 mg/dL, in order to avoid hypoglycemia and glucose variability (similar to the conclusive results from NICE-SUGAR)<sup>[17]</sup>. The following year, the American Diabetes Association recommended a very similar glycemic control, ranging from 140-180 mg/dL<sup>[18]</sup>. These recommendations are consistent with current critical care guidelines that support the use of insulin infusions in values that exceed 150 mg/dL, with the aim of maintaining a glycemia of 180 mg/dL in an attempt to avoid hypoglycemic episodes<sup>[19,20]</sup>. The Society of Critical Care Medicine guidelines recommended to keep a BG between 150 mg/dL and 180 mg/dL<sup>[19]</sup>.

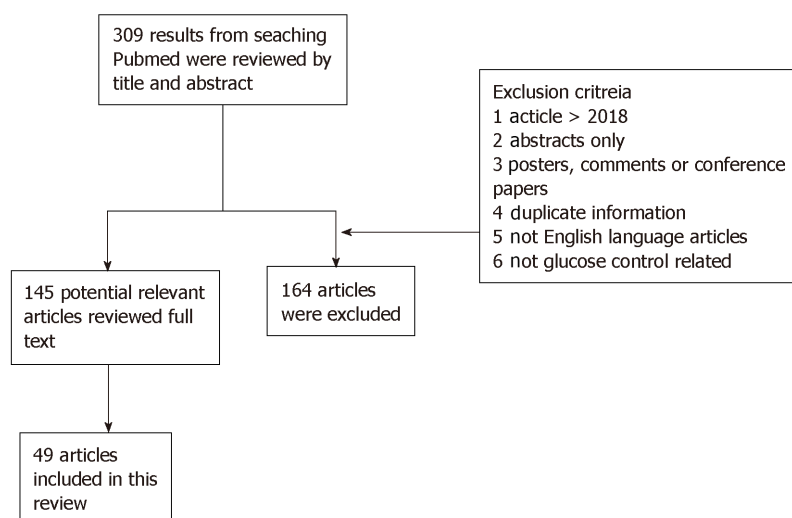
Despite these recommendations, some studies have reported results that have different outcomes. For example, the COITSS study investigators ran a multicenter randomized clinical trial involving 509 adult patients with septic shock, revealing no significant mortality difference in patients with a target BG of 80-110 mg/dL compared to those with a target BG of 150 mg/dL<sup>[21]</sup>.

In many studies, preexisting diabetes mellitus has remained a significant cause for bias in terms of glucose management, as prior studies have shown variability in the response to therapy and different mortality from other patients in the ICU<sup>[10]</sup>. These diabetic patients can develop resistance to glucose fluctuations and can actually benefit from higher BG ranges, avoiding BG variability and hypoglycemic episodes. Marik *et al*<sup>[22]</sup> suggested the necessary target BG ranges based on the hemoglobin A1c (referred to commonly as HbA1c; 160-220 mg/dL in patients with HbA1c > 7%, and 140-200 mg/dL in patients with HbA1c < 7%). Table 1 summarizes some of these guidelines and recommendations for critically ill patients.

## INSULIN THERAPY IN CRITICALLY ILL PATIENTS AND NEWER TECHNOLOGIES FOR BG MONITORING

Prior to 2001, no randomized controlled trials had assessed specific BG targets among critically ill patients. More recently, a variety of studies have focused on management criteria for BG in critically ill patients via glycemic monitoring, use of IV insulin, and computerized processes. Krinsley *et al*<sup>[23]</sup>, in a study of 1600 critically ill patients managed with insulin therapy, reported a 75% reduction in acute kidney injury, 19% decrease in the number of patients transfused with packed red blood cells, 11% decrease in length of ICU stay, and a drop of 29% in mortality. This study aimed to decrease glucose levels to < 140 mg/dL with IIT. However, in a systematic review and meta-analysis by Marik *et al*<sup>[15]</sup> reviewing TGC (80-110 mg/dL) in ICU patients and including seven randomized controlled trials with more than 11000 patients, no reduction was found in 28-d mortality, blood stream infections, or requirement for renal replacement therapy. These investigators concluded that there is no evidence to support the use of IIT in ICU patients. These findings have since been replicated by other studies<sup>[3,24]</sup>. In one such, continuous insulin infusion via central venous catheter led to hypoglycemia<sup>[24]</sup>.

Other studies have shown less of a risk of hypoglycemia. In 2014, Amrein *et al*<sup>[25]</sup> conducted a nurse-driven trial with the Space Glucose Control System™ involving 40 critically ill patients and utilizing a computer-assisted device combined with an infusion pump for glycemic control. The target values were set at 80-150 mg/dL and it was noted that the adherence to the given insulin dose advised by the computer program was 98.2%; only one severe hypoglycemic episode occurred (0.03% of glucose readings)<sup>[25]</sup>. In a similar study of 210 patients in four different ICUs, monitoring BG was followed by management with a computerized insulin infusion program that had been programmed to a moderate glycemic range of 120-160 mg/dL in surgical ICUs and 140-180 mg/dL in medical ICUs<sup>[26]</sup>. The mean BG was 147 mg/dL in the surgical ICUs and 171 mg/dL in the medical ICUs. Only 17% had one or more glycemic episodes between 60-79 mg/dL and 9.8% < 70 mg/dL<sup>[26]</sup>.



**Figure 1** Flowchart describing the methodology for this review.

The Food and Drug Administration (commonly known as the FDA), in 2014, recommended that the use of point-of-care (POC) BG monitors were not suitable for critically ill patients<sup>[27]</sup>. In addition, the Centers for Medicare and Medicaid Services indicated that “off-label” use of such glucometers in the ICU could be subject to citations and fines during site evaluations<sup>[28]</sup>. The main reasons for the FDA and Centers for Medicare and Medicaid Services concerns was that ICU patients are unstable and that might cause erroneous BG readings.

In general, POC glucose monitors cost less, require smaller blood samples, and provide almost instant results. For years, they have been the preferred bedside glucose monitoring devices for glycemic management<sup>[29]</sup>. In a study of a large academic hospital, POC showed significant accuracy<sup>[30]</sup>. Results from glycemic POC paired to results of central laboratory testing of samples drawn no more than 60 min and passed the FDA’s 98% criteria<sup>[30]</sup>.

New software incorporating current guidelines may be just as beneficial for glycemia control<sup>[31]</sup>. Some studies have used the Clinical Notification System that relies on specific criteria and notifies nursing staff of imminent hypoglycemia and persistent hyperglycemia, defined as two consecutive readings  $> 150$  mg/dL<sup>[32,33]</sup>. The sensitivity and specificity of this system are excellent, being 98.1% and 99.1% respectively<sup>[32,33]</sup>.

Continuous BG monitoring is now available<sup>[34-36]</sup>. In a single-center study comparing the benefits of continuous with intermittent glucose monitoring, a peripheral venous catheter was inserted with the GlucoClear™ probe<sup>[35]</sup>. These monitors were flushed with heparin, calibrated, and began BG monitoring every 5 min using a glucose oxidase-based method. Target glycemic ranges for this study were between 90-150 mg/dL. The number of patients with BG  $< 70$  mg/dL in continuous versus the intermittent groups was 8/39 (20.5%) and 15/38 (39.5%) respectively. The time spent with BG  $< 70$  mg/dL was calculated with a continuous glucose monitoring device, and resulted in  $0.4\% + -0.9\%$  versus  $1.6\% + -3.4\%$  ( $P < 0.05$ ) in intermittent glucose monitoring group<sup>[35]</sup>.

In a study by Flower *et al*<sup>[36]</sup>, utilizing a novel intravascular continuous glucose monitoring with chemical fluorescence sensing mechanism, 92.4% (404/437) were in target glycemic control (108-180 mg/dL), with no values  $< 72$  mg/dL.

There are now subcutaneous continuous glucose monitoring sensors in case intravenous access is not available<sup>[37]</sup>. In a small cohort of 14 surgical ICU patients, the Sentrino continuous glucose monitoring glucometer (Medtronic, Dublin, Ireland) was used<sup>[38]</sup>. The study showed that the sensor provided good accuracy, overestimating glycemia by only 1.5 mg/dL<sup>[38]</sup>.

## BG CONTROL IN DIABETIC PATIENTS IN THE ICU

The glycemic control protocols vary among different institutions and according to whether the patient has preexisting diabetes mellitus or not. The effects of IIT, for example, have been more noticeable in nondiabetic critical patients<sup>[39,40]</sup>. In one study, the mortality rates for nondiabetic patients undergoing IIT was 36.8%, as compared to

**Table 1 Glycemic range recommendations**

Study	Glycemic range	Ref.	Comments
American College of Physicians	140-200 mg/dL	Qaseem <i>et al</i> <sup>[17]</sup> , 2014	Recommend use of moderate glucose control to avoid hypoglycemic episodes
American Diabetes Association	140-180 mg/dL	American Diabetes Association <sup>[18]</sup> , 2012	Intensive insulin therapy in TGC can cause severe hypoglycemia
Society of Critical Care Medicine	150-180 mg/dL	Jacobi <i>et al</i> <sup>[19]</sup> , 2012	Recommend the use of moderate use of glucose control
COITSS study	80-110 mg/dL	Annane <i>et al</i> <sup>[21]</sup> , 2010	No significant mortality in patients with TGC compared to MGC
Standards of medical care in diabetes	Nondiabetic HbA1c < 7% 140-200 mg/dL HbA1c > 7% 160-220 mg/dL	Marik <i>et al</i> <sup>[22]</sup> , 2014	Different approach between diabetics and nondiabetics, due to glucose variability in tolerance

MGC: Moderate glucose control; TGC: Tight glucose control.

40.9% in the control group<sup>[39]</sup>. In addition, when compared to patients with diabetes, the interventional group mortality was 39.6% versus 36.8% in the diabetic group<sup>[39]</sup>. In fact, some authors have also suggested that diabetes may be “protective” in the ICU<sup>[40]</sup>.

Mortality is lower for the ICU diabetic population when it comes to hyperglycemia and glucose variability, as compared to nondiabetics. However, hypoglycemia and severe hypoglycemia have an equal mortality rate for both types of patients<sup>[10,41]</sup>. In a study evaluating both nondiabetic patients and diabetic patients with tight and moderate glycemic control (80-110 mg/dL and 90-140 mg/dL), nondiabetic mortality was 11.9% in the moderate glycemic control group when compared to 8.1% in the TGC group<sup>[42]</sup>. In contrast, patients with diabetes had a 12.3% mortality with TGC compared to 9.8% for the moderate glycemic control group<sup>[42]</sup>.

## COST-EFFECTIVENESS

Cost analysis in the ICU remains an important topic. In one study, an economic analysis reported a cost-saving of 2638 Euros per patient in the group that was treated with intensive glycemic control<sup>[43]</sup>. Some have suggested that blood gas analyzers capable of monitoring continuous BG levels are the best option for accuracy and cost-saving, if they are in proximity to the ICU, even when the cost per device is \$40000. The single test cost is very similar to a POC meter (\$100) and the accuracy is equal to a central laboratory device<sup>[44]</sup>. It is clear that euglycemia and avoidance of hypoglycemia decreases the length of stay in the hospital (from 29 d to 24 d) and has a lower health-care cost (mean \$5847), showing a notable amount of money-saving in 5 d<sup>[45]</sup>.

Another factor to consider when analyzing cost savings is the role of TGC in reducing blood stream infections. Some studies have reported that decreasing 5% of hospital-acquired infections could improve cost savings considerably; in fact, one of these studies showed a cost-saving of \$1580 per patient, driven by the decreased length of stay in the ICU<sup>[46,47]</sup>. Such goals can be achieved by attempting to control BG with avoidance of hypoglycemia.

## FUTURE APPROACHES

As noted above, dysregulation of glycemia is a significant factor in the poor prognosis of an ICU patient<sup>[48]</sup>. There are other contributing factors that can change the glycemic status, such as age (older), underweight condition, and type of feeding that is managed in the ICU, since these are labile and can create fluctuations in a more noticeable way compared with the rest of the patients. Critical care clinicians may not be fully aware of these findings. Indeed, some survey studies have shown that clinicians vary significantly in how they manage glycemic index in the ICU and very few are aware that hypoglycemia is associated with an increased hospital mortality<sup>[49]</sup>. Educational programs aimed at understanding these important risk factors are needed. The development of professional awareness of current guidelines and introduction of new technologies are the first step for improving patient care outcomes.

We believe that computerized, protocol-driven and continuous BG monitoring will become the standard of care in ICUs across the world.

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## Treatment of early stage (T1) esophageal adenocarcinoma: Personalizing the best therapy choice

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### Abstract

Esophagectomy is considered the primary form of management for esophageal adenocarcinoma (EAC); however, the surgery is associated with high rates of morbidity and mortality. For patients with early-stage EAC, endoscopic resection (ER) presents a potential curative treatment option that is less invasive and carries fewer risks procedure related risks, but it is associated with higher rates of cancer recurrence following the procedure. For some patients, age and comorbidities may prevent them from having esophagectomy as a treatment option, while other patients may be operative candidates but do not wish to undergo esophagectomy for a variety of reasons related to their values and preferences. Furthermore, while anxiety of cancer recurrence following ER may significantly diminish a patient's quality of life (QOL), so might the morbidity surrounding esophagectomy. In addition to considering health status, patient preferences, and impacts on QOL, physicians and patients must also consider what treatments would be both beneficial and available to the patient, considering esophagectomy methods-minimally invasive *vs* open-or the use of chemoradiotherapy in addition to ER. Our article reviews and summarizes available treatment options for patients with early EAC and their potential effects on the health and wellbeing of patients based on the current data. We conclude with a request for more research of available options for early EAC patients, the conditions that determine when each option should be employed, and their effects not only on patient health but also QOL.

**Key words:** Esophageal cancer; Adenocarcinoma; T1b; Esophagectomy; Endoscopic resection; Chemoradiotherapy; Quality of life

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**Core tip:** This paper is an important source of information for patients and clinicians faced with a diagnosis of T1b esophageal adenocarcinoma (T1b EAC). This paper explores and then outlines the potential benefits and risks of the numerous treatment options for T1b EAC, highlighting the integral role a patient's individual wishes and values play into making a treatment decision that achieves the greatest outcome for that patient. The review advocates for further research regarding the effects of T1b EAC treatment options on a patient's quality of life.

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## INTRODUCTION

Esophageal carcinoma is the eighth most common cancer and the sixth most deadly cancer worldwide<sup>[1]</sup>. While esophageal squamous-cell carcinoma (SCC) is the most prevalent type of esophageal carcinoma globally, esophageal adenocarcinoma (EAC) has a higher incidence than SCC within the United States and much of the Western world<sup>[2]</sup>. The incidence rate of EAC has increased rapidly since the 1970s, which has corresponded with a rise in the incidence of risk factors for EAC such as obesity, a high-fat diet, chronic gastroesophageal reflux, and Barrett's esophagus (BE)<sup>[3-5]</sup>. For the purposes of this review, we focus primarily on treatments for early (T1) EAC.

Esophageal cancer is staged by tumor-node-metastasis based on depth of invasion of the primary tumor, lymph node involvement, and extent of metastatic disease (see [Figure 1](#))<sup>[6,7]</sup>. Tumor staging in T1 EAC is further subdivided and reflects the increasing likelihood of lymph node involvement with increasing lesion depth<sup>[7,8]</sup>. Specifically, T1 EAC is divided into Tis (carcinoma *in situ*; also known as high-grade dysplasia), T1a (confined to the mucosa, without lympho-vascular involvement), and T1b (involvement of submucosa)<sup>[9-11]</sup>. T1b can be substaged further into T1bsm1, T1bsm2, and T1bsm3, each of which represent further invasion into the submucosa, although the reliability of correctly substaging T1b tumors is limited by the precision and accuracy of staging techniques<sup>[12]</sup>. Despite the rising incidence of EAC in recent years, 5-year survival rates have improved for a sub-set of patients, specifically those diagnosed with early-stage EAC, for whom the cancer is confined only to the esophagus<sup>[13]</sup>. This suggests the importance of examining which treatment modalities for T1 EAC confer the most benefit relative to the trade-off between quality of life (QOL) and risk of recurrence.

Until recently, esophagectomy was recommended for all stages of EAC tumors; however, given high rates of morbidity (30%-50%) and mortality (1%-10%) associated with esophagectomy, less aggressive treatment such as endoscopic resection (ER) are increasingly utilized, especially for T1a tumors<sup>[7,8,10,14]</sup>. For patients considering esophagectomy, minimally invasive esophagectomy (MIE) is becoming a more frequently used alternative to the traditional and morbid open esophagectomy (OE). MIE is associated with fewer respiratory complications, intensive care unit stays, faster recovery time, and improved patient satisfaction compared to OE; however, the procedure requires an experienced surgeon due to its steep learning curve, and the clinical relevance of MIE's benefits relative to OE has been contested<sup>[15-17]</sup>. By contrast, endoscopic therapies offer a promising alternative for patients with stage T1a and, potentially, T1bsm1 lesions<sup>[7,8,10,18]</sup>. In fact, according to National Comprehensive Cancer Network 2018 guidelines, endoscopic resection (ER) is the preferred treatment for patients with T1a and T1bsm1 adenocarcinoma without signs of lymph node involvement<sup>[19]</sup>. ER may also offer an alternative for patients ineligible for surgery due to advanced age and/or high comorbidity, or those who wish to pursue a less invasive treatment<sup>[10,18,20,21]</sup>. Aligned with this view, a recent decision analysis found that for T1b patients older than 70 or patients with high comorbidity, ER, rather than esophagectomy, is the most cost-effective treatment<sup>[22]</sup>. However, ER therapies cannot address nodal involvement and therefore carry greater risk of incomplete resection for patients with T1bsm2, T1bsm3, or later tumors, which are more likely to involve lymph nodes<sup>[7]</sup>. Additionally, it is challenging to assess submucosal substaging using ER. A full thickness resection of the submucosa is necessary to accurately identify the

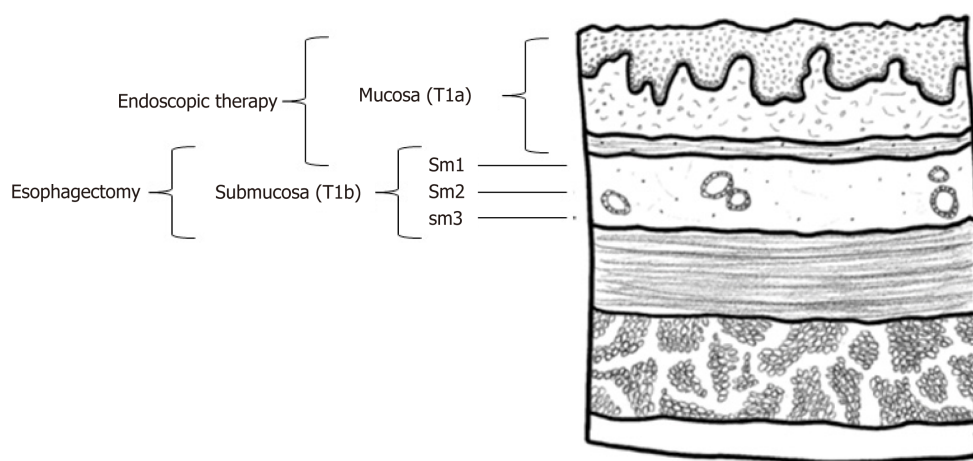


Figure 1 Esophageal adenocarcinoma therapies by tumor stage.

depth of submucosal invasion; ER resections often do not include the full submucosa, and when they do, these specimens may be compromised by saline injection and resection<sup>[23]</sup>.

Several factors complicate treatment decision-making. Age and age-related comorbidities must be considered as 30% of EAC patients are 75 and older at the time of diagnosis<sup>[24]</sup>. Concerns about treatment morbidity and negative side effects are important factors for patients regardless of age<sup>[25]</sup>. Imaging techniques such as computerized tomography, positron-emission tomography scan, and endoscopic ultrasound (EUS), run the risk of understaging T1 and T2 lesions, which should be considered when weighing treatment options with varying levels of risk of recurrence<sup>[6,11,25,26]</sup>. The extant literature, reviewed in detail below, suggests that the choice of treatment for early-stage (particularly T1b) EAC patients is highly complex; it depends not only on stage and sub-stage, but also on patient preferences for treatment aggressiveness versus cancer risk, and patient characteristics such as age and comorbidity. Table 1<sup>[16,22,25,27-29]</sup> summarizes the different treatment options for patients with T1 EAC.

Due to the variability in outcomes associated with different treatment modalities, decisions in this realm are preference-sensitive; that is, decision-making is contingent on the intersection of patients' values and understandings of treatment options<sup>[30]</sup>. As such, the primary aim of this review is to overview the current landscape of early-stage EAC treatment options, including their associated risks of cancer recurrence and morbidity.

## ENDOSCOPIC THERAPIES

### Endoscopic resection

As a diagnostic technique, ER is more accurate and precise at staging early EAC than imaging methods such as EUS, reducing the risk of under- or over-staging the disease<sup>[31]</sup>. ER is a crucial component of treatment decisions for early EAC, as treatment plans are highly dependent on tumor staging and appearance, a topic more thoroughly addressed below.

Curative ER is an option for patients at low risk of lymph node involvement. These patients are characterized by having no or minimal submucosal invasion, no lympho-vascular involvement, negative deep margins, and well- to moderately-differentiated tumor biology; the majority of patients with these characteristics are staged as T1a<sup>[32,33]</sup>. ER offers these patients a potentially curative treatment while preserving the esophagus. For later-stage patients, ER serves primarily as a staging procedure, more accurate than available imaging methods<sup>[34]</sup>. In T1a patients, the risk of lymph node metastases is comparable to the mortality rate associated with esophagectomy, suggesting that esophagectomy is not appropriate for this population<sup>[9]</sup>. Additionally, ER has been shown to yield local control rates exceeding 95% in T1a patients, with survival times comparable to age and sex-matched adults without cancer<sup>[7]</sup>. A decision analysis found that ER resulted in more quality-adjusted life years than esophagectomy for T1a patients regardless of age or comorbidity, but for T1b patients, ER was only cost-effective relative to esophagectomy for older patients or patients with high comorbidity<sup>[22]</sup>. Thus, evidence suggests that endoscopic therapy is an

**Table 1 Treatment of early stage esophageal adenocarcinoma treatment options**

Treatment option	Potential benefits	Potential disadvantages
Endoscopic resection with or without radiofrequency ablation	Organ preserving; very low mortality; low morbidity; small, transient effect on quality of life	Does not address potential lymph node metastases; higher risk of recurrence and lower rate of complete response (particularly in T1b patients)
Esophagectomy	High curative rate; higher disease related survival rate; lower recurrence rates; addresses lymph node metastases	High rates of early and long-term morbidity; considerable rates of surgical mortality; large decrement in quality of life; high post-operative pain; complicated surgical procedure with high operative times and financial costs

acceptable first-line treatment with curative intent for T1a patients.

The low (0%-3.9%) risk of nodal metastases for T1a tumors is sufficient to justify using ER as a primary curative treatment, which is up to 98% effective for patients with Barrett's Esophagus (BE) and early neoplasia<sup>[35-37]</sup>. T1b tumors, by contrast, carry a 20% risk of lymph node metastasis, indicating that ER alone leaves a patient at considerable risk of unaddressed lymph node metastasis and cancer progression<sup>[36,37]</sup>.

### **Endoscopic resection of T1b tumors**

The mortality and morbidity associated with esophagectomy may reach unacceptable levels even for those with riskier T1b lesions, especially older patients with comorbidities. Consequently, T1b patients unwilling or unable to undergo esophagectomy may elect to undergo ER. While ER is ineffective for T1b patients with lymph node metastases, it can potentially be curative for T1b patients without nodal involvement.

Lymph node metastasis is a robust predictor of recurrence and disease-specific survival, and tumor depth is a predictor of lymph node metastasis<sup>[14,38]</sup>. A study ( $n = 69$ ) of T1b patients found that deeper submucosal invasion was associated with poorer outcomes from ER<sup>[39]</sup>. Of those at low risk of nodal involvement (T1bsm1) managed with radical ER, there was only one local recurrence; this recurrence was treated with repeat ER, suggesting that ER is a viable option for T1b patients with minimal submucosal invasion. However, of the 30 high-risk patients with deeper submucosal invasion who opted for ER, six developed metastatic disease during follow-up, and five died of cancer. Among higher-risk patients who did undergo esophagectomy, two of 25 patients went on to develop metastases and die of cancer. Because a greater portion of high-risk patients developed metastatic cancer under ER than under surgical management, the authors concluded that esophagectomy remains the optimal treatment for T1b tumors with deep invasion of the submucosa<sup>[39]</sup>.

Another study ( $n = 107$ ) also demonstrated the curative potential of ER for EAC patients with only mucosal or superficial submucosal involvement and found a 5-year recurrence-free rate of 97% and only one case of lymph node metastasis. However, 18 out of 41 patients with T1bsm2-3 tumors showed lymph node metastases and only 57% were recurrence-free after 5 years<sup>[40]</sup>. These data indicate that, on one hand, ER is a valuable curative treatment for T1bsm1 patients with lower morbidity and mortality rates than esophagectomy, but, on the other hand, ER potentially confers higher cancer risk.

For some T1bsm1 patients, the QOL benefit of organ-preserving ER may be worth the potential risk of untreated nodal metastasis, assuming initial staging and substaging were correct. However, once tumor depth reaches T1bsm2-3, rates of lymph node metastases and cancer recurrence become much higher, and esophagectomy should be the primary form of curative treatment<sup>[40]</sup>. It is important to reiterate that accurate, precise EAC staging and substaging of T1a and T1b is difficult<sup>[6,11,25,26]</sup>. Consequently, the risk of potentially understaging patients should be communicated by the clinician and considered when deciding between conservative ER and esophagectomy, as patients may desire a more aggressive treatment when presented with the possibility that their cancer may be understaged.

As previously mentioned, tumor depth is not the only predictor of lymph node involvement; there are other factors to consider when determining risk of lymph node metastases, including tumor morphology, histologic grade, and lymphovascular invasion<sup>[41,42]</sup>. In one study ( $n = 85$ ), T1b tumors were grouped according to depth of submucosal invasion in conjunction with histological grade and lymphovascular invasion. Patients with tumors that were well- or moderately-differentiated and had no lymphovascular invasion had rates of overall and disease-specific survival closer to T1a tumors than to T1b tumors with poor differentiation and T1b tumors with



lymphovascular invasion<sup>[43]</sup>.

Another study ( $n = 66$ ), examined the effects of ER on T1bsm1 patients defined as low risk, characterized by having macroscopically polypoid or flat lesions and good to moderate tumor differentiation (G1-G2), and found that out of the 61 patients whose remissions were assessed, 53 achieved complete endoluminal remission (CER) and fifty-one achieved long-term remission<sup>[44]</sup>. When focal lesions were smaller than 2 cm, the CER rate jumped from 87% to 97%. There were no associated tumor deaths. The rate of major complications from ER was 1.5% with a 0% mortality rate, and biopsy and imaging results showed that only one patient had lymph node metastases<sup>[44]</sup>. Ishihara *et al.*<sup>[45]</sup> found no metastasis in any of the 32 T1b lesions smaller than 30 mm without lymphovascular involvement and a poorly differentiated component in their study of the risk of metastasis of EAC, concluding such lesions as good candidates for ER.

These studies highlight that T1b tumors have similar prognostic outcomes to T1a tumors if their histology and morphology do not indicate nodal involvement. Furthermore, they identify the importance of considering multiple tumor characteristics to determine the risk of lymph node metastasis. As previously stated, ER techniques may not always accurately stage submucosal involvement; therefore, examining multiple tumor characteristics to inform tumor classification and treatment decisions is important<sup>[23]</sup>.

Despite the benefits associated with organ preservation, the impact of fear of cancer recurrence on QOL is a factor to consider for T1b patients eligible for ER. One study ( $n = 91$ ) examined QOL and fear of cancer recurrence following endoscopic versus surgical treatment for early-stage EAC (defined as BE with high-grade dysplasia, T1, T1sm, and T2N0M0 tumors) and found no differences in health-related or cancer-specific QOL between treatment groups<sup>[46]</sup>. While the surgical group reported significantly more reflux and eating problems, the ER group reported greater fear of cancer recurrence and anxiety<sup>[46]</sup>. The authors of the study noted that leaving the esophagus intact may prompt cancer anxiety among patients treated with ER and especially among T1b patients, for whom the chance of incomplete eradication and nodal metastases are higher<sup>[27]</sup>. Additionally, a study ( $n = 20$ ) eliciting health state utility values associated with dysplastic BE-related health states from non-dysplastic BE patients reported that the utility of states associated with potential cancer recurrence were comparable to the utility associated with esophagectomy<sup>[47]</sup>. Along these lines, a decision analysis comparing ER and esophagostomy outcomes for T1 EAC patients found that the optimal treatment strategy depended most heavily on the post-treatment health state utility values, indicating that for patients with T1b EAC, treatment decisions should be centered around patient preferences<sup>[22]</sup>. Together, these studies suggest that patient perceptions of and preferences for cancer risk are important to consider when making treatment decisions for T1b EAC.

### **Endoscopic eradication therapy: Radiofrequency ablation and cryotherapy**

Patients who have early-stage EAC that has developed within a large segment of BE have the option of ER followed by radiofrequency ablation (RFA) or other ablative therapies to eliminate the remaining BE<sup>[34,48-50]</sup>. Ablative techniques are important as they reduce the risk of metachronous neoplasia developing in residual BE<sup>[51]</sup>.

Research with BE patients suggests that RFA is an effective method for eradicating areas with dysplasia or early intramucosal adenocarcinoma<sup>[52]</sup>. Although RFA carries a 7.8%-11.3% risk of adverse events (predominantly strictures and bleeding) these events are often low grade, with a low rate (0.6%) of severe adverse events (e.g. perforation)<sup>[53,54]</sup>. The impact of RFA on QOL in EAC patients has not been specifically studied. However, one study reported the effects of RFA on patients with dysplastic BE and found that performing RFA improved QOL by reducing anxiety about developing cancer, worry about needing an esophagectomy, stress, impact on daily QOL, dissatisfaction with the condition of their esophagus, and impact on work and family life<sup>[55]</sup>. These findings indicate a potential benefit of RFA for patients with T1a or T1bsm1 EAC and for patients with T1bsm2 or sm3 EAC who are unable or unwilling to undergo esophagectomy.

Research suggests cryotherapy is also an effective method of eradication of dysplasia<sup>[50,56]</sup>. Cryotherapy is also utilized as treatment for patients with EAC who are unwilling or unable to undergo more aggressive treatments<sup>[57-60]</sup>. A 2017 analysis of the safety and efficacy of liquid nitrogen spray for EAC patients who were not candidates for conventional therapy demonstrated complete response rates for 76.3% of T1a patients, 45.8% of T1b patients, and 66.2% for all T1 patients, with a low rate of low-grade strictures (13.6%)<sup>[57]</sup>. A 2013 assessment of patients ineligible for conventional EAC therapy found that 75% of patients with T1a EAC and 60% of patients with T1b EAC showed complete endoscopic response, with benign strictures occurring in 13% of patients<sup>[58]</sup>. These data suggest that cryotherapy is tolerable and effective for T1



EAC patients who cannot undergo conventional treatments; however, data regarding long-term outcomes are lacking<sup>[59,60]</sup>.

## SURGICAL TREATMENTS

### **Esophagectomy**

Esophagectomy is the mainstay of treatment for resectable esophageal cancer and is indicated for T1b tumors with more than minimal submucosal invasion or later-stage tumors due to increased risk of nodal involvement<sup>[9,10]</sup>. The pain and discomfort experienced by patients after surgery has a significant negative effect on a patient's QOL<sup>[61]</sup>. Specifically, a 2014 meta-analysis found significant and lasting detrimental effects of surgery on QOL: Social functioning, fatigue, pain, reflux, dyspnea, and coughing problems were significantly worse than pre-operation for at least nine to twelve months after surgery, at times extending beyond one year<sup>[62]</sup>.

Regardless of the modality for esophagectomy (*e.g.*, open or minimally-invasive), optimal surgical outcomes occur in high-volume centers with highly-skilled and well-practiced surgeons. Esophagectomy in high-volume surgical centers reduces the morbidity, mortality, length of hospital stay, and cost of the procedure<sup>[63]</sup>. Additionally, the long-term prognosis of a patient following the surgery is associated with the case-volume of the surgical center, with a higher volume predicting a better prognosis<sup>[64,65]</sup>. These data indicate that, when possible, patients may benefit from assuming the additional burden of seeking high-volume hospitals with highly experienced staff and the necessary resources to prevent and manage potential complications.

### **Open versus minimally-invasive**

Treatment esophagectomy consists of two primary surgical techniques: OE and MIE. MIE is performed laparoscopically or thoracoscopically, where access to the abdominal and thoracic cavity is granted via small abdominal incisions. OE, on the other hand, requires a right thoracotomy and laparotomy, which involves large incisions where the ribs and abdominal wall are opened widely. Both OE and MIE carry significant risk of complications; one meta-analysis found complication rates of 48.2% and 41.5% in patients undergoing OE and MIE, respectively<sup>[66]</sup>. Although each of these rates are high, the difference between the two was significant and favored MIE. This meta-analysis also found a post-operative mortality risk that again significantly favored MIE, with an average mortality risk of 3.8% and 4.5% for MIE and OE respectively<sup>[66]</sup>. MIE has also been shown to result in superior short-term outcomes relative to OE, with reduced blood loss, fewer pulmonary and respiratory complications, lower total morbidity rates, and shorter post-operative hospital stays<sup>[66-71]</sup>. Owing to the complexity of the procedure, the operative time for MIE is, however, longer than that of OE<sup>[16]</sup>. MIE and OE have not been shown to differ in oncologic outcomes, with comparable lymph node retrieval and overall survival<sup>[72]</sup>.

Regarding the impact of each procedure on QOL, a systematic review comparing MIE and OE found that, while overall health and social and emotional function more frequently improved following MIE relative to OE, other QOL outcomes were comparably and negatively associated with both surgery types<sup>[73]</sup>. Another meta-analysis found that MIE patients reported higher QOL than OE patients immediately after surgery, but evidence for this disparity was less robust 1-year post-operation<sup>[74]</sup>. Thus, while MIE may not have as large of an immediate decrement on QOL, MIE, like OE, remains an aggressive procedure that carries risks and can produce complications.

Important to note is the varying accessibility of MIE versus OE. Although there is evidence to suggest superiority of MIE over OE, the choice between surgical techniques may not be available to some patients because of geographic and logistical barriers such as the steep learning curve of MIE and the low availability of surgical tools necessary for MIE<sup>[16,75,76]</sup>. Therefore, OE may be the only option for patients who do not have access to centers equipped for MIE or do not have the resources to seek such centers.

## EMERGENT TREATMENT STRATEGIES

### **Chemoradiotherapy and endoscopic resection**

As mentioned above, ER is primarily recommended for T1a patients due to concerns regarding the increased rate of lymph node metastases following submucosal invasion<sup>[28]</sup>. Lymph node metastases can be addressed through lymphadenectomy

during esophagectomy and/or chemoradiotherapy (CRT). CRT alone is sometimes used as a non-operative treatment in place of surgery for older patients with locally advanced EAC who are unable, unwilling, or not referred to undergo esophagectomy despite the risk of nodal involvement. However, current data show that, compared with esophagectomy, definitive CRT for stage I-III EAC patients is ineffective<sup>[77-83]</sup>. Additionally, CRT's impact on QOL and the differences in severe adverse events relative to esophagectomy have not been well documented. With this in mind, ER in combination with chemotherapy or CRT warrants further exploration as a potential organ-preserving alternative to esophagectomy that can address lymph node metastases, particularly in patients who are older and/or have comorbid conditions who are poor operative candidates<sup>[84]</sup>.

Existing research regarding CRT + ER is limited for early-stage EAC patients. Minashi *et al*<sup>[85]</sup> found that ER and selective CRT provided to patients with T1b (sm1-2) resulted in a 3-year survival rate of 90.7%, which is comparable to that of surgery. A review of six studies ( $n = 168$ ) in which all patients had superficial esophageal SCC treated with CRT + ER found promising rates of control of local recurrence following treatment, ranging from 0%-9%, and 3-year overall survival rates ranging from 87%-100%<sup>[86]</sup>. Patients who developed metachronous esophageal lesions after ER and adjuvant CRT were all successfully treated with salvage ER<sup>[86]</sup>. The major limitation of these findings is that the patients in this review had SCC, which tends to have a better response to CRT than EAC; therefore, these results alone cannot be used to justify the use of ER and CRT for T1 EAC<sup>[82]</sup>. Another study ( $n = 32$ ) compared outcomes of ER alone, CRT + ER, and esophagectomy in patients with T1b EAC<sup>[87]</sup>. This study found an EAC recurrence rate of 11% with CRT + ER, compared to a 38% EAC recurrence rate with ER alone, and a 29% EAC recurrence rate with esophagectomy. Although there was a trend toward better outcomes for CRT + ER, differences in EAC recurrence rates were not statistically significant, potentially due to a lack of statistical power; however, these findings suggest that CRT + ER could be a viable treatment option for T1b EAC patients unable or unwilling to undergo esophagectomy<sup>[87]</sup>. Another report assessed the efficacy of salvage ER following CRT in two patients with T2N0M0 EAC who were unfit for esophagectomy<sup>[88]</sup>. Both patients achieved complete endoscopic and histological remission after removing residual lesions with ER following treatment with CRT regimen. While this sample size is too small to generalize the results to the early EAC patient population, these results suggest the utility of future work examining the efficacy of CRT + ER in early-stage EAC patients<sup>[88]</sup>.

### **Biomarkers and precision medicine**

Another avenue of research with promising therapeutic potential is the identification of prognostic and predictive biomarkers of EAC and tailored treatment plans that target these biomarkers. These targeted therapies work by acting on molecular characteristics of a patient's tumor, rather than applying a systemic conventional chemotherapy. Prognostic biomarkers of overall survival for patients with EAC have been identified, and include *SPARC*, *SPP1*, and *MET* gene expression, COX-2 angiogenic factor expression, and HER2 positivity<sup>[89-92]</sup>. Some molecular profiles are more common in EACs than others; EGFR (16% of EACs), HER2 (19% of EACs), and MET (6% of EACs), some of the more common biomarkers in EAC, have available targeted therapies, although they have mostly been explored in adenocarcinoma of the gastroesophageal junction<sup>[93]</sup>. HER2 positivity and EGFR overexpression are prognostic biomarkers, the presence of which indicate a poorer EAC prognosis as they promote cancer growth<sup>[94,95]</sup>. Patients with HER2 positivity are considered for treatment with trastuzumab, which acts on HER2 cells to inhibit tumor cell growth<sup>[96]</sup>. Those with EGFR overexpression may be candidates for treatment with cetuximab with chemotherapy, which has shown a trend toward improved survival relative to chemotherapy alone<sup>[93]</sup>. Because targeted therapies work only on cells that express a given biomarker, therapies such as trastuzumab and cetuximab are most likely to yield positive outcomes only in the subset of EAC patients with HER2 positivity and EGFR overexpression, respectively<sup>[93,97]</sup>. The efficacy of these targeted treatments, which offer the benefits of superior outcomes for a subset of patients and lower toxicity than conventional CRT, merit further study as a potential definitive and/or neoadjuvant treatment for EAC patients.

## **CONCLUSION**

A thorough understanding of available treatment options for early-stage EAC and their effects on survival, health, and QOL is paramount for informing treatment-

related decisions and improving patient outcomes. Many patients diagnosed with early-stage EAC are older, have comorbid conditions, or are eligible to undergo esophagectomy but choose not to have it. Future research concerning these patients' preferences and effects of different treatments on QOL are warranted. Additional exploration of T1b tumor characteristics that can accurately predict whether the tumors are at high or low risk of lymph node metastasis would also aid in treatment choice optimization for T1b patients. The potential for the combination of ER and CRT to provide an effective, organ-preserving treatment for some early-stage EAC patients is important and requires further investigation.

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## Mechanisms of action of aqueous extract from the *Hunteria umbellata* seed and metformin in diabetes

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### Abstract

The plant kingdom is an important potential source of effective treatment for various diseases. Most herbs have long been used for medicinal purposes, and plant metabolites with their derivatives had been used in ethnomedicine. However, concerns exist about the quality and safety of herbal medicine products, particularly relating to safety, dosage, and mechanism of action. This mini review reveals some insights about the *Hunteria umbellata* seed, which is similar to that of insulin secretagogue metformin. Studies have validated its beneficial role in hyperglycemic, insulin resistance and obesity conditions, which are components of metabolic syndrome. However, none of these studies evaluated the mechanisms by which this plant extract performs its anti-hyperglycemic, insulin resistance and anti-obesity actions in metabolic syndrome. This understanding would provide considerable progress toward drug design using this plant material. Hence the need for this awareness to sensitize the researchers in this field who are passionate about drug design to consider the pathways discussed below for *Hunteria umbellata* seeds. *Hunteria umbellata* seed extract may represent a new therapeutic strategy for type-2 diabetes in place of metformin if it is well-studied.

**Key words:** Insulin; Diabetes; *Hunteria umbellata*; Metformin; Metabolic syndrome

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**Core tip:** Herbs have been used for medicinal purposes since time is immemorial, although concerns exist about quality, safety, dosage and mechanism of action of herbal medicinal products. This mini review reveals some insights about the *Hunteria umbellata* seed, which is similar to metformin insulin secretagogue but with less side effects in diabetic subjects. Based on its beneficial roles that have been documented, none of the studies have evaluated the mechanisms involved. Therefore, researchers in this field who are passionate about drug design need to consider the pathways discussed below for the *Hunteria umbellata* seed. This may serve as a new therapeutic strategy for



type-2 diabetes in place of metformin.

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## INTRODUCTION

A systematic review published by Herman *et al*<sup>[1]</sup> revealed that eight out of ten of the world population diagnosed with type-2 diabetes are within developing and developed countries; this indicates the role of socioeconomic factors played in the demography and dynamics of type-2 diabetes. According to the International Diabetes Federation projection, one in every 10 adults will be diagnosed with diabetes by 2030<sup>[2]</sup>. A year following this press release, type-2 diabetes was described as a global epidemic requiring attention and urgent action<sup>[3]</sup>. These data clearly support that expedited actions should be directed towards these countries. Pathophysiologically, failed insulin secretion and insulin resistance drive type-2 diabetes<sup>[4]</sup>; thus, type-2 diabetes is amenable to insulinotropic drugs<sup>[5]</sup>. Due to socioeconomic constraints, low- and medium-income countries have no access to insulinotropic drugs; therefore cheaper, more accessible therapeutic options must be considered to offset current statistics and future projections.

*Hunteria umbellata* (K. Schum.) Hallier f. belong to the Apocynaceae family, a West African glabrous tree known as Abeere in Yoruba, Southwest Nigeria<sup>[6]</sup>. It has been widely used by different folk, which include the management of infections, diseases, treatment of pain and metabolic disorders such as diabetes mellitus and obesity<sup>[7]</sup>. Many genera in the Apocynaceae family have been well-studied, especially their chemical composition and economic importance. Studies have revealed that the aqueous seed extract of *Hunteria umbellata* has been tested on different experimental models of diabetes<sup>[8]</sup> for its anti-hyperglycemic effect coupled with anti-obesity and anti-hyperlipidemic potentials<sup>[9]</sup>, which are well-documented. Likewise, oral toxicity studies have been conducted that authenticate its safety prior to oral administration in experimental animals<sup>[10]</sup>. Biometric analysis of the *Hunteria umbellata* seed conducted by Ajibola and co-researchers revealed that aqueous extract of the *Hunteria umbellata* seed performed well in reducing fasting blood glucose in type-2 diabetic patients in a few short weeks compared with metformin, coupled with lesser side effects. The patients placed on metformin exhibited symptoms such as abdominal pains, belching, chest pain, diarrhea, headache, nausea and vomiting, runny nose and weakness; but the only complaint recorded from patients treated with the *Hunteria umbellata* seed was extreme bitterness, which caused one of the patients to have an allergic reaction to the extract, causing seldom vomiting<sup>[11]</sup>.

Furthermore, hypoglycemic activity of its seed extract has been documented in normal, high glucose and nicotine-induced hyperglycemic rats, mediated *via* intestinal uptake of glucose coupled with adrenergic inhibition, respectively<sup>[6]</sup>. Similarly, other animal models were also used and reported to ascertain the efficacy of *Hunteria umbellata* seed extract as an anti-diabetes and anti-obesity plant<sup>[9]</sup>. It has been documented by Boone *et al*<sup>[12]</sup> that the *Hunteria umbellata* seed possesses eburnamine, eburnamonine, hunteriamine, hunterine, vincamine and corymine as part of its phytoconstituent, with eburnamonine and eburnamine coupled with hunterine that have been indicated to possess strong and lasting hypotensive action. Cerebrovascular activity of the *Hunteria umbellata* seed has been traced to eburnamonine, which is reported to be more abundant<sup>[12]</sup>. This has a positive effect on general blood circulation, while anti-hypertensive and sedative properties have been attributed to vincamine<sup>[12]</sup>. Likewise, Adeneye and fellow researchers<sup>[13]</sup> suggested that anti-hyperglycemic potential posed by the *Hunteria umbellata* seed was attributed to an isolated, newly extracted bisindole alkaloid called erinidine, with the *in vitro* and *in vivo* anti-hyperglycemic studies conducted using erinidine confirming that the *Hunteria umbellata* seed can mediate its anti-hyperglycemic action *via* intestinal glucose uptake inhibition. Findings have revealed that none of these studies have delved into the mechanisms by which this plant extract performs its anti-hyperglycemic actions in experimental diabetes models, which would provide strong progress towards drug design using *Hunteria umbellata* seeds.

## SYSTEMIC ACTIONS OF AQUEOUS EXTRACT OF *HUNTERIA UMBELLATA* SEED IN DIABETICS

A study revealed that aqueous extract of *Hunteria umbellata* seeds were capable of ameliorating metabolic syndrome associated with high fructose-induced rats, (Figure 1) thereby acting as a scavenging agent for reactive oxygen species and increased enzymes activities that detoxify reactive oxygen species, which invariably revealed *Hunteria umbellata* seeds as a source of nutraceuticals for treating metabolic syndrome<sup>[14]</sup>.

Meanwhile, metformin is known as a potent anti-hyperglycemic agent specific for type-2 diabetes, but exerts its pharmacologic actions on type-2 diabetes in routes different from any other class of drugs<sup>[15]</sup>.

The apparent volume of distribution (V/F) of metformin following single oral doses of 850 mg immediate release metformin hydrochloride averaged  $654 \pm 358$  L. Metformin is negligibly bound to plasma proteins. Metformin partitions into erythrocytes, most likely as a function of time. At usual clinical doses and dosing schedules of metformin, steady state plasma concentrations of metformin are reached within 24-48 h and are generally  $< 1 \mu\text{g/mL}$  during controlled clinical trials, which serve as the basis of approval for metformin. Notably, maximum metformin plasma levels did not exceed  $5 \mu\text{g/mL}$ , even at maximum doses.

Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine, and does not undergo hepatic metabolism or biliary excretion. Renal clearance is approximately 3.5 times greater than creatinine clearance, which indicates that tubular secretion is the major route of metformin elimination<sup>[15]</sup>.

## METFORMIN MECHANISMS OF ACTION IN DIABETES MELLITUS

The liver is the main site of action for metformin, where it has been shown to reduce hepatic glucose output by 75%<sup>[15]</sup>, and reduce hepatic glucose production mainly by inhibiting liver gluconeogenesis<sup>[16]</sup>, which is the formation of glucose by the liver from non-carbohydrate sources, such as amino acids. In addition, the uptake of gluconeogenic substrates (alanine and lactate) is reduced by metformin<sup>[15]</sup>. Insulin sensitivity is increased in skeletal muscles because of an increase in the tyrosine kinase activity of the insulin receptor along with increased GLUT1 (glucose transporter) transport activity by increasing translocation to the plasma membrane<sup>[15]</sup>. Metformin altered endocrine function in the pancreas by stimulating the expression of the glucagon-like peptide 1 (GLP-1) receptor and GLP-1 protein in the pancreas. GLP-1 is responsible for increasing the secretion of insulin and lowering the secretion levels of glucagon (a hormone that raises blood glucose levels)<sup>[15]</sup>. Metformin slightly delays the absorption of glucose through the gastrointestinal (GI) tract<sup>[15]</sup>.

## CONCLUSION

This short communication is put together for researchers working on type-2 diabetic drug design to explore the potential of *Hunteria umbellata* seed extract via this pathway (Figure 2).



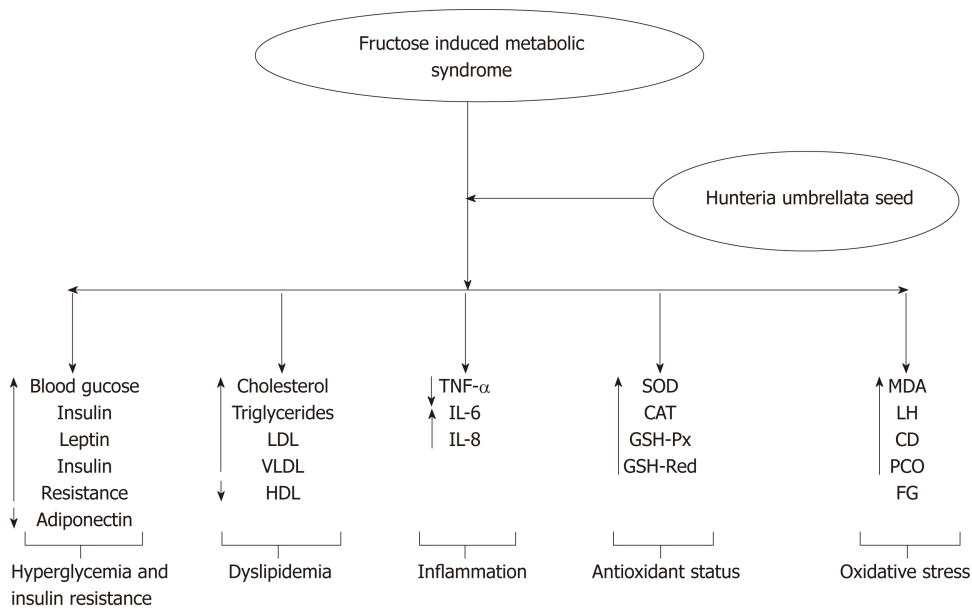


Figure 1 Systemic actions of aqueous extract from the *Hunteria umbellata* seed in diabetics. Adopted from<sup>[14]</sup>.

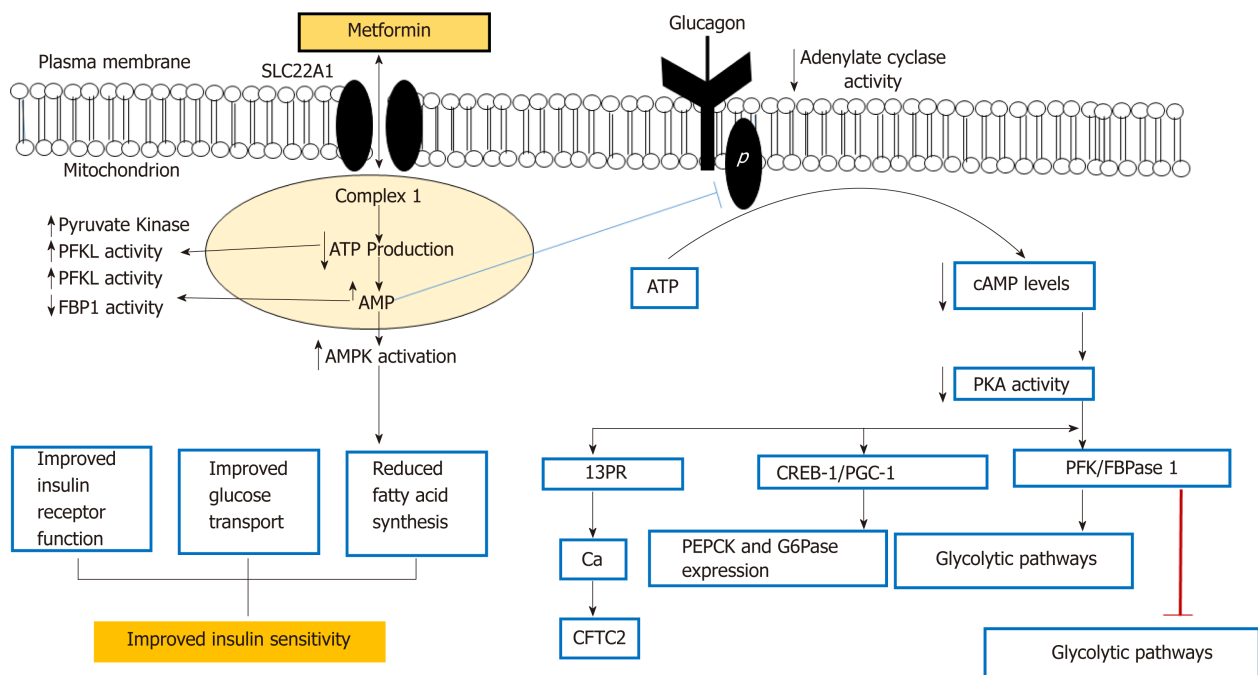


Figure 2 Mechanistic action of metformin in hepatocytes of the liver in diabetic patients. Adopted from<sup>[8]</sup>.

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## Fecal microbiota transplantation: Historical review and current perspective

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### Abstract

There is a growing interest in the use of fecal transplantation for chronic intestinal conditions. We aim to review the methodology and safety of fecal microbiota transplantation and the evidence to support its use in treating a variety of diseases. We reviewed the history of fecal transplantation in China and found that there were varieties of fecal material used in ancient China. The first written record on fecal treatment was found in an ancient tomb in Middle China. This paper explores the historical and current perspectives of fecal microbiota transplantation. The ancient fecal transplantations did not have any background support from life science. In those ancient days, short of knowledge about bacteria, clinicians were aiming at a change of intestinal environment. Today, we aim at a change of the intestinal microbiome.

**Key words:** Fecal transplantation; Microbiota; Intestinal microbiome; Microenvironment

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**Core tip:** There is a growing interest in the use of fecal transplantation for chronic intestinal conditions. In the article, we reviewed the history of fecal transplantation in China. The first written record on the oral use of fecal matter was in 770 BC. Although the ancient fecal transplantations did not have any evidence from life science, the ancient healers were fully aware of the acting value of gastrointestinal variety. Today, researchers in the field are working on various ways to change the microbiome at different levels of the gut.

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## INTRODUCTION

The gastrointestinal tract harbors a diversity of microflora, and any alterations may contribute to problems like chronic gastrointestinal infections and inflammatory bowel diseases<sup>[1,2]</sup>. Recently, the microflora has also been shown to be potentially responsible for cardiac, metabolic and autoimmune conditions and some neoplasms<sup>[3-5]</sup>. In 1958, Eisman *et al*<sup>[6]</sup> reported the first four cases of fecal transplantation for the control of pseudomembranous enterocolitis. The successful control of chronic diarrhea was assumed to be due to a change of the intestinal microflora.

In recent years, there is a growing interest in the use of fecal transplantation for chronic intestinal conditions, including ulcerative colitis, celiac disease, irritable bowel syndrome and *Clostridium difficile* infection<sup>[7-10]</sup>. Many assumed that fecal transplantation was new technique not realizing that it was an established practice in ancient China. We aim to review the historical methodology and safety of fecal microbiota transplantation and the evidence to support its use in treating a variety of diseases.

## HISTORICAL REVIEW OF FECAL TRANSPLANTATION IN CHINA

The first written record on the oral use of fecal matter was contained in one of the oldest Text of Chinese Medicine excavated in an ancient tomb in Middle China, called "Fifty-two Treatment Formulae"<sup>[11]</sup>. It was estimated that the document was written in 770 BC. Some details of the preparation of a fecal product called "golden juice" were given, and it was indicated for detoxication<sup>[12]</sup>. The authors did not encounter stories about medicinal use of fecal material in other ancient cultures while searching through relevant literature (British Encyclopedia).

The next record appeared in an important classic for medical teaching in the Han Dynasty (206 BC to AD 220), and the indications given were gastrointestinal emergencies<sup>[13]</sup>. Fecal material was reported to be used during epidemics.

Ge Hong (AD 284-364), a well-known Taoist healer, compiled a treatment guide "Handbook of Emergency Conditions" in which many medicinal items and formulae containing fecal matter of human, chicken, dog, cattle, horse, *etc.* were described<sup>[12]</sup>.

Subsequently, classics compiled in the following dynasties, Song (AD 960-1279), Ming (AD 1368-1644) and Qing (AD 1644-1912), all contained supplemented versions of fecal preparations, which had already been described or were new inventions<sup>[14]</sup>. Clinical applications ranged from detoxication in emergency events, removal of harmful causes in infections, treatment of severe gastrointestinal problems like uncontrolled diarrhea and vomiting and most recently as an anti-allergic decoction in severe anaphylaxis-like emergencies<sup>[15]</sup>.

Many different types of "fecal medicine" have been described. A table of eleven frequently described items is given (Table 1). Over 1550 recorded prescriptions containing different fecal substances are available for scrutiny<sup>[16]</sup>.

The unfavorable stigma attached to the use of fecal matter in the past is unavoidable. Obviously, the majority of the items described have become less popular, although they remain respectable. Herb Books and some senior traditional practitioners still favor these applications<sup>[16]</sup>. While it is easy to be skeptical about ancient practices and label them as simple superstition or folk lore, one may objectively analyze the logic behind the historical uses. In return to this objective exercise, lessons can be learned that may serve the current field and help the further development of fecal transplantation.

## VARIETIES OF FECAL MATERIAL USED IN ANCIENT CHINA

Looking at the variety of fecal products and their ancient applications, their correlation with the ancient logic of Traditional Chinese Medicine could be identified as follows.

**Table 1 Eleven Chinese Medicine fecal compositions**

No.	Official name	Classic medical monograph	Fecal origin	Property and flavor	Clinical indications
1	White clove	Yunnam Bencas (Herb Book)	Sparrow	Bitter, warm	Gastrointestinal disorder
2	Silk worm sand	Bencao Gangmu (Herb Dictionary)	Silk worm	Sweat, bitter, warm	Vomit, diarrhea, rheumatism
3	Chicken white	Ancient Bencao (Ancient Herb Book)	Chicken	Bitter, salty, cold	Detoxicate, diuretic
4	Golden juice	Handbook of Emergency Conditions	Human male	Slight-bitter, cold	Detoxicate, severe fever
5	Bipolar pin	Handbook of Distinguished Clinician	Rat	Bitter, salty	Abdominal cramp, fever
6	Perfume of dragon	Ancient Bencao (Herb Book) extension	Whale	Sweet, sour, warm	Analgesia, diuretic, bronchial spasm
7	Human yellow	Special Bencao (Herb Book)	Human	Bitter, salty, cold	Detoxicate, severe infection
8	Moon sand	Original classic	Rabbit	Bitter, cold	External infection
9	Penta crease	Original Bencao (Herb Book)	Small bat	Sweat, bitter, warm	External use
10	Moonlight sand	Special Bencao (Herb Book)	Bat	Bitter, cold	External use, eye infection
11	Flying dragon	Bencao Gangmu (Herb Dictionary)	Pigeon	Bitter, warm	Infection

**Principle of detoxication**

This principle advocates the use of a toxic agent to counteract an intoxicated state. Ge Hong used “golden juice” in emergency gastrointestinal disorders presenting with high fever<sup>[17]</sup>.

**Principle of homeopathic medicine**

This principle advises the use of fecal material in a situation of uncontrolled repeated gastrointestinal upset. It was believed that in an event of uncertain pathological cause, pushing the clinical problem to the extreme would allow a natural defense to better develop<sup>[18]</sup>.

**Principle resembling vaccination**

Life substances from the guts, *i.e.* fecal matter, were used for severe gastrointestinal problems resistant to standard treatment<sup>[19]</sup>.

**Principle of anti-allergy or anti-poisonous invasion**

Skin allergy and infections caused by insects and small animals were treated with their fecal material<sup>[20]</sup>.

## PROCEDURES OF FECAL FORMULATION IN ANCIENT CHINA

The procedures described in the classic literature describing the preparation of fecal material is illustrated below.

**Simple collection and drying of fecal material**

This crude method was reserved for the droppings of insects and small animals. The products were to be used externally<sup>[20]</sup>.

**Adding special herbal components to initiate specific effects**

Prescriptions of Traditional Chinese Medicine demanded one champion herb to be supported by one or more partners. Glycyrrhiza was the component widely used with human fecal matter in the most ancient description<sup>[12]</sup>.

**Creating an acceptable outlook of the fecal preparation**

Detailed instructions for maintaining cleanliness and filtering out unfavorable components were given. As an example, Ge Hong's well respected “golden juice” followed this procedure: (1) Feces were collected from healthy boys; (2) Clean spring water was used to form a suspension; (3) The suspension was put into a red earth vase to be buried underground for up to 12 mo; and (4) On maturity, the fecal fluid already separated into three layers: the surface yellow layer was the “golden juice,” and the middle brownish layer and the bottom debris were to be discarded.

This way of preparation must have involved fermentation and the fecal matter would have influenced the replication and selection of the microbiome involved. If “golden juice” were providing any bioactive influence, it is to be speculated whether the effect was biotic or antibiotic. Whether the juice could just be providing a special environment for the gastrointestinal microbiome to change, adapt and reorganize



deserves careful speculations<sup>[12,20]</sup>.

## DISCUSSION

### *What do we learn from the ancient history of fecal transplantation?*

The ancient fecal transplantations did not have any evidence related to modern life science. However, healers of those days were fully aware that fecal matter may be toxic or harmful. However, under special circumstances the fecal matter could provide unexpected and favorable outcomes. This plausible explanation matches quite well with today's practice of fecal transplantation. Today, we aim at a change of the intestinal microbiome. In those ancient days, clinicians were aiming at a change of the intestinal environment (without the knowledge about bacteria)<sup>[21,22]</sup>. The concept and benefits from the old practice were not linked with today's microbiome. Ancient fecal treatment could only be the provision of a specific gastrointestinal environment through the return of unwanted metabolized food and various forms of artificial treatment have been completed (like to "golden juice") before its application as a drug.

Today, we are working on various ways to changes the microbiome at different levels of the gut for a variety of gastrointestinal disorders, which are likely to be related to odd bacterial flora or infection<sup>[23,24]</sup>. Because the microbiome in the gut represents a healthy symbiosis between the human body and the organisms, a microenvironment suitable for a stable healthy symbiotic situation is of vital importance to maintain the stability. While we may still need fecal transplant in situations when immediate results are needed, the research direction should include studies on the provision of a favorable microenvironment for the usual symbiotic microbiome. Provision of the vital microenvironment could be preventive against the loss of the normal microbiome in inflammatory conditions. A suitable micro-environment for a healthy microbiome should also be the result after fecal transplantation. In the future, creating suitable oral prescriptions acceptable to all users with the aim of maintaining a favorable microenvironment could be the research direction. Obviously, more details about the symbiotic microbiomes at different levels of the gastrointestinal tract will need to be defined first<sup>[25]</sup>.

Transplantation of a living microbiome has the intention of providing active, beneficial organisms to the gut, which for various reasons has failed to maintain their satisfactory survival. An unsatisfactory microenvironment necessary for microbial replication could be the cause. After all, it has been reported that as much as 20% to 60% of the human associated microbiome is uncultivable<sup>[26]</sup>. Instead of reintroduction of the microbiome, which is difficult to control and lacks standards, a satisfactory restoration of the microenvironments in the gut might be an alternative. With a suitable microenvironment, the spontaneous replication of the original microbiome that should have remained in suitable quantities would become possible<sup>[27]</sup>.

## CONCLUSION

Ancient fecal transplantations were not supported by life science evidences. However, healers of those days were fully aware that even though fecal matter may be toxic or harmful, under special circumstances it could provide unexpected and beneficial outcomes. Today, we aim to change the intestinal microbiome. In those ancient days without knowledge of bacteria, clinicians aimed to change the intestinal environment. Current research on intestinal microbiomes could include study of the intestinal environment that normally sustain their healthy growth.

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## Use of music during colonoscopy: An updated meta-analysis of randomized controlled trials

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### Abstract

#### BACKGROUND

Music seems to be beneficial in multiple clinical areas. Colonoscopy is a stressful event for patients, especially with conscious sedation. Music during colonoscopy has been evaluated in multiple randomized controlled trials (RCTs) with varied results. Even meta-analyses on the subject over the years have yielded inconsistent conclusions. Therefore, we conducted an up-to-date meta-analysis regarding music during colonoscopy.

#### AIM

To assess the effects of music played during colonoscopy on patients' perspectives and sedation requirements.

#### METHODS

Multiple large databases were aggressively searched (November 2018). RCTs comparing music to without music during colonoscopy on adult patients were included. Pooled estimates were calculated for sedative medication doses, total procedure time, and patients' experience, willingness to repeat procedure, and pain scores using odds ratio (OR) and mean difference (MD) with random effects model.

#### RESULTS

Eleven studies ( $n = 988$ ) were included. Music during colonoscopy showed a

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statistically significant reduction in procedure times (MD: -2.3 min; 95%CI: -4.13 to -0.47;  $P = 0.01$ ) and patients' pain (MD: -1.26; 95%CI: -2.28 to -0.24;  $P = 0.02$ ) while improving patients' experience (MD: -1.11; 95%CI: -1.7 to -0.53;  $P < 0.01$ ) as compared to no music. No statistically significant differences were observed between music and no music during colonoscopy for midazolam (MD: -0.4 mg; 95%CI: -0.9 to 0.09;  $P = 0.11$ ), meperidine (MD: -3.06 mg; 95%CI: -10.79 to 4.67;  $P = 0.44$ ), or patients' willingness to repeat the colonoscopy (OR: 3.89; 95%CI: 0.76 to 19.97;  $P = 0.1$ ).

## CONCLUSION

Music appears to improve overall patient experience while reducing procedure times and patient pain. Therefore, music, being a non-invasive intervention, should be strongly considered during colonoscopy.

**Key words:** Colonoscopy; Music; Relaxation; Meta-analysis

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**Core tip:** Music during stressful events has been shown to improve patient experience. Colonoscopy is a stressful event for many patients. Music during colonoscopy has been studied by many randomized controlled trials and meta-analyses with varying results. Therefore, given new studies available for analysis, we performed an updated meta-analysis. This meta-analysis demonstrated that music during colonoscopy reduces patients' pain while improving patients' experience and procedure times. With these results and extremely limited adverse effects of music, music should be strongly considered during colonoscopy.

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## INTRODUCTION

Colonoscopy is an important procedure with screening, diagnostic, and therapeutic indications, but it is associated with significant patient anxiety. Stress and discomfort encountered both pre- and intra-operatively are associated with delays in proceeding with screening colonoscopy, increased medication use during the procedure, decreased patient satisfaction, and increased patient refusal to repeat colonoscopy<sup>[1-3]</sup>.

Utilization of music during gastrointestinal procedures is a common approach to reduce patient anxiety, as it has been in many fields of medicine, including radiology, gynecology, urology, and pulmonology<sup>[4-11]</sup>. Multiple randomized control trials (RCTs) have attempted to quantify the effects of music on various aspects of undergoing colonoscopy. A previous meta-analysis of RCTs demonstrated increased patient willingness to repeat the procedure when music was utilized in the endoscopy suite; however, no significant differences in levels of dosage of administered sedative, patient reported pain level, nor procedure time<sup>[12]</sup>. Other meta-analyses have also come to differing conclusions regarding the utility of music during colonoscopy<sup>[13-15]</sup>. Over time, many other RCTs have been undertaken, demonstrating variable findings in regards to significant differences in these aforementioned parameters. Some studies demonstrate reduced anxiety scores and improved satisfaction<sup>[16-25]</sup>. Some studies showed reduced pain scores<sup>[19,26-27]</sup> and reduced sedative requirements<sup>[18-19,28-30]</sup>. Furthermore, some studies demonstrated little significant difference amongst anxiety levels nor sedation requirements, though variable improvements in patient experience and willingness to repeat the procedure<sup>[31-35]</sup>. Given this variation in results and sedative medication utilized, this meta-analysis sought to include novel data points by selecting only studies using moderate sedation to ascertain any significant differences in patient reported pain, satisfaction, procedure time, sedating medication requirements, and patient willingness to repeat exam when music is utilized in the endoscopy suite.

## MATERIALS AND METHODS

### Data acquisition

Medline, PubMed, Scopus, Cumulative Index for Nursing and Allied Health Literature, Cochrane Central Register of Controlled trials, and Embase were searched for articles (search date November 2018) using “music” and “colonoscopy”. Studies included were RCTs with adult subjects (age  $\geq 18$  years) comparing music *vs* no music during colonoscopy and only moderate sedation. Two independent reviewers extracted data using standard forms. Pooled estimates were calculated for the effects of music for dose of sedative medications (midazolam and meperidine), total procedure time, and patient’s self-reported pain scores, experience, and willingness to repeat the same procedure using odds ratio (OR) and mean difference (MD) with random effects model.

### Statistics

The impact of music on patients having colonoscopy was analyzed by calculating pooled estimates of sedative medication doses (meperidine and midazolam), total procedure time, and patients’ pain scores, experience, and willingness to repeat the colonoscopy using OR and MD. A random effects model was utilized to calculate the summary estimate with significance was indicated by  $P$ -value  $< 0.05$ .  $I^2$  measure of inconsistency was used to assess heterogeneity.

### Quality assessment of studies

The Cochrane’s Collaboration Risk of Bias Tool was used to assess the quality of included studies<sup>[36]</sup>. In this tool, each outcome was given a GRADE (very low, low, moderate, or high) based on the quality of evidence. The parameters evaluated in each study were as follows: Precision, consistency of results, effect magnitude, and potential bias (publication and other forms)<sup>[37]</sup>.

## RESULTS

The initial search identified 177 articles. **Figure 1** of these articles, 11 RCTs ( $n = 988$ ) met the inclusion criteria<sup>[18,19,25,26,28,29,32,33,35,38,39]</sup>. **Table 1** all RCTs were published from 2002-2016. Studies were global, including many countries (United States, Germany, Spain, Japan, Italy, China, Turkey, India, Australia, and Sri Lanka). Most of the studies were deemed high quality studies based on the Cochrane’s Collaboration Risk of Bias Tool (**Table 2**).

Procedure times were evaluated in nine studies<sup>[19,25,26,28,29,32,35,38,39]</sup>. Music during colonoscopy demonstrated a statistically significant reduction in procedure times (MD: -2.3 min; 95%CI: -4.13 to -0.47;  $P = 0.01$ ). **Figure 2** Patient pain scores were evaluated in six studies<sup>[18,19,28,29,33,35]</sup>. The use of music during colonoscopy showed statistically significant decrease in patient pain levels as compared to no music (MD: -1.26; 95%CI: -2.28 to -0.24;  $P = 0.02$ ). **Figure 3** Furthermore, patient experience was improved using music as compared to no music (MD: -1.11; 95%CI: -1.7 to -0.53;  $P < 0.01$ ) in four studies<sup>[18,28,29,35]</sup>. **Figure 4** No statistically significant differences were observed between music and no music during colonoscopy for midazolam (MD: -0.4 mg; 95%CI: -0.9 to 0.09;  $P = 0.11$ ), meperidine (MD: -3.06 mg; 95%CI: -10.79 to 4.67;  $P = 0.44$ ), or patients’ willingness to repeat the procedure (OR: 3.89; 95%CI: 0.76 to 19.97;  $P = 0.1$ ).

## DISCUSSION

Undergoing colonoscopy is a stressful experience for many patients. The ease of introducing music into the endoscopy suite makes its use an attractive modality to enhance the patient experience. Multiple studies demonstrate that use of music not only subjectively improves patient experience during medical procedures, but improves objective measures of patient stress including heart rate, blood pressure, and measured levels of salivary cortisol<sup>[16,27,39,40]</sup>. As noted above, multiple RCTs have attempted to demonstrate possible benefits of music during colonoscopy with variable results. Ten years ago, many authors of this study conducted a meta-analysis yielding the observation that while music does increase patient willingness to repeat the procedure, it did not necessarily reduce need for sedating medication, reduce patient reported pain score, nor reduce procedure time<sup>[12]</sup>. However, many RCTs conducted over the ensuing decade supplied new data points which suggest the benefits of music during colonoscopy may be greater than previously observed, with



**Table 1** Description of studies included in the meta-analysis

Ref.	Publication year	Number of patients	Type of study	Type of music
De silva <i>et al</i> <sup>[26]</sup>	2016	118	RCT	Variety per patient
Martindale <i>et al</i> <sup>[33]</sup>	2013	119	RCT	Classical
Costa <i>et al</i> <sup>[19]</sup>	2010	110	RCT	Variety per patient
Bechtold <i>et al</i> <sup>[35]</sup>	2006	29	RCT	Watermark by Enya
Ovayolu <i>et al</i> <sup>[18]</sup>	2006	32	RCT	Turkish classical
Harikumar <i>et al</i> <sup>[28]</sup>	2006	166	RCT	Choice of 6 styles (headphones)
Uedo <i>et al</i> <sup>[39]</sup>	2004	60	RCT	Easy-listening
López-Cepero Andrada <i>et al</i> <sup>[25]</sup>	2004	78	RCT	Classical
Smolen <i>et al</i> <sup>[32]</sup>	2002	34	RCT	Variety per patient
Schiemann <i>et al</i> <sup>[38]</sup>	2002	133	RCT	Variety radio station
Lee <i>et al</i> <sup>[29]</sup>	2002	109	RCT	Variety per patient

RCT: Randomized controlled trial.

possible statistically significant reduced procedure times, patient reported pain scores, and enhanced overall patient experience.

This meta-analysis concludes that music played during colonoscopy improved patient experience and procedure times while reducing patient pain. This meta-analysis is unique from the others given the use of the newest RCTs and minimizing confounding variables by only using moderate sedation rather than moderate and deep sedation.

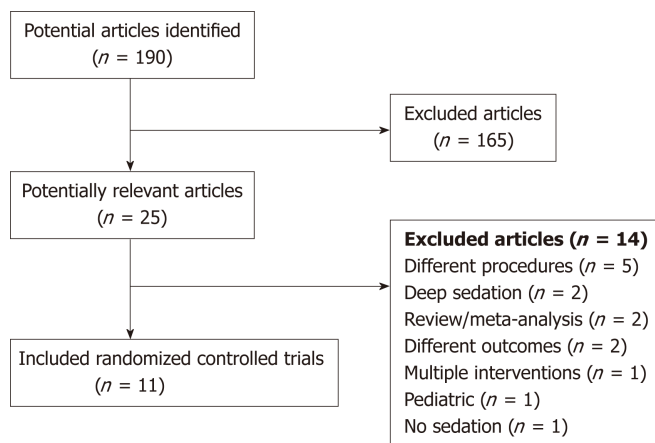
This updated meta-analysis has many strengths. This meta-analysis includes only RCTs to limit selection and observation bias, more patients than prior meta-analyses, and global studies. This meta-analysis also focused on only one type of sedation. However, all meta-analyses have limitations as well. First, music was initiated at different times during the procedure process, in some studies initiated pre-procedurally while initiated later in others. Second, the delivery method also differed amongst studies, with some patients receiving music *via* headphones and others *via* a radio in the room. Third, the genre of music varied widely amongst these studies with some studies utilized classical or easy listening selections, while other studies allowed patients to select their own music. The inevitable variation of any given individual patient's response to different music selections, particularly when considering cultural and generational preferences as well as response to stressful stimuli, must be considered when translating these results into one's own clinical practice. Naturally, music selection likely also alters the behavior of the performing endoscopist with new evidence that selection of music can affect adenoma detection rate<sup>[41]</sup>.

In conclusion, given the low cost and relative ease of introducing music during colonoscopy, these results suggest it is reasonable to include music to both improve patient pain and experience as well as possibly productivity given reduced procedure times.

**Table 2** Quality assessment summary of all included studies

Ref.	Study design	Random sequence generation	Allocation concealment	Blinding	Blinding outcome assessment	Incomplete outcome data	Selective reporting	Other bias	Quality assessment
De silva <i>et al</i> <sup>[26]</sup> , 2016	RCT	Adequate	Adequate	Double-blinded	Adequate	None	None	None	High
Martindale <i>et al</i> <sup>[33]</sup> , 2013	RCT	Adequate	Adequate	Double-blinded	Adequate	None	None	None	High
Costa <i>et al</i> <sup>[19]</sup> , 2010	RCT	Adequate	Inadequate	Single-blinded	Adequate	None	None	None	Moderate
Bechtold <i>et al</i> <sup>[35]</sup> , 2006	RCT	Adequate	Not described	None	Inadequate	None	None	None	Low
Ovayolu <i>et al</i> <sup>[18]</sup> , 2006	RCT	Adequate	Adequate	Double-blinded	Adequate	None	None	None	High
Harikumar <i>et al</i> <sup>[28]</sup> , 2006	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	Moderate
Uedo <i>et al</i> <sup>[39]</sup> , 2004	RCT	Not described	Not described	Double-blinded	Adequate	None	None	None	Low
López-Cepero Andrada <i>et al</i> <sup>[25]</sup> , 2004	RCT	Not described	Adequate	Double-blinded	Adequate	None	None	None	Moderate
Smolen <i>et al</i> <sup>[32]</sup> , 2002	RCT	Not described	Adequate	Double-blinded	Adequate	None	None	None	Moderate
Schiemann <i>et al</i> <sup>[38]</sup> , 2002	RCT	Not described	Adequate	Double-blinded	Adequate	None	None	None	Moderate
Lee <i>et al</i> <sup>[29]</sup> , 2002	RCT	Not described	Adequate	Double-blinded	Adequate	None	None	None	Moderate

RCT: Randomized controlled trial.

**Figure 1** Details of search algorithm.

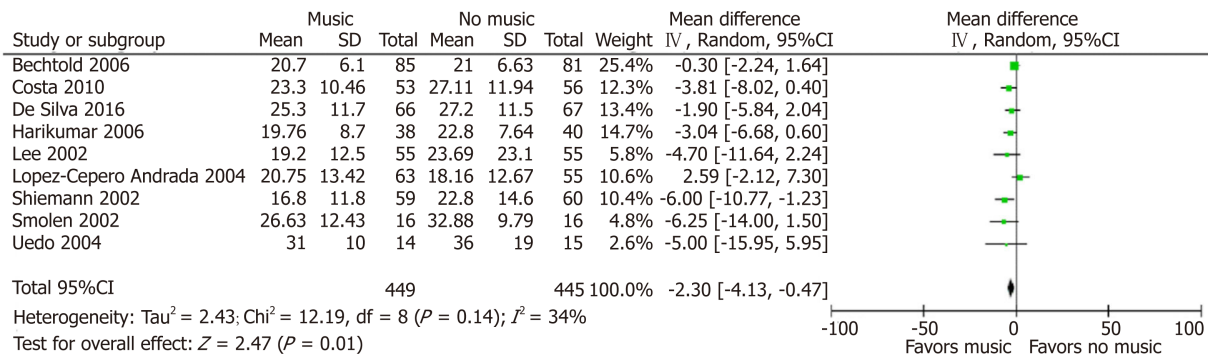


Figure 2 Forest plot showing comparison between music and no music during colonoscopy for procedure time.

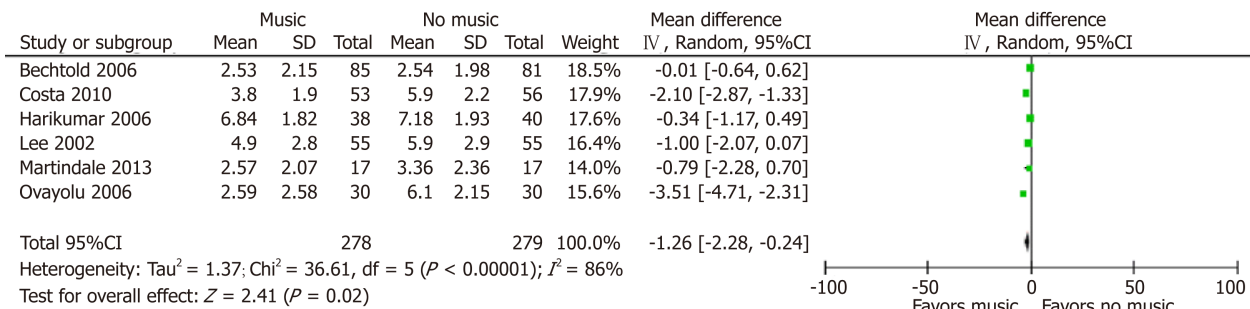


Figure 3 Forest plot showing comparison between music and no music during colonoscopy for patients' pain.

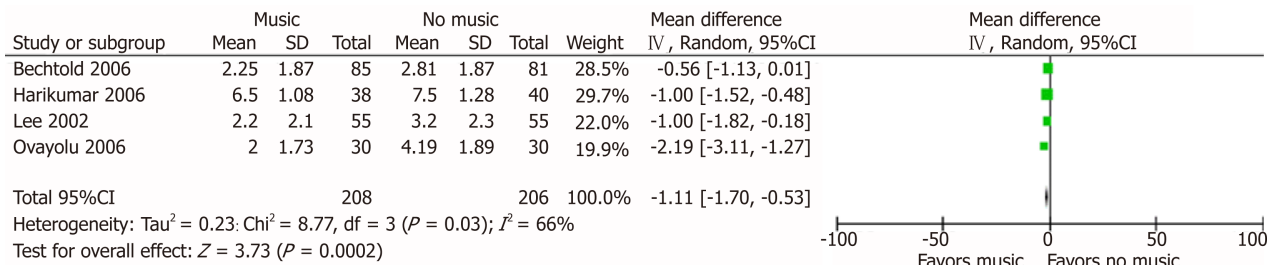


Figure 4 Forest plot showing comparison between music and no music during colonoscopy for patients' experience.

## ARTICLE HIGHLIGHTS

### Research background

Music during colonoscopy has been a controversy subject despite multiple randomized controlled trials and meta-analyses. Studies vary from music during colonoscopy helping reduce need for sedative medications and enhancing patient experience to offering little to no benefit. Given this variability, we conducted this meta-analysis to include all studies to-date and limiting them to only conscious sedation.

### Research motivation

To determine if music is beneficial to patients undergoing colonoscopy. If beneficial, music would be a very low-cost intervention to improve patients' experience and pain during a very stressful procedure.

### Research objectives

The objectives of this research were to fully assess the effects of music during colonoscopy sedative medication doses (meperidine and midazolam), total procedure time, and patients' pain scores, experience, and willingness to repeat the colonoscopy.

### Research methods

A meta-analysis was performed by calculating pooled estimates of sedative medication doses (meperidine and midazolam), total procedure time, and patients' pain scores, experience, and willingness to repeat the colonoscopy using odds ratio and mean difference using a random effects model.

### Research results

This research showed that music during colonoscopy improved patient experience and procedure times while reducing patient pain.

### Research conclusions

Music is a benefit to patients undergoing the stressful procedure of colonoscopy. Music during colonoscopy improves the patient experience while reducing pain. In addition, procedure times are improved with music playing during colonoscopy. Music is a low-cost intervention that shows significant benefit and should strongly be considered in endoscopy suites. In the future, more endoscopy suites should be equipped with music.

### Research perspectives

This meta-analysis shows that music has a role in the endoscopy suite. Also, this meta-analysis demonstrates that with more studies, the results of any meta-analysis may be significantly altered as these results differ from some prior meta-analyses.

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