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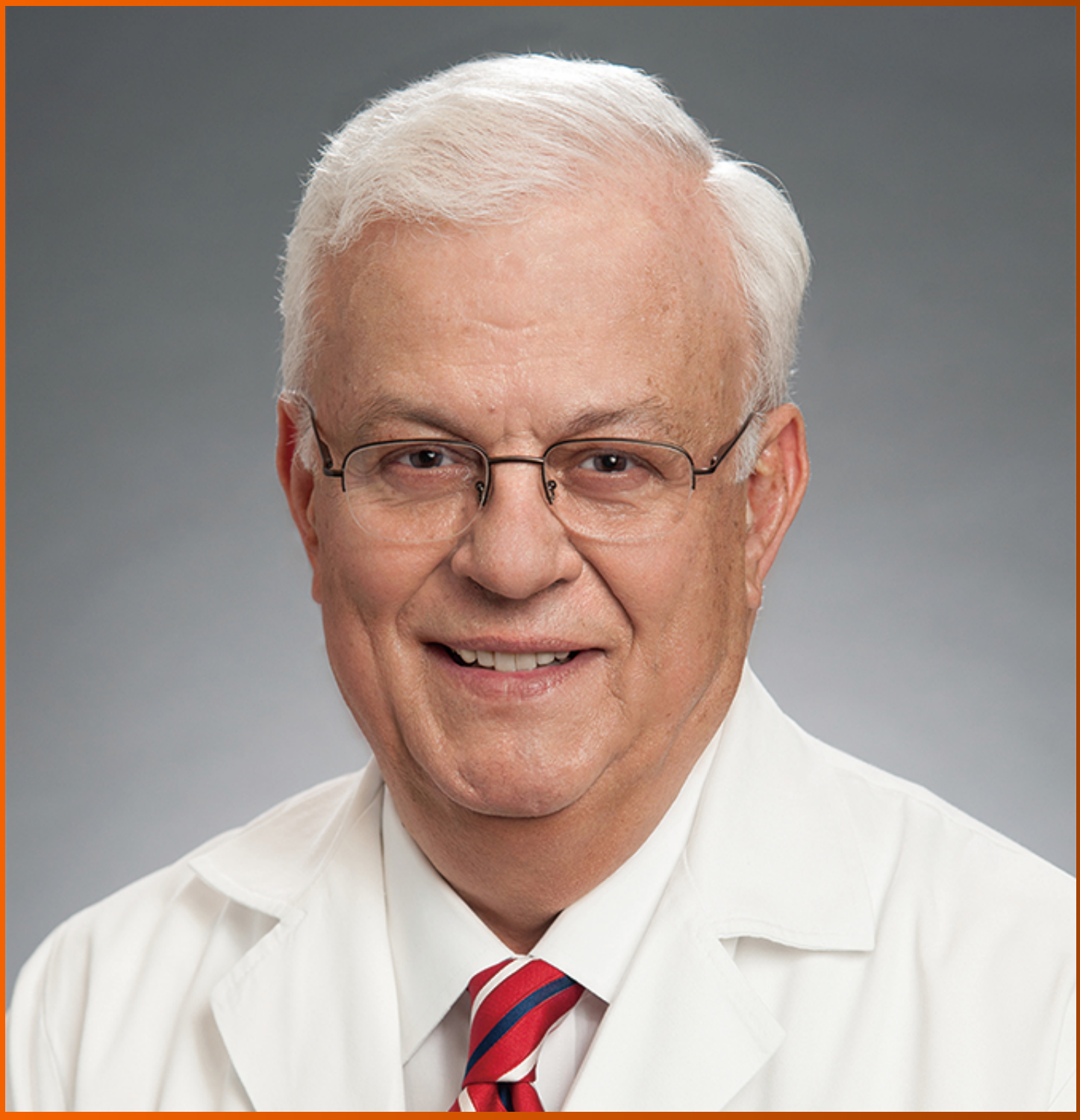

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REVIEW

Precision medicine in inflammatory bowel disease: Individualizing the use of biologics and small molecule therapies

Eric Cheah, James Guoxian Huang

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Eric Cheah, Department of Gastroenterology and Clinical Nutrition, The Royal Children's Hospital Melbourne, Parkville, VIC 3052, Australia

James Guoxian Huang, Department of Paediatrics, Khoo Teck Puat-National University Children's Medical Institute, National University Health System, Singapore 119228, Singapore

James Guoxian Huang, Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228, Singapore

Corresponding author: James Guoxian Huang, MBBS, Assistant Professor, Attending Doctor, Staff Physician, Department of Pediatrics, Khoo Teck Puat-National University Children's Medical Institute, National University Health System, NUHS Tower Block Level 12, 1E Kent Ridge Road, Singapore 119228, Singapore. james_huang@nuhs.edu.sg

Abstract

The advent of biologics and small molecules in inflammatory bowel disease (IBD) has marked a significant turning point in the prognosis of IBD, decreasing the rates of corticosteroid dependence, hospitalizations and improving overall quality of life. The introduction of biosimilars has also increased affordability and enhanced access to these otherwise costly targeted therapies. Biologics do not yet represent a complete panacea: A subset of patients do not respond to first-line anti-tumor necrosis factor (TNF)-alpha agents or may subsequently demonstrate a secondary loss of response. Patients who fail to respond to anti-TNF agents typically have a poorer response rate to second-line biologics. It is uncertain which patient would benefit from a different sequencing of biologics or even a combination of biologic agents. The introduction of newer classes of biologics and small molecules may provide alternative therapeutic targets for patients with refractory disease. This review examines the therapeutic ceiling in current treatment strategies of IBD and the potential paradigm shifts in the future.

Key Words: Precision medicine; Therapeutic ceiling; Inflammatory bowel disease; Biologics; Small molecules

Core Tip: Precision medicine and individualizing patient care has been the holy grail in the management of inflammatory bowel disease (IBD). A one-size-fits-all approach, utilizing the current armamentarium of biologics and small molecules, still yields less than ideal clinical outcomes, with significantly high non-response rates. Multiple challenges remain in breaking this therapeutic ceiling: Achieving an early diagnosis of IBD ideally even in the pre-clinical phase; accurately prognosticating the disease course; and tailoring an appropriately sequenced therapy regime to a patient's disease severity, pharmacokinetic and pharmacodynamic profile.

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INTRODUCTION

The incidence of inflammatory bowel disease (IBD) has seen a rise within Asia Pacific. Overall incidence and prevalence rates in Asia are lower than the West, but are on the rise[1,2]. Treatment of IBD has progressed rapidly over the past several decades. A new era in the treatment of IBD began with the development of the chimeric monoclonal anti-tumor necrosis factor (TNF)-alpha antibody cA2 in the early 1990's. cA2 was subsequently renamed infliximab, and was first licensed by the United States Food and Drug Administration (FDA) in August 1998 for the treatment of Crohn's disease (CD)[3]. Since the introduction of infliximab, there has been an advent of newer biologics and small molecule agents, along with paradigm shifts in the treatment goals of IBD. The agents currently FDA approved for use include the anti-integrin (vedolizumab), anti-interleukin (IL)-12/23 p40 (ustekinumab), anti-IL-23p19 (risankizumab), oral Janus kinase (JAK) inhibitors (tofacitinib, upadacitinib), and sphingosine-1-phosphate (S1P) receptor modulator (ozanimod). As of 2019, mesenchymal stem cell therapy (darvadstrocel) received a regenerative medicine advanced therapy designation for complex perianal fistulas in adult patients with CD. New targets and treatments being explored in Phase II/III trials include anti-integrin (etrolizumab, ontamalimab), IL-23p19 inhibitors (mirikizumab, brazikumab, guselkumab), oral JAK inhibitor (filgotinib), and S1P modulator (etrasimod)[4].

Current treatment goals in IBD aim to more than just achieve clinical remission. Deep remission, the combination of clinical remission and mucosal healing, represents an important therapeutic target that is now increasingly attainable with the timely use of biologics[5]. Expert consensus statements in the STRIDE[1]/STRIDE-II guidelines, with evidence from the CALM study, have helped us define treat-to-target strategies in adults and children utilizing clinical indices, biomarkers, and endoscopic parameters [6-8]. Aspirational targets include transmural healing in CD and histologic healing in ulcerative colitis (UC).

There is an increasing need to develop newer biologics and small molecules targeting novel cytokine pathways, as current therapeutic options are far from perfect in achieving the above-mentioned treatment targets. A meta-analysis of real-world deep remission rates with anti-TNF agents demonstrated that deep remission was only achieved in 48.6% of CD patients and 43.6% of UC patients at 1 year[9]. In a review by Papamichael *et al*[10], the rates of primary non-response and non-remission to anti-TNF agents in IBD were between 10%-40% and 50%-80%, respectively. A further 23%-46% of those initial responders or those who achieve remission have a secondary loss of response over time [11]. The clinical remission rates with second-line biologics are also poorer in patients, who have had a prior loss of response to anti-TNF agents, especially those who had a primary non-response[12-14]. Such data distinctly highlight the therapeutic ceiling in current IBD management: how can we optimize current therapies to go beyond this therapeutic ceiling?

BIOMARKERS IN IBD

Predictive and prognostic biomarkers, pharmacogenomics, and response to therapy

Precision and personalized medicine has long been a discussed topic in the management of IBD. It is an aspirational goal to accurately predict those with a complicated and aggressive clinical course, and to administer timely targeted therapy to the individual's molecular inflammatory profile[15]. Particularly for CD, the emphasis is for appropriately early management within the window of opportunity, before permanent digestive damage is done[16,17]. However, management decisions are still currently made using a one-size-fits-all approach. The conventional strategy is the step-up approach, which will inevitably undertreat patients who are destined to run a more aggressive disease course. With easier

access to biologics and small molecules, a top-down approach may be used, which has been shown to improve clinical outcomes in prognostically severe CD[18,19] but this may otherwise expose patients destined to have mild disease to unnecessary risks and overtreatment. This top-down strategy would also be unaffordable in financially constrained health-care settings particularly in the Asia-Pacific region, and may pose a challenge in health jurisdictions that limit the use of expensive novel therapies.

Furthermore, the heterogeneity and variability of the clinical course of IBD between different individuals would mean a suboptimal approach in a substantial group of patients. There is a general consensus that a tailored approach is required, with a need for accurate biomarkers that enable the right patient to be matched to the right treatment (Table 1).

Stratification of treatment approaches can help identify those of a more complicated course and hence tailoring treatment accordingly. Among prognostic biomarkers at diagnosis predicting a more complex CD phenotype and worse outcomes including stricturing phenotype and need for surgery, identified microbial predictors include circulating antibodies against bacterial antigens such as anti-outer membrane protein C, anti-*Saccharomyces cerevisiae* antibody, perinuclear anti-neutrophil cytoplasmic antibodies, and anti-CBir1 flagellin. However, it is unclear if these represent a cause or effect of severe disease[20-24].

A CD8+ T-cell clonal signature was identified to predict worse outcomes and relapse in IBD patients [25,26]. This genomic biomarker was subsequently validated in independent cohorts of newly diagnosed CD and UC patients in the United Kingdom[27]. There is now a trial in progress in the United Kingdom to assess this whole-blood biomarker to guide treatment for newly diagnosed CD patients[28]. It is currently available in clinical use: PredictSure IBD; PredictImmune, Cambridge, United Kingdom.

Other predictive tools include the use of fecal calprotectin as a predictor of endoscopic disease activity as well as histologic inflammation, and fecal calprotectin values have been shown to be predictive of relapse in asymptomatic patients with IBD[29-33]. In a biomarker discovery trial, the EMBARK study, serum matrix metalloproteinase 9 and serum IL-22 were found to be associated with inflammatory disease activity for patients with UC and CD respectively[34].

Pharmacogenomic testing has also become common place in IBD. A commonly used predictor of risk of adverse drug reactions and pharmacologic response is the utility of thiopurine methyltransferase (TPMT) genotyping and metabolite testing, as well as nudix hydrolase 15 (NUDT15) genotyping. Thiopurine use is associated with adverse effects (AEs) in up to 40% of patients[35]. TPMT genotype testing is cost effective, and heterozygous and homozygous TPMT genotypes correlate with AEs. Dose reduction in the TPMT variants significantly reduce adverse hematologic effects without reducing treatment efficacy[36]. NUDT15 variants, first elucidated in a Korean population, have also been more recently described as associated with thiopurine induced myelosuppression and is more predictive of myelosuppression in East Asians[37-39]. Furthermore, thiopurine metabolite testing can aid in dose optimization and compliance[40] and prevents hepatotoxicity by identifying a subgroup of thiopurine-'shunters' who preferentially produce the hepatotoxic metabolite 6-methylmercaptopurine.

Apart from this, several biomarkers as predictors of non-response to anti-TNF include higher oncostatin M expression[41,42] and low expression of triggering receptor expressed on myeloid cells 1 [43,44]; antibody formation to anti-TNF is associated with the HLA-DQA1*05 genotype[45]. Higher baseline concentrations of serum cytokine IL-22, whose expression is induced by IL-23, is associated with greater likelihood of response to brazikumab[46].

OPTIMIZING AND MAXIMIZING CURRENT BIOLOGIC AGENTS

Therapeutic drug monitoring and drug dosing strategies

Introduction of therapeutic drug monitoring (TDM) has guided our approach in going beyond the therapeutic ceiling. Multiple studies have demonstrated an association with serum drug concentration of biologics, mainly in anti-TNF agents, and outcomes of patients[47-55]. It has assisted us in guiding dose modification (dose escalation or reduction), and informed us of primary or secondary loss of response, thus avoiding persistence of potentially ineffective therapy[56]. The approach of proactive *vs* reactive therapeutic drug monitoring remains a hotly debated topic[57].

Dashboard systems are clinical decision support tools utilizing computer software modelling to predict ideal personalized medication dosing[58,59]. This 'model-based dosing' has long been used by pharmacists and pharmacologists, for example, to dose antibiotics such as aminoglycosides. For anti-TNF dosing, dashboard driven pharmacokinetic (PK) dose optimization considers individual patient covariates including C-reactive protein, albumin, body-weight, sex and also serum drug levels. The PRECISION trial demonstrated that dashboard driven personalized dosing resulted in a significantly higher proportion of patients maintaining clinical remission after 1 year of treatment compared with patients that continued treatment without proactive adjustments: 88% *vs* 64%, respectively[60]. Utilizing the same PK dashboard system during Infliximab induction, Dubinsky *et al*[61] recently showed improved infliximab durability; and at 52 wk, 119/123 patients remained on infliximab in steroid free remission. The OPTIMISE trial is underway to evaluate the safety and efficacy of proactive TDM

Table 1 Selected list of biomarkers in inflammatory bowel disease

Biomarker class	Biomarker	Clinical utility
Prognostic biomarkers	Anti-ompC, ASCA, ANCA, anti-CBir1, flagellin	Prediction of more severe CD phenotype- particularly stricturing and need for surgery
	CD 8+ T cell clonal signature	Prediction of more severe disease course and relapse in CD and UC
Surveillance of disease activity	Fecal calprotectin	Predictor of endoscopic disease activity as well as histologic inflammation, and relapse in asymptomatic patients with IBD
	MMP-9	Associated with disease activity in UC
	IL-22	Associated with disease activity in CD
Pharmacogenomics and prediction of safety	TPMT	Risk of thiopurine adverse reaction
	NUDT15	Risk of thiopurine adverse reaction, more common in East Asian/Asian populations
	Thiopurine metabolites (6TG, 6MMP)	Levels associated with adverse drug reaction: myelosuppression, hepatotoxicity. 6TG range also associated with therapy response
Prediction of response to therapy	Oncostatin M	Higher levels predictor of non-response to anti-TNF
	TREM-1	Low levels predictor of non-response to anti-TNF
	HLA-DQA1*05	Expression associated with risk of antibody formation to anti-TNF
	IL-22	Higher level associated with response to anti-IL23p19 (brazikumab)

ANCA: Anti-neutrophil cytoplasmic antibody; ASCA: Anti-Saccharomyces cerevisiae antibody; CD: Crohn's disease; IBD: Inflammatory bowel disease; IL: Interleukin; MMP: Matrix metalloproteinase; NUDT15: Nudix hydrolase 15; ompC: Outer membrane protein C; TG: Thioguanine; TNF: Tumor necrosis factor; TPMT: Thiopurine methyltransferase; TREM: Triggering receptor expressed on myeloid cells; UC: Ulcerative colitis.

combined PK dashboard-driven infliximab dosing compared with standard of care dosing in patients with CD[62].

"Supratherapeutic" anti-TNF dosing

Numerous exposure-response relationship studies including post-hoc analyses of randomized controlled trials show a positive correlation between biologic drug concentrations and favorable therapeutic outcomes in IBD and other immune-mediated inflammatory diseases; higher drug concentrations are typically associated with improved therapeutic outcomes[63]. Conversely, lower drug concentrations, with or without anti-drug antibodies, are associated with treatment failure and drug discontinuation[64,65].

Multiple clinical studies have provided various TDM targets, especially with the anti-TNF agents infliximab and adalimumab, at various time points that are associated with outcomes of clinical remission[51,66]. Several observational studies have suggested that higher median infliximab concentrations are associated with superior clinical and biochemical remission rates. Given the wide variation in observed concentrations among responders, one may even wonder if the "therapeutic threshold" is identical for all patients and for the different phases of the treatment (induction *vs* maintenance and active *vs* quiescent disease).

Yarur *et al*[67], found that that levels of infliximab ≥ 10 mcg/mL were best associated with fistula healing, though surprisingly, a small number of patients required levels of ≥ 20 mcg/mL to achieve fistula healing. Feng *et al*[68] demonstrated that on incremental gains analysis, mucosal healing rates gradually increased as infliximab levels went up and reached a brief plateau (> 85%) when the infliximab trough level was 10 μg/mL. However, there was still a small proportion that seemingly benefited from an anti-TNF levels > 12 mcg/mL to achieve mucosal healing. Ungar *et al*[69] similarly demonstrated in a retrospective study a significant association between serum levels of anti-TNF agents and level of mucosal healing. They went on to propose that serum levels of 6-10 μg/mL for infliximab and 8-12 μg/mL for adalimumab are required to achieve mucosal healing in 80%-90% of patients with IBD.

Several other studies demonstrated that higher trough levels of infliximab and adalimumab are associated with those achieving mucosal healing in CD[68-79]. In a substudy of the TAILORIX trial, Bossuyt *et al*[71] found that infliximab trough level of 7.8 μg/mL at the end of induction (week 14) was associated with both radiologic response and remission. Continuously high infliximab exposure (infliximab > 5 μg/mL at all time points) was associated with radiologic response.

Low infliximab trough concentrations and the presence of antibodies to infliximab are associated with worse outcomes. Trough concentrations of > 3 μg/mL during maintenance is associated with sustained clinical outcomes[54,70,80,81]. Vande Casteele *et al*[82] concluded that an appropriate infliximab therapeutic window is between 3 and 7 μg/mL for IBD responders during maintenance therapy based on previous studies, and prospectively validated it in a randomized controlled trial (TAXIT).

The optimal "therapeutic window" for biologics remains to be elucidated, and the upper limit is unclear. While the abovementioned studies observed an association between certain trough concentration ranges and a corresponding degree of disease remission, the 'ideal' trough concentration to induce remission may vary between individuals. This may be due to a variety of factors such as an individual's disease burden and therefore mucosal TNF burden, early *vs* advanced disease, and an individual's unique pharmacodynamic makeup. There may be a subset of patients who might benefit from dose escalation to 'supratherapeutic' trough concentrations and reassuringly, there is little evidence to indicate greater toxicity with higher infliximab levels.

Sequencing of biologics

While it remains a conventional strategy to offer an anti-TNF agent as the first-line biologic, it has been well established from network meta-analyses that anti-TNF non-responders do not have an optimal response after switching to second-line biologics[12-14]. It thereby raises the question whether certain individuals would benefit from receiving these traditionally second-line biologics as first-line therapy options, and highlights the importance of optimal biologic sequencing.

There are limited head to head trials between biologic agents: In the VARSITY trial (adalimumab *vs* vedolizumab) for moderate to severe UC, vedolizumab demonstrated superiority in clinical and endoscopic remission but not corticosteroid free clinical remission[83]. In the SEAVUE study (adalimumab *vs* ustekinumab) for moderate to severe Crohn's, ustekinumab failed to demonstrate superiority over adalimumab[84].

In the Galaxi-1 study involving participants with moderately to severely active CD, guselkumab was compared to placebo and ustekinumab. It was a phase 2, dose-ranging study[85], not powered to evaluate potential differences in efficacy and safety between guselkumab and ustekinumab.

From the HIBISCUS and GARDENIA trials[86,87] Etrolizumab *vs* adalimumab or infliximab in moderate to severe UC- failed to demonstrate superiority.

While offering novel alternate pathway biologics as first-line therapy may gain traction in the near future, this approach is often limited by government access in many jurisdictions. Access to newer therapies for adult patients is already limited by licensing authorities, but the pathways remain even more restricted for pediatric patients. Access to biologics in pediatric patients is often on compassionate grounds, due to a significant lag in clinical trials and therefore delaying official approval from medical licensing authorities such as the European Medicines Agency and United States FDA. There is a need for this cohort of patients to have better access to new/emerging therapies through more advanced pharmacogenomic, pharmacokinetic and pharmacodynamic modelling. This is to allow earlier initiation of trials or better ways to "extrapolate" adult data for presentation to licensing authorities to allow for use in children.

Dual biologics and combinations of newer advanced therapies

Combinations of biologic agents and recently combinations of biologics with newer small molecule agents have been attempted to go beyond our current therapeutic ceiling. This concept is not new, however, and was first attempted by Sands *et al*[88] as a randomized controlled trial comparing the safety and tolerability of patients on infliximab not in remission and adding natalizumab *vs* a placebo arm. Although the main trial ran for 10 wk and was not powered to assess for differences in efficacy between the groups, there was a higher proportion of patients achieving a clinical response at each time point and this proportion continues to increase over time in the combination biologic group, compared to response rates for the monotherapy arm which remained unchanged.

Since then, there has been much in the literature of dual biologics or with newer small molecule therapies, but as case reports or case series and observational cohort studies (Table 2). The data is largely heterogenous but reassuringly has demonstrated acceptable safety for patients with refractory IBD with no new concerning signals[89,90].

In the pipeline, there are several randomized controlled trials evaluating the efficacy of combination biologics. The EXPLORER trial (ClinicalTrials.gov Identifier: NCT02764762) in high-risk CD patients involved triple combination therapy with vedolizumab, adalimumab and methotrexate which has completed but not yet reported.

The phase 2a VEGA study evaluated the safety and efficacy of combination induction therapy with guselkumab plus golimumab (GOL) *vs* monotherapy with guselkumab or GOL in adults with moderately to severely active UC through to week 12, most recently presented at the European Crohn's and Colitis Organization 2022 congress[91,92]. A greater proportion of patients who received combination therapy achieved clinical response as judged by Mayo score at week 12 (83.1%) *vs* guselkumab (74.6%) or GOL (61.1%). Similarly, the proportion of patients who achieved clinical remission in the combination group (36.6%) was greater than that in the monotherapy group (21.1% and 22.2%, respectively). The DUET UC (ClinicalTrials.gov Identifier: NCT05242484) is a phase 2b study of

Table 2 Publications of dual biologics

Ref.	Type	Number of participants/IBD type	Biologic combinations	Therapy duration or follow up (mo)	Outcomes
Buer *et al* [97], 2018	Case series, prospectively followed	Adult: 10 (4 CD, 6 UC)	Anti-TNF, adding on vedolizumab. Combination was intended as a bridging therapy	12-20	Clinical: HBI, PMS, 100 % CRem, 50% endoscopic remission. No serious AE (3 minor infections)
Olbjørn *et al*[98], 2020	Case series	Pediatric: 13 (9 CD, 4 UC)	Anti-TNF + vedolizumab (8), anti-TNF + ustekinumab (5), (for anti-TNF side effects)	N/A	3/8 (37.5%) Clinical and biochemical remission
Kwapisz *et al*[99], 2021	Case series	Adult: 15 (14 CD, 1 UC)	8 vedolizumab + anti-TNF, 2 ustekinumab + anti-TNF, 5 vedolizumab + ustekinumab	24	73% CRes, 44% ERes, 27% SE, 20% surgery
Yang *et al* [100], 2020	Retrospective cohort	Adult: 22 (CD)	24 combinations: 13 vedolizumab + anti-TNF, 8 vedolizumab + ustekinumab, 3 ustekinumab + anti-TNF	1	Endoscopic, PRO2 response/remission, CRP, 50% CRes, 36% SF CRem, 43% ERes, 4% SE (1 SLE-1 cancer), 33% surgery
Glassner *et al*[101], 2020	Retrospective cohort	50	53 combinations: 25 vedolizumab + ustekinumab, multiple other combinations	5.5-13	50% CRem, 34% ERem, 16% SE, 12% surgery
Privitera *et al*[102], 2020	Case series, indication active IBD and active EIM	Adult: 16 (11 CD, 4 UC)	Variety of combinations. Most frequent: 3 vedolizumab + adalimumab, 3 vedolizumab + ustekinumab	0.5	At 6 mo: Response IBD/EIM: 42.8%, 11%; Remission IBD/EIM: 14.2%, 55.5%, AE: 3/16 (18.8%)
Dolinger *et al*[103], 2021	Case series	Pediatric: 16: (CD 7, UC 8, IBD-U 1)	Vedolizumab + ustekinumab, vedolizumab + tofacitinib, ustekinumab + tofacitinib	6	SF remission at 6 mo 12/16 (75%)
Goessens *et al*[104], 2021	Retrospective cohort, hetero-genous, active IBD and/or EIM	Adult: 98 (CD 58, UC 40)	Anti-TNF + vedolizumab, anti-TNF + anti-IL, anti-IL + vedolizumab, tofacitinib + anti-TNF, tofacitinib + vedolizumab, anti-IL + anti-IL, others	5-16	PGA: Complete or partial improvement was observed in 21/80 (26%) and 35/80 (44%); Mean clinical disease activity for IBD: Significantly higher prior to combination than during combination (2.2 +/- 0.7 *vs* 1.2 +/- 1.1; $P < 0.0001$). Simple clinical activity scores (quiescent scores 0, mild scores 1, moderate scores 2 and severe scores 3

AE: Adverse events; CD: Crohn's disease; CRes: Clinical response; CRem: Clinical remission; EIM: Extraintestinal manifestations; ERem: Endoscopic remission; ERes: Endoscopic response; HBI: Harvey Bradshaw index; IBD: Inflammatory bowel disease; IL: Interleukin; N/A: Not applicable; PGA: Physician global assessment; PMS: Partial Mayo score; PRO2: Patient reported outcome scores; SE: Serious infection; SF: Steroid free; TNF: Tumor necrosis factor; UC: Ulcerative colitis.

combination therapy with guselkumab and GOL (JNJ-78934804) in participants with moderately to severely active UC that is planned; the DUET CD (ClinicalTrials.gov Identifier: NCT05242471) is a phase 2b study of the same biologic combination in individuals with moderately to severely active CD, and is currently recruiting trial participants.

The other considerations to overcome current therapeutic plateaus with biologic agents include added adjunctive therapies such as vitamin D, curcumin, microbiome alteration *via* dietary modification, exclusive enteral nutrition and probiotics[93].

CONCLUSION

Ongoing efforts in adding to and optimizing IBD treatments must be commended. However, remission rates are still far from optimal with current treatment approaches. The urgent need to develop new therapeutics also brings us to the challenge that we must meet: Improving the design and delivery of clinical trials, allowing generalizability, be of clinical equipoise and to factor in biomarker discovery. Advances in basic science, translational and clinical aspects of drug development is essential to achieve breakthroughs in IBD therapeutics that meet the needs of patients, physicians and health regulators[94-96]. In conclusion, in addition to the strategies as aforementioned, to go beyond our current therapeutic

ceiling requires not only early diagnosis or early stratification but early treatment. This entails incorporating a multiomics approach to better personalize treatment, sequence or combine our therapies, and incorporate the ever-advancing artificial intelligence technology, rather than a one-size-fits all approach[95]. These goals remain attainable and we continue to have a sense of optimism.

FOOTNOTES

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Country/Territory of origin: Australia

ORCID number: Eric Cheah 0000-0002-9444-1119; James Guoxian Huang 0000-0002-5869-4194.

Corresponding Author's Membership in Professional Societies: Gastroenterological Society of Singapore.

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MINIREVIEWS

Systemic treatment for unresectable hepatocellular carcinoma

Wattana Leowattana, Tawithep Leowattana, PathompThep Leowattana

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Wattana Leowattana, PathompThep Leowattana, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

Tawithep Leowattana, Department of Medicine, Faculty of Medicine, Srinakharinwirot University, Bangkok 10110, Thailand

Corresponding author: Wattana Leowattana, BMed, MD, MSc, PhD, Full Professor, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajavithi Road, Rachatawee, Bangkok 10400, Thailand. wattana.leo@mahidol.ac.th

Abstract

Hepatocellular carcinoma (HCC) is most commonly found in the context of liver cirrhosis and, in rare cases, in a healthy liver. Its prevalence has risen in recent years, particularly in Western nations, due to the increasing frequency of non-alcoholic fatty liver disease. Advanced HCC has a poor prognosis. For many years, the only proven therapy for unresectable HCC (uHCC) was sorafenib, a tyrosine kinase inhibitor. Recently, the synergistic effect of an immune checkpoint inhibitor, atezolizumab, and bevacizumab outperformed sorafenib alone in terms of survival, making it the recommended first-line therapy. Other multikinase inhibitors, lenvatinib and regorafenib, were also recommended as first and second-line drugs, respectively. Intermediate-stage HCC patients with retained liver function, particularly uHCC without extrahepatic metastasis, may benefit from trans-arterial chemoembolization. The current problem in uHCC is selecting a patient for the best treatment while considering the preexisting liver condition and liver function. Indeed, all study patients had a Child-Pugh class A, and the best therapy for other individuals is unknown. Additionally, in the absence of a medical contraindication, atezolizumab could be combined with bevacizumab for uHCC systemic therapy. Several studies are now underway to evaluate immune checkpoint inhibitors in combination with anti-angiogenic drugs, and the first findings are encouraging. The paradigm of uHCC therapy is changing dramatically, and many obstacles remain for optimum patient management in the near future. The purpose of this commentary review was to give an insight into current systemic treatment options for patients with uHCC who are not candidates for surgery to cure the disease.

Key Words: Hepatocellular carcinoma; Unresectable hepatocellular carcinoma; Non-alcoholic fatty liver disease; Tyrosine kinase inhibitor; Sorafenib; Lenvatinib; Immune checkpoint inhibitor; Atezolizumab; Bevacizumab

Core Tip: Hepatocellular carcinoma (HCC) is a major health problem that is the fourth leading cause of cancer-related mortality worldwide. The 5-year survival rate was nearly 19%, but only 2% in metastatic HCC. The first oral multikinase inhibitor for the systemic treatment of advanced or unresectable HCC (uHCC) was sorafenib. However, when compared to sorafenib, the combination of atezolizumab and bevacizumab increased survival rates and was authorized as first-line treatment for uHCC. Regorafenib and cabozantinib are suggested for use as second-line drugs in the event that the disease progresses. Transarterial chemoembolization for palliative care or downstaging is also suggested. This review focused on systemic therapy for uHCC patients who are not appropriate for liver-directed therapy.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common kind of primary liver cancer and a serious public health concern globally. With 905677 new cases and 830180 fatalities in 2020, liver cancer is the sixth most common malignancy and the third major cause of cancer mortality. The age-adjusted global incidence is 9.5 deaths per 100000 and the mortality rate is 8.7 per 100000 people[1]. HCC typically develops in the presence of cirrhosis, less frequently in chronic liver disease that is not cirrhotic, and very rarely in a healthy liver. The risk factors for HCC are well-known and vary by geographic location. In Asia and Africa, viral hepatitis is the main cause of HCC, but in North America and Western Europe, fatty liver disease and obesity are the main causes. The length of time that these risk factors have been detected in human populations is strongly correlated with the global increase in HCC incidence[2-4]. Depending on the tumor stage and liver function, the prognosis and treatment plan for HCC in cirrhotic livers are determined (Child–Pugh score). With a 5-year relative survival rate of 18.4%, overall survival (OS) is poor. Patients with localized, regional, and metastasis have 5-year survival rates of 33%, 10%, and 2%, respectively[5]. HCC may be treatable in its early stages by resection, liver transplantation, or ablation. However, patients are typically identified at intermediate or advanced stages due to a lack of symptoms.

For patients with HCC, the Barcelona Clinic Liver Cancer (BCLC) staging system employs many factors to direct the treatment course of action. The Child-Pugh score is comprised of the Eastern Cooperative Oncology Group (ECOG) performance status (PS), tumor burden (including portal invasion status and hepatic spread), and an estimation of the underlying liver function that should be estimated in addition to the Child-Pugh score using the Model for End-Stage Liver Disease score in cases of decompensated cirrhosis or the alpha-fetoprotein (AFP) concentration and albumin-bilirubin score in cases of compensated liver disease. HCC patients are classified into four stages: Very early (BCLC 0), early (BCLC A), intermediate (BCLC B), unresectable (BCLC C), and end-stages (BCLC D). Nearly 40% of HCC patients have an early diagnosis, making them eligible for curative procedures such as local radiofrequency ablation or surgery (liver transplantation or hepatic resection). A systemic, chemoembolization, or radioembolization treatment is necessary for more than half of them, which is in the intermediate stage and is unresectable (BCLC B and C)[6].

Unfortunately, systemic therapy is the only treatment available for individuals with HCC because more than 50% of cases are discovered at an advanced stage. In addition, recurrences occur in around 70% of individuals who have had their main tumor surgically removed[7]. When liver-directed medicines are not a possibility or the patient has experienced a significant recurrence, systemic therapy is chosen. Systemic treatments, including tyrosine kinase inhibitors (TKIs), monoclonal antibodies, and immune checkpoint inhibitors, have been made possible by recent technological advancements. The FDA has officially authorized sorafenib and lenvatinib as first-line therapies for advanced HCC. Second-line treatments for individuals who advanced or did not tolerate sorafenib include cabozantinib, regorafenib, ramucirumab, nivolumab, and pembrolizumab. The United States FDA recently approved the use of neurotrophic tyrosine receptor kinase (NTRK) inhibitors, larotrectinib or entrectinib, in HCC patients with NTRK fusion-positive solid tumors. Although cytokine treatment [interferon alpha-2b, interleukin (IL)-12] produced disappointing results, nivolumab and pembrolizumab have shown promising outcomes in phase II studies in terms of progression-free survival (PFS)[8-11]. Nivolumab in first-line treatment and pembrolizumab in second-line therapy phase III trials' primary endpoints, however, were not achieved[12,13]. The preliminary findings of a phase III study showed that atezol-

izumab with bevacizumab improved OS and PFS when compared to sorafenib as first-line therapy for HCC[14].

The development of effective HCC therapeutics is complicated by tumor heterogeneity caused by multiple risk factors. Greater understanding of the heterogenic tumorigenic pathways should provide information on tumor biomarkers, genomes, and other tumor characteristics that predict response to targeted treatment in HCC. In this review, we aimed to address the carcinogenesis of HCC, clinical trials that assessed medications targeting these tumorigenic pathways, and future directions of targeted treatment in advanced HCC.

PATHOGENESIS

Several pathways have been associated with the development of HCC. These pathways have served as the primary targets for systemic therapy development.

Mitogen-activated protein kinase pathway

In order to activate or deactivate their target, the mitogen-activated protein kinases (MAPK) phosphorylate either their own dual serine and threonine residues or those present on their substrates. As a common downstream pathway for numerous tyrosine kinase receptors, the MAPK pathway is a biological signaling system that controls crucial physiological processes such as cell proliferation, cell differentiation, stress responses, apoptosis, and immune defense. When external growth stimuli bind to these tyrosine kinase receptors, the MAPK cascade is initiated. An MAP3K stimulates an MAP2K, which in turn activates an MAPK, in an MAPK module. MAPK protein phosphatases, which dephosphorylate both phosphothreonine and phosphotyrosine residues on MAPKs, can inhibit MAPK phosphorylation processes. The most prevalent tyrosine kinase receptors are the insulin-like growth factor receptor, platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and epidermal growth factor receptor (EGFR). Growth factors that bind to these receptors cause a phosphorylation cascade, which in turn activates the adaptor molecular complex (growth factor receptor-bound protein 2-Src homology and collagen-son of sevenless). The proto-oncogenes rat sarcoma virus (Ras), rapidly accelerated fibrosarcoma (Raf), and guanosine triphosphatase are subsequently activated by this complex, leading to the activation of mitogen-activated protein kinase (MEK) 1/2 and extracellular signal-regulated kinase (ERK) 1/2 downstream. Eventually, this pathway results in the upregulation of gene transcription, which encourages cell proliferation, through the transcriptional activators c-Jun and c-Fos[15,16]. It has also been demonstrated that ERK phosphorylates proteins are involved in cell proliferation, apoptosis resistance, and angiogenesis in HCC[17,18]. Ras mutations were discovered to occur often in HCC, according to several studies, and overexpression of this pathway, particularly in high-grade tumors, has been reported in HCC[19,20]. Sorafenib, which inhibits the Raf serine/threonine kinase as well as several other receptors, was developed on the basis of this pathway[21,22].

Phosphoinositide 3-kinase–Ak strain transforming–mammalian target of rapamycin pathway

Cell development and regulation also depend on the phosphoinositide 3-kinase (PI3K)-Ak strain transforming (AKT)-mammalian target of rapamycin (mTOR) signaling pathway. Growth factors including epidermal growth factor and IL-2 bind to their associated tyrosine kinase receptors to activate PI3K. Phosphoinositol triphosphate, a lipid second messenger produced by PI3K, activates the serine/threonine kinase AKT (protein kinase B). After that, AKT phosphorylates a number of proteins, including the proapoptotic protein Bcl-2 opponent of cell death, to activate the mTOR family of proteins (BAD). To promote cell cycle progression and unrestricted growth and proliferation, mTOR regulates the phosphorylation of the translational repressor proteins ribosomal protein S6 kinase beta-1 (P70S6 kinase) and eukaryotic translation initiation factor 4E-binding protein 1 (EIF4EBP1 or 4E-BP-1)[23-25]. Studies have shown that cancers with abnormal expression of the pathway including phosphatase and tensin homolog (PTEN), AKT, and PS6 are more severe and have a poorer prognosis overall than tissues with normal expression[26,27]. In approximately half of all HCC patients, expression of the tumor suppressor gene product PTEN, which generally suppresses PI3K activity, is significantly decreased. Due to the gene deletion process, PTEN is inactivated, which results in uncontrolled PI3K activity and downstream phosphorylation of proteins that inhibit apoptosis and encourage tumor growth[28,29].

Wingless and Int-1/β-catenin pathway

The Wingless and Int-1 (WNT)/β-catenin signaling system regulates embryonic development, cellular proliferation, and differentiation. It is a highly conserved and carefully regulated molecular process. Notably, growing data suggests that abnormal WNT/β-catenin signaling increases the formation and progression of HCC. The two distinct WNT signaling pathways, known as non-canonical and canonical, with the latter including β-catenin activation. Comprehensive genomic analyses have revealed that β-catenin-encoding CTNNB1 and AXIN1 gain-of-function mutations are present in about 35% of human HCC samples. Human HCCs with activated WNT/β-catenin pathways display unique gene expression

patterns and malignant properties. Through its downstream effectors, activated WNT/β-catenin interacts with a variety of signaling pathways to encourage the development of HCC. As a result, medications that target WNT/β-catenin have being looked into as possible HCC treatments[30,31].

SYSTEMIC TREATMENT FOR UNRESECTABLE HCC

First-line systemic therapy

Single drug multikinase inhibitor: Sorafenib-A multikinase inhibitor (MTKI), sorafenib blocks tyrosine kinases and pathways essential for angiogenesis and cell proliferation. VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β, KIT, RET, RAS/RAF/MAPK, FLT-3, and Janus kinase/signal transducer and activator of transcription protein are among the receptors that it inhibits (STAT)[32]. The multicenter phase III European Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) research and the Asia-Pacific trial on OS benefit compared with placebo both supported the efficacy of first-line sorafenib for uHCC patients with Child-Pugh class A cirrhosis. The inclusion criteria for both studies were the same: Advanced HCC with detectable illness, mostly Child-Pugh class A cirrhosis, no prior systemic therapy, adequate hematological, renal, and hepatic function, and a life expectancy of at least 12 wk. Six hundred and two untreated uHCC patients from Europe, North America, South America, and Australia were included in the SHARP study. Two groups of patients were randomly treated with either sorafenib 400 mg (n = 299) twice daily or a placebo (n = 303). Treatment with sorafenib was continued until the illness became worse, the toxicity was too much, or someone passed away. Patients receiving sorafenib experienced response rates of just 2%, compared with only 1% in the placebo group. Clinical outcomes were achieved in 43% and 32% of patients in the sorafenib and placebo groups, respectively (P = 0.002). The project was halted after a second planned interim analysis showed that the median OS with sorafenib was significantly longer at 10.7 mo compared to 7.9 mo with placebo (P < 0.01). With sorafenib, 1-year survival rates were 44%, while with placebo, they were 33% (P < 0.01). The most common treatment-related side effects in people taking sorafenib were diarrhea, weight loss, hand-foot syndrome (HFS), and hypophosphatemia[33] (Table 1).

The Asia-Pacific study, which ran alongside the SHARP trial, was designed to look at the safety and activity of sorafenib in uHCC patients. They randomly assigned 150 of the 226 participants who were drawn from 23 locations in China, South Korea, and Taiwan to treat with sorafenib 400 mg twice daily or a placebo (n = 76). Similar to the SHARP study, sorafenib had a low response rate (3.3% *vs* 1.3% for placebo). Sorafenib had a disease control rate of 35.3% whereas a placebo had a rate of 15.8% (P < 0.001). The median OS periods for sorafenib and the placebo were 6.5 mo and 4.2 mo, respectively (P = 0.014). Despite the fact that both studies utilized the identical eligibility standards and treatment strategy, the Asia-Pacific trial's patients had more extrahepatic disease, more hepatic lesions, a worse PS, more advanced disease, and a higher rate of AFP elevation. Because of this, their sorafenib-related survival in the Asia-Pacific study (6.5 mo) was lower than in the SHARP trial (10.7 mo). In the subgroup analysis, sorafenib showed higher efficacy in patients without extrahepatic spread [hazard ratio (HR): 0.55 *vs* 0.84], with hepatitis C-associated illness (HR: 0.47 *vs* 0.81), and with a low neutrophil-to-lymphocyte ratio (HR: 0.59 *vs* 0.84)[34] (Table 2).

In a subset analysis of the SHARP trial, Bruix *et al*[35] looked at how the etiology of the illness, the tumor load, PS, the tumor stage, and past treatments affected survival, disease control, time to progression (TTP), and safety. Compared to placebo, sorafenib treatment significantly enhanced survival and disease control regardless of the etiology, tumor load, PS, tumor stage, or previous therapy. Except for individuals who were hepatitis B virus (HBV)-positive, sorafenib also reliably reduced the median time to progression (MTP). The relationship between the pre-study liver state and the effects of sorafenib on liver function were investigated in a second subgroup analysis of the SHARP trial data. Individuals with baseline transaminase, AFP, or bilirubin levels elevation showed shorter survival periods than those with normal baseline values, regardless of therapy. Patients with normal or high liver markers experienced the same level of safety. The investigators came up with the conclusion that sorafenib was safe and efficient independent of the liver biomarkers present at baseline and that bilirubin levels, which are used to evaluate hepatic function, were consistent during sorafenib treatment. It should be highlighted that the majority of the trial participants had Child-Pugh class A cirrhosis. Therefore, individuals with more severe cases (Child-Pugh class B and C) should not extrapolate from these findings[36].

Sorafenib was also further studied in two large prospective observational trials, GIDEON and INSIGHT. The GIDEON research, or Global Investigation of Therapeutic DEcisions in HCC and of its Treatment with Sorafenib, examined the safety and tolerability of sorafenib in patients with advanced HCC in actual clinical settings. All treatment choices in this study were made by the patient's attending physician. When therapy first started, out of 2708 people, 72.7% were categorized as Child-Pugh class A, 24.5% as class B, and 2.7% as class C. Sorafenib's starting dosage was 400 mg twice daily. Child-Pugh class A, B, and C patients experienced adverse events (AEs) at rates of 69%, 64%, and 39%, respectively, whereas 9%, 14%, and 3% of patients experienced serious medication responses. The most frequent AEs were HFS (32%, 17%, and 5%), diarrhea (28%, 26%, and 11%), and exhaustion (16%, 14%, and 14%) in

Table 1 Summary of phase 3 trials of sorafenib and lenvatinib for treatment of unresectable hepatocellular carcinoma

Ref.	No. of patients (Child-Pugh A/B)	Treatment or placebo	Median OS in mo	Response rate, %	Control rate, %	Median TP in mo	Dose reduction, % patients	Discontinuation due to AE, % patients
Llovet *et al*[33] (SHARP)	298 (284/14)	Sorafenib (400 mg × 2/d)	10.7	2	43	5.5	26	11
	303 (297/6)	Placebo	7.9	1	32	2.8	7	5
Cheng *et al*[34] (Asia-Pacific)	150 (146/4)	Sorafenib (400 mg × 2/d)	6.5	3.3	35.3	5.5	30.9	19.5
	76 (74/2)	Placebo	4.2	1.3	15.8	2.8	2.7	13.3
Kudo *et al*[47] (REFLECT)	478 (478/0)	Lenvatinib (12 mg/d)	13.6	40.6	73.8	7.4	37	9
	476 (476/0)	Sorafenib (400 mg × 2/d)	12.3	12.4	56.4	3.7	38	7

AE: Adverse event; OS: Overall survival; SHARP: Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol; TP: Time to progression.

Table 2 Summary of the 2 large prospective observational studies of sorafenib for treatment of unresectable hepatocellular carcinoma

Ref.	No. of patients (Child-Pugh A or Child-Pugh B)	Treatment	Median OS in mo	Response rate, %	Control rate, %	Median TP in mo	Dose reduction, % patients	Discontinuation due to AE, % patients
Abou-Alfa *et al*[68]	98	Sorafenib (400 mg × 2/d)	10.7	2	43	5.5	26	11
	38	Sorafenib (400 mg × 2/d)	7.9	1	32	2.8	7	5
Cheng *et al*[34] (Asia-Pacific)	150 (146/4)	Sorafenib (400 mg × 2/d)	6.5	3.3	35.3	5.5	30.9	19.5
	76 (74/2)	Placebo	4.2	1.3	15.8	2.8	2.7	13.3

AE: Adverse event; OS: Overall survival; TP: Time to progression.

patients with Child-Pugh classes A, B, and C scores. Patients in Child-Pugh class A had a longer median OS (13.6 mo) than those in Child-Pugh class B or C (5.2 mo) (2.6 mo). The GIDEON study showed that the most prevalent drug-related side effects, as well as sorafenib tolerance, were comparable between Child-Pugh class A and Child-Pugh class B patients. They discovered that some individuals with Child-Pugh class B cirrhosis might receive sorafenib safely. Because of the variety of individuals with Child-Pugh class B conditions, patients should be carefully selected for sorafenib treatment based on a thorough evaluation of their hepatic condition[37].

The INSIGHT study was a prospective multicenter trial that recruited 788 uHCC patients who were treated with sorafenib. The main objective was to assess sorafenib's safety and efficacy, especially TTP and OS. Sorafenib was generally given at a dosage of 800 mg daily. The prevalence of cirrhosis among the patients was 56.7% Child-Pugh class A, 23.3% Child-Pugh class B, and 3.3% Child-Pugh class C. Patients in Child-Pugh classes A, B, and C experienced an OS of 17.6, 8.1, and 5.6 mo, respectively ($P < 0.01$). Sorafenib-related AEs occurred in 64.9% of patients and were regarded as severe in 9.8%. The most frequent serious adverse medication event (5.2%) was diarrhea. HFS (16.5%), nausea (8.0%), and exhaustion (7.9%) were other notable medication side effects. Both MTP and survival decreased dramatically when Child-Pugh scores increased. Patients having a Child-Pugh score of 7 and those with a score of 8 had comparable MTP and survival rates. Patients with a 9 on the Child-Pugh scale had substantially reduced MTP and survival ($P < 0.01$ and $P = 0.003$, respectively)[38].

McNamara *et al*[39] performed a meta-analysis of sorafenib therapy in patients with Child-Pugh class B cirrhosis, including 30 trials and 8678 participants. Most of the investigations were retrospective or prospective single-institution studies. Child-Pugh class A participants had an objective response rate of 4.6%, whereas Child-Pugh class B participants had an objective response rate of 4.2%. The distribution of Child-Pugh status was 79% Child-Pugh class A and 19% Child-Pugh class B. The assessed median OS for Child-Pugh class A patients was 8.8 mo and 4.6 mo for Child-Pugh class B patients. There was an inverse relationship between OS and Child-Pugh class B hepatic dysfunction. Child-Pugh class B

patients had considerably lower survival, according to four studies that compared patients with Child-Pugh classes A and B using several variables ($P < 0.001$). In 35% of individuals with Child-Pugh class A or B, there was grade 3 or 4 toxicities. The rates of treatment termination without progress and treatment-related mortality were comparable. The study's findings revealed that while sorafenib response, safety, and tolerability were unaffected by the Child-Pugh score, survival was strongly impacted by these factors. No research involving patients with Child-Pugh class B cirrhosis were controlled trials, despite the large number of such studies that have been done, and the information that is now available is insufficient to draw firm conclusions on the use of sorafenib in such individuals. This meta-analysis proved that certain individuals with Child-Pugh class B cirrhosis can get sorafenib safely. Patients with Child-Pugh scores of 7 or less seem to be the safest group to treat with sorafenib, and patients with Child-Pugh scores ≥ 8 should be cautioned. Selected Child-Pugh class B patients may benefit from sorafenib even if Child-Pugh class A patients have much higher survival rates than Child-Pugh class B patients.

Other MTKIs such as brivanib, sunitinib, linifanib, erlotinib, and everolimus were also investigated, but none of them demonstrated superiority over sorafenib[40-44]. Prior to regorafenib's approval for second-line therapy in April 2017 and lenvatinib's approval for first-line therapy in August 2018, sorafenib was the sole FDA-approved therapeutic option for uHCC[45,46].

Lenvatinib-Lenvatinib is a MTKI that targets VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β, FGFR-1, FGFR-2, FGFR-3, FGFR-4, KIT, and RET. Lenvatinib is approved as the first-line treatment for uHCC patients. Patients who weigh 60 kg or more should take 12 mg daily, while those who weigh less should take 8 mg. Patients with uHCC who have Child-Pugh class A cirrhosis should not have their doses reduced, and there is no suggested dose for those with Child-Pugh class B or C cirrhosis. Based on the REFLECT trial, a noninferiority phase 3 research, lenvatinib was authorized. In this trial, there were 954 untreated patients with uHCC with Child-Pugh class A cirrhosis from 154 locations in 20 nations throughout the Asia-Pacific, European, and North American continents. Lenvatinib at a daily dose of 12 mg ($n = 478$) (for patients' weight > 60 kg) or 8 mg (for patients weighing 60 kg) was given to patients at random, while sorafenib ($n = 476$) was given at a dose of 400 mg twice daily. OS was the primary aim, and patients were treated up until their radiological state worsened or they developed significant toxicity. Response rate, TTP, and PFS were secondary endpoints. Lenvatinib was comparable to sorafenib in terms of survival, with a median OS of 13.6 mo and 12.3 mo, respectively [HR: 0.92; 95% confidence interval (95%CI): 0.79-1.06]. According to baseline features, these effects were discovered to be constant across all patient groupings. In terms of all secondary objectives, lenvatinib was considerably more successful than sorafenib, according to an independent imaging analysis. Response rates were 40.6% *vs* 12.4% ($P < 0.001$) for lenvatinib *vs* sorafenib; disease control rates were 73.8% *vs* 58.4%; MTP rates were 7.4 mo *vs* 3.7 mo ($P < 0.001$); and median PFS rates were 7.3 mo *vs* 3.6 mo ($P < 0.001$). When compared to sorafenib, lenvatinib was related with more incidences of hypertension associated with treatment (42% *vs* 30%), proteinuria (25% *vs* 11%), and hypothyroidism (16% *vs* 2%), and lower rates of alopecia (3% *vs* 25%), HFS (27% *vs* 52%), and diarrhea (39% *vs* 46%). The reductions of dosage, treatment suspensions, and drugs discontinuations were reported by 40%, 37%, and 9% of lenvatinib-treated patients, respectively, and 32%, 38%, and 7% of patients who were treated with sorafenib, respectively. During the REFLECT trial's follow-up period, 33% of patients who were treated with lenvatinib and 39% of patients who were treated with sorafenib received antineoplastic drugs. The median survival time among patients who did not get further treatment with lenvatinib was 11.5 mo and 9.1 mo with sorafenib. Patients who underwent further therapy following lenvatinib therapy and sorafenib therapy had median survival lengths of 20.8 mo and 17.0 mo, respectively. This was one of the earliest signs that sequential treatment might enhance survival[47].

Finn *et al*[48] investigated the relationships between serum or tissue biomarkers and effectiveness outcomes from the REFLECT study. ELISA was used to assess serum biomarkers [VEGF, angiopoietin-2 (ANG2), FGF19, FGF21, and FGF23]. The nCounter PanCancer Pathways Panel assessed gene expression in tumor tissues. Pharmacodynamic variations in serum biomarker levels from baseline were investigated, as were clinical outcome relationships with baseline biomarker levels. They included 407 people in the serum analysis group (lenvatinib $n = 279$, sorafenib $n = 128$) and 58 people in the gene-expression analysis group (lenvatinib $n = 34$, sorafenib $n = 24$). They observed that, whereas both treatments were associated with increases in VEGF, only lenvatinib was associated with increases in FGF19 and FGF23 across the whole study period. Responders had greater levels of FGF19 and FGF23 on cycle 4, day 1 than non-responders (FGF19: 55.2% *vs* 18.3%, $P = 0.014$; FGF23: 48.4% *vs* 16.4%, $P = 0.002$, respectively). In both therapy groups, higher baseline VEGF, ANG2, and FGF21 Levels were associated with a shorter OS. With greater baseline FGF21, lenvatinib had a longer OS than sorafenib [10.9 mo *vs* 6.8 mo, respectively; HR: 0.53; 95%CI: 0.33-0.85; $P = 0.0397$]. In a biomarker examination of tumor tissue, VEGF/FGF-enriched groups outlived the intermediate VEGF/FGF group (HR: 0.39; 95%CI: 0.16-0.91; $P = 0.0253$). They concluded that shorter OS may be predicted by increased baseline levels of VEGF, FGF21, and ANG2. Lenvatinib's superior OS *vs* sorafenib may be predicted by higher baseline FGF21, but further research is required to prove this.

Now, uHCC has an additional first-line MTKI option in lenvatinib. In March 2018, it was approved in Japan. It was also approved in the United States in August 2018. The European Association for the Study of the Liver, European Society for Medical Oncology (ESMO), National Comprehensive Cancer

Network (NCCN), American Society of Clinical Oncology (ASCO), and all agreed that lenvatinib should be used in the first-line setting but only for patients with Child-Pugh class A cirrhosis[49-52].

Combination therapy of immune check-point inhibitor and VEGF inhibitor

Atezolizumab plus bevacizumab: The importance of VEGF and VEGFR signaling pathways in angiogenesis and tumor formation has been highlighted through research into the etiology of HCC. Both healthy and pathological angiogenesis are regulated by the VEGF protein family[53]. When VEGF overexpression was observed in these tumors, their function as therapeutic targets in uHCC was found. It is not unexpected that the idea of targeting tumor angiogenesis as a potential therapeutic method was offered as early as 1971 given the knowledge that a significant phase in tumor development requires oxygen and nourishment supply for sustained growth. As previously stated, VEGF expression is increased in HCC. Sorafenib targets the VEGF signaling system *via* MTKI, resulting in the therapeutic advantage already documented. However, the tangible benefit of sorafenib in terms of VEGF inhibition prompted researchers to look for other pathways targeting tumor angiogenesis and VEGF inhibition.

Because it develops in persistently inflamed livers from both viral and non-viral origins, HCC is usually referred to as an immunogenic malignancy. Furthermore, tumor-associated antigen expression and particular gene alterations that result in unique neoantigens lead to HCC immunogenicity. Innovative immunomodulation treatment options in uHCC have emerged as a result of investigations into the tumor microenvironment (TME) in HCC. Hepatitis viruses' persistent inflammation and the parenchyma's production of cytokines and growth factors coexist in a complex microenvironment. Due to restricted T cell activation that results in the generation of tumor-related antigens, the liver is also known to have an intrinsic immunosuppressive environment. The majority of the immune system's anti-tumor defenses are T cells, and tumor cells that have overexpressed the programmed cell death ligand 1 (PD-L1) have created an immunosuppressive milieu. Immunological checkpoints are coinhibitory membrane glycoproteins that largely block T cell immune overactivation during inflammatory and infectious conditions. Normally, this avoids collateral tissue damage, but in the TME, their expression plays a critical role in encouraging T cell exhaustion and immunological tolerance. The presence of Cytotoxic T Lymphocyte-Associated protein 4 (CTLA-4) and PD-1, immunological checkpoints involved in T cell activation and other inflammatory responses in malignancies, is well understood. In tumor expression on T cell activation and other inflammatory responses, CTLA-4 and PD-1 are immunological checkpoints that have been thoroughly studied. Additionally, expressed on regulatory T cells, CTLA-4 prevents T cells from co-stimulating when antigen is given. This is accomplished by the competitive binding of CD80 and CD86 receptors on antigen-presenting cells, which results in decreased CD28 stimulation and immune escape. As PD-1 binds to its ligands, PD-L1 and PD-L2, the CD28 pathway is also affected, which prevents CD8+ T cell activation and results in immunological inactivation. By expressing PD-L1 and PD-L2, cancer cells use this strategy to escape immune monitoring. Enduring antigen T cell tiredness is caused by exposure to the TME and is demonstrated by an increase in PD-L1 in tumor cells and antigen-presenting cells, which is induced by reactive T cells that express PD-1. As a result, the prognosis is poorer and the tumor grows larger with less effective tumor suppression[54-56].

Bevacizumab, a VEGF monoclonal antibody and atezolizumab, an anti-PD-L1 antibody, have been used in combination therapy for uHCC patients, which has resulted in the most recent and notable advancement in the treatment of HCC[14]. In the TME, VEGF overexpression has been seen to support immunological tolerance and evasion in malignancies. The main function of VEGF is angiogenesis, which paradoxically results in a hypoxic and acidotic TME and attracts immune-suppressive cells like regulatory T cells. VEGF also increases the expression of PD-1 on tumor-infiltrating T cells[57-59]. Targeting VEGF lowers immune suppression, and combining immune checkpoint inhibitors leads in enhanced immunological reactivation *via* increased T cell activity and tumor cell penetration. The phase III IMbrave150 research randomly allocated 501 patients who had not previously undergone systemic treatment to atezolizumab-bevacizumab or sorafenib in a 2:1 ratio. The study found that atezolizumab-bevacizumab improved OS by 67.2% (95%CI: 61.3%-73.1%) at 12 mo while sorafenib improved OS by 54.6% (95%CI: 45.2%-64.0%)[59]. Atezolizumab-bevacizumab had an objective response rate (ORR) of 27.3% (95%CI: 22.5%-32.5%) and sorafenib had an ORR of 11.9% (95%CI: 7.4%-18.0%) according to Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1). This combination's adverse event profile was consistent with the established safety profiles of each medication and the underlying condition. Proteinuria, tiredness, and an increase in aspartate aminotransferase (AST) levels were also observed in 15% of patients with grade 3-4 hypertension. In the atezolizumab-bevacizumab arm, approximately 15% of patients withdrew therapy due to side effects, compared to 10% in the sorafenib arm. The most common reported reason for withdrawal was gastrointestinal side effects. Bleeding is a recognized consequence of bevacizumab due to its anti-angiogenic properties. A typical consequence of cirrhosis is upper gastrointestinal bleeding, which has the potential to be a life-threatening hemorrhage. This was observed in 7% of patients in the atezolizumab-bevacizumab group versus 4.5% in the sorafenib group. Due to the increased risk of catastrophic bleeding with bevacizumab, patients must not have esophagogastric varices prior to beginning treatment. This encouraging advancement has rendered this combination, the FDA-approved preferred strategy for treating uHCC and is currently advised as the first-line treatment in uHCC following the 2021 recommendations[60].

Atezolizumab plus cabozantinib: Kelley *et al*[61] conducted an open-label, randomized, phase 3 study (COSMIC-312) in 837 uHCC patients from 178 centers in 32 countries to compare cabozantinib + atezolizumab against sorafenib as first-line systemic therapy. Patients required to have Child-Pugh class A, ECOG PS 0 or 1, detectable illness as defined by RECIST 1.1, BCLC stage B or C, and adequate organ and marrow function. Through a web-based interactive response system, they were randomly assigned (2:1:1 = 432:217:188) to receive cabozantinib 40 mg once daily plus atezolizumab 1200 mg intravenously (IV) every 3 wk, sorafenib 400 mg twice daily, or cabozantinib 60 mg once daily. Intention to treat (ITT) population had a median follow-up of 13.3 mo, whereas the PFS ITT group had a median follow-up of 15.8 mo (IQR: 14.5-17.2), (IQR: 10.5-16.0). The authors showed that median PFS in the combination therapy arm was 6.8 mo (99%CI: 5.6-8.3) *vs* 4.2 mo (99%CI: 2.8-7.0) in the sorafenib arm (HR: 0.63, 99%CI: 0.44-0.91, P = 0.0012). In the combination treatment group, the median OS was 15.4 mo, compared to 15.5 mo in the sorafenib group (HR: 0.90, 96%CI: 0.69-1.18; P = 0.44). The most frequent grade 3 or 4 AEs included a 9% (38/429) increase in alanine aminotransferase in the combination treatment arm, compared to 3% (6/207) in the sorafenib arm, and 6% (12/188) in the cabozantinib arm. Hypertension was found in 9%, 8%, and 12%, HFS was found in 8%, 8%, and 9%, serious treatment-related AEs occurred in 18%, 16%, and 13% of the combination treatment arm, sorafenib arm, and cabozantinib arm, respectively. Treatment-related grade 5 events occurred in 1% of the combination treatment arm, < 1% of the sorafenib arm, and < 1%) of the cabozantinib arm. They suggested that cabozantinib in combination with atezolizumab might be a therapy option for some uHCC patients[62].

Combination therapy of anti-PD-L1 and CTLA-4 antibody

Durvalumab and tremelimumab: Several studies have shown that extended exposure to CTLA-4 inhibitors may not be required for long-term anti-tumor effects. In metastatic melanoma, Eroglu *et al*[63] found that a single dosage of the CTLA-4 inhibitor tremelimumab might result in exceptionally extended durations of objective anti-tumor responses lasting more than 12 years. In advanced non-small cell lung cancer, a phase Ib research discovered that tremelimumab 1 mg/kg plus durvalumab 20 mg/kg every 4 wk provided an anti-tumor action while being tolerated. In a recent phase I/II study, durvalumab 20 mg/kg with tremelimumab 1 mg/kg every 4 wk for 4 doses was evaluated. The results showed acceptable tolerability and encouraging early efficacy in the second-line setting. The T-300/D-1500 high-dose had the highest risk and benefit outcome with a median OS and ORR of 18.7 mo and 22.7 mo, respectively, in the expanded phase 2 study that examined combinations of 75 mg tremelimumab with 1500 mg durvalumab (T-75/D-1500) and T-300/D-1500. In the phase 3 HIMALAYA study, the T-300/D-1500 group was examined as first-line treatment. Patients with uHCC were divided into four groups: (1) T-300/D-1500 followed by D-1500 every 4 wk (STRIDE); (2) D-1500 every 4 wk; (3) sorafenib 400 mg twice daily; and (4) T-75 every 4 wk followed by D-1500 every 4 wk (T-75/D). T-75/D enrollment was halted due to a planned analysis found no significant difference between D-1500 and T-75/D. D was noninferior to sorafenib alone in terms of OS (16.6 mo *vs* 13.8 mo; HR: 0.86; 96%CI: 0.73-1.03). The T-300/D-1500 ORR was 20.1%, 17% with durvalumab alone, and 5.1% with sorafenib alone. Nevertheless, no statistically significant change in PFS was seen. Durvalumab showed a good safety profile and wasn't worse than sorafenib. Durvalumab generated grade 3/4 AEs in 12.9% of patients treated with the combo group, and sorafenib in 25.8% of patients[64-66]. As a result, the combination of durvalumab plus tremelimumab is a realistic first-line option for patients who are not candidates for atezolizumab or bevacizumab, such as those with a high risk of bleeding[67].

For uHCC patients with a Child-Pugh class A cirrhosis without anticoagulant therapy, an ECOG score of 0 or 1, and treatment for esophageal varices, current ASCO and ESMO guidelines recommend atezolizumab-bevacizumab combination therapy rather than lenvatinib or sorafenib monotherapy. Tremelimumab plus durvalumab is an alternative to bevacizumab for patients who are unsuitable to receive it. Monotherapy with sorafenib or lenvatinib is an alternative treatment for Child-Pugh class B cirrhosis patients with no worse than score 7, or when double immunotherapy is considered unsafe, or when the clinical condition of the patients is less fit, or with multiple comorbidities with predicted poor acceptance to combined immunotherapy. Only individuals with cirrhosis that is no worse than Child-Pugh class A are advised to take lenvatinib. Lenvatinib often causes fewer HFS and alopecia than sorafenib and has a lower overall toxicity profile. According to the REFLECT study, it also had a higher ORR, better PFS, and a longer TTP. As a result, if monotherapy is indicated, the majority of physicians now choose to begin with lenvatinib. However, considering the extended amount of experience and the noninferior median OS observed in the REFLECT study, sorafenib may still be preferable (Figure 1).

Second-line systemic therapy

Single drug-multikinase inhibitor: Regorafenib-Regorafenib was proposed as a possible second-line therapy for uHCC in the RESORCE study in patients who had progressed on sorafenib. The EGFR and VEGF receptors are the targets of the MTKI regorafenib. The phase III, randomized, double-blind trial compared OS to placebo in 567 Child-Pugh class A patients who had been receiving sorafenib and tolerated treatment for at least 20 of the 28 d prior to discontinuation. After enrollment, patients were randomized to receive regorafenib 160 mg daily or a placebo. When compared to the placebo, the median OS with regorafenib was 10.6 mo as opposed to 7.8 mo (HR: 0.63, 95%CI: 0.50-0.79; P < 0.01).

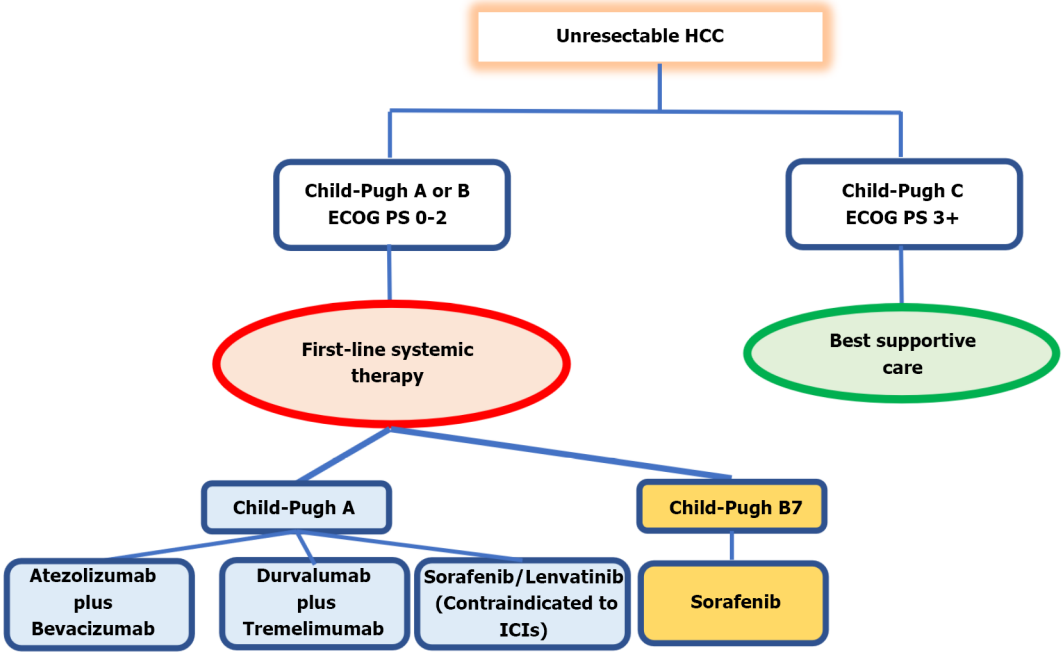

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Figure 1 First-line systemic therapy in unresectable hepatocellular carcinoma. ECOG PS: Eastern Cooperative Oncology Group Performance Status; ICIs: Immune checkpoint inhibitors; HCC: Hepatocellular carcinoma.

Hypertension, hand-foot skin reactions, tiredness, and diarrhea were all grade 3 or 4 AEs. They stated that regorafenib is the only systemic therapy that has been demonstrated to improve survival in HCC patients who are advancing on sorafenib treatment. Regorafenib should be studied in combination with more systemic medications in the future, as well as third-line therapy for patients who do not respond to or tolerate the sorafenib-regorafenib regimen[45].

Cabozantinib-in uHCC patients who had previously been treated with sorafenib and had suffered disease progression on at least one systemic therapy, the MTKI cabozantinib has demonstrated clinical effectiveness with good outcomes. Cabozantinib acts by targeting the VEGF, MET, and AXL receptors. Antiangiogenic resistance, epithelial mesenchymal transition, invasion, and metastasis have all been related to MET and AXL receptors. High levels of MET and AXL expression have been associated with a poor prognosis in HCC, and higher levels of MET activity have been observed in previously treated patients who develop sorafenib resistance. In this double-blind, randomized phase III study (CELESTIAL), 707 patients who had previously taken sorafenib, who had progressed on at least one systemic therapy for HCC, or who had received up to two prior systemic therapies, were randomly assigned to receive cabozantinib 60 mg daily as opposed to a placebo. OS was the main objective, with PFS and ORR as additional endpoints. Patients who received cabozantinib showed a longer OS (10.2 mo *vs* 8.0 mo; HR: 0.76; 95%CI: 0.63-0.92; P = 0.005). When cabozantinib and placebo have been used, PFS was 5.2 mo *vs* 1.9 mo, and ORR was 4% and 1%, respectively. Sixty-eight percent of cabozantinib patients developed grade 3 or 4 adverse effects, with hand-foot skin reactions, hypertension, fatigue, diarrhea, and an increase in liver AST levels being the most frequent. They concluded that in patients with advanced HCC who had already had treatment, cabozantinib therapy produced longer OS and PFS than placebo. Nearly twice as many major side events occurred in the cabozantinib group than they did in the placebo group[68].

Single drug-VEGF inhibitor

Ramucirumab: For the treatment of uHCC patients who have previously had sorafenib therapy and have an AFP of 400 ng/mL or more, ramucirumab has a license. Inhibiting ligand-stimulated VEGFR2, cell proliferation, and angiogenesis, ramucirumab binds to VEGFR2 and blocks the binding of VEGFR ligands VEGF-A, VEGF-C, and VEGF-D. The dose for uHCC patients is 8 mg/kg IV every 2 wk until the condition progresses or there is significant toxicity. For those with mild to moderate hepatic impairment, the manufacturer advises against dose adjustment, but it also notes that clinical deterioration has been observed in patients with Child-Pugh class B and class C cirrhosis who received ramucirumab. Child-Pugh class B or class C cirrhosis patients should only consider ramucirumab if the potential benefits are deemed to exceed the risks of clinical worsening. In a phase 2 trial including 42 uHCC patients, ramucirumab was used as a first-line treatment. Ramucirumab was administered to patients at a dose of 8 mg/kg every 2 wk until the condition progressed or unacceptable toxicity developed. PFS was the primary objective, with response and survival serving as additional goals. The

median PFS, MTP, and survival were each 4.0, 4.2, and 12.0 mo, respectively. The median response time was 14.1 mo, and the disease control rate was 69.0%. There were 4 partial responses (9.5% of patients) [69]. The main ramucirumab trial was the phase 3 REACH trial, which included uHCC patients who were unresponsive to locoregional treatment. All of the patients had previously undergone sorafenib treatment. In addition to best supportive care (BSC), patients were randomly treated with ramucirumab 8 mg/kg IV (n = 283) or a placebo (n = 282) every 2 wk, or until disease progresses, intolerable toxicity, or death. PFS, response, and disease control were secondary goals, with OS serving as the primary objective. The median survival time with ramucirumab was 9.2 mo compared to 7.6 mo with placebo (P = 0.14). In the ramucirumab and placebo groups, the median PFS times were 2.8 mo and 2.1 mo, respectively (P < 0.01). MTP for ramucirumab was 3.5 mo and 2.6 mo for the placebo (P < 0.01). In comparison to the placebo group, which only saw two partial responses (1% of patients), the ramucirumab group showed a full resolution and 19 partial responses (7% of patients) (P < 0.01). Fifty six percent of ramucirumab-treated individuals and 46% of placebo receivers achieved disease control (P = 0.011). The reductions of dosage were required in 7% of patients who were treated with ramucirumab and less than 1% of placebo patients, with dose omission rates of 22% and 10%, respectively; 10% and 3% of patients in the ramucirumab and placebo groups were discontinued due to unfavorable drug side effects. The most frequent grade 3 or 4 AEs were ascites (5% of patients who were treated with ramucirumab *vs* 4% of placebo patients), AST elevation (5% *vs* 8%), thrombocytopenia (5% *vs* 1%), hypertension (12% *vs* 4%), asthenia (5% *vs* 2%), and hyperbilirubinemia (1% *vs* 5%). Investigations were conducted on a predetermined sample of individuals having a baseline AFP level of 400 ng/mL. With ramucirumab, the median survival time was 7.8 mo as opposed to 4.2 mo with a placebo (P = 0.006). Survival was 11.8 mo with placebo and 10.1 mo with ramucirumab in patients with an AFP value less than 400 ng/mL (P = 0.51). These results led the researchers to propose that a high baseline AFP level may be a predictor of who may respond favorably to ramucirumab treatment[70].

The objective of the phase 3 study (REACH-2) was to address the efficacy of ramucirumab treatment in baseline AFP levels of 400 ng/mL or above patients. They were recruited because they had an ECOG PS of 0 or 1, intolerance to sorafenib, Child-Pugh class A cirrhosis, a history of disease progression, and they weren't candidates for locoregional therapy or resistant to it. Every 2 wk, patients were treated with ramucirumab 8 mg/kg (n = 197) or a placebo (n = 95), along with BSC, until the illness progressed or the side effects became unbearable. When ramucirumab was used instead of a placebo, the response rates were 5% and 1%, respectively (P = 0.1697), while the rates of disease control were 59.9% and 38.9%, respectively (P < 0.001). When compared to placebo, treatment with ramucirumab was related with significantly prolonged median PFS (2.8 mo *vs* 1.6 mo, P < 0.001) and median survival (8.5 mo *vs* 7.3 mo, P = 0.019). The REACH-2 study revealed that ramucirumab gave significantly better survival than placebo when taken as follow-up treatment following sorafenib in patients with an AFP value of 400 ng/mL or more. Ramucirumab did not cause HFS, a well-known side effect of other targeted medications[71]. Post hoc analysis of the REACH and REACH-2 studies confirmed the relevance of AFP as a predictive indicator, with AFP response considerably greater in individuals treated with ramucirumab than placebo (P < 0.0001). Survival was considerably enhanced with an AFP response (13.6 mo *vs* 5.6 mo; HR: 0.45; P < 0.0001)[72]. Ramucirumab is advised by NCCN as a category 1 medication for further therapy following sorafenib usage in individuals with an AFP level of 400 ng/mL or more[52].

Single drug-immune check-point inhibitor

Pembrolizumab: In the phase II KEYNOTE-224 research, pembrolizumab, an anti-PD1 monoclonal antibody, demonstrated comparable clinical antitumor effectiveness and safety when given to patients who had previously taken sorafenib for uHCC patients[11]. Finn *et al*[13] performed a phase 3 randomized, double-blind trial at 119 healthcare centers in 27 countries. With a follow-up length of 13.8 mo for pembrolizumab and 10.6 mo for placebo, they randomly allocated 413 patients to receive pembrolizumab plus BSC or placebo plus BSC. The median OS for pembrolizumab was 13.9 mo compared to 10.6 mo for placebo. At the end of the study, the median PFS for pembrolizumab was 3.0 mo *vs* 2.8 mo for placebo. Pembrolizumab was used in 147 (52.7%) and 62 (46.3%) patients, respectively; treatment-related events occurred in 52 (18.6%) and 10 patients (7.5%), respectively. No new cases of hepatitis C or B were discovered. They concluded that OS and PFS did not satisfy the criteria for statistical significance. Pembrolizumab offers a positive risk-to-benefit ratio in this population, according to the results, which are similar to those of KEYNOTE-224.

Drug-combination of PD-1 and CTLA-4 antibody

Nivolumab and ipilimumab: The CTLA-4 molecule, a crucial signaling checkpoint required for T-cell activation, is the target of the immune check-point inhibitor (ICI) ipilimumab. They successfully target two different immunological checkpoints when combined with nivolumab, inducing the modify immune response. The United States FDA approved nivolumab with ipilimumab as a second-line therapy in March 2020. The efficacy of combination treatment was proven in the phase 1/2 CHECKMATE-040 trial, which included 148 patients who were treated with sorafenib and clinically were better than Child-Pugh class A. The study evaluated 3 different regimens: Arm A: Nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 wk for 4 cycles, followed by biweekly nivolumab 240 mg;

Arm B: Nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 wk for 4 cycles, followed by biweekly nivolumab 240 mg; Arm C: Nivolumab 3 mg/kg every 2 wk plus ipilimumab 1 mg/kg every 6 wk. The suggested regimen is Arm A. According to the findings, the proposed regimen had the best ORR of 32%. CR was obtained by 8% of patients, and PR by 24%. The duration of response was 17 mo on average. The disease control rate was comparable among the three groups. However, larger sample size experiments are required to corroborate this conclusion[73]. In this CHECKMATE-040 cohort, individuals with or without hepatitis B or C had similar patterns of AEs, however Arm A was associated with higher TRAEs. Due to TRAEs, treatment was stopped in 18% of Arm A patients, 6% of Arm B patients, and 2% of Arm C patients. Rashes, adrenal insufficiency, hypothyroidism or thyroiditis, colitis, pneumonitis, and infusion-related complications were all observed in 35%, 18%, 22%, 10%, 10%, and 8% of patients, respectively.

In conclusion, the appropriate second-line therapy regimen and sequencing are not well established and rely on the patient's PS, liver function, and choice of first-line therapy. TKIs like sorafenib, lenvatinib, or cabozantinib are suggested as second-line treatments for patients who have previously received atezolizumab plus bevacizumab or durvalumab plus tremelimumab. Given the possibility for a greater ORR than single medications, combination immunotherapy with nivolumab-ipilimumab is preferred for patients who have become worse on TKIs such sorafenib or lenvatinib. Pembrolizumab is an alternative if the patients are unable to take double ICIs. Regorafenib or cabozantinib may be considered as second-line options if sorafenib or lenvatinib have been chosen as the first-line therapy and the patients have a contraindication to ICIs. Patients with an AFP level > 400 ng/mL are advised to use ramucirumab[74] (Figure 2).

FUTURE TRENDS IN SYSTEMIC COMBINATION THERAPY

Since the rapid FDA approval of atezolizumab and bevacizumab, combining checkpoint inhibitors with multikinase inhibitors-especially anti-angiogenesis therapy-has gained widespread acceptance. Numerous studies have shown that the synergistic interaction of ICIs and TKIs promotes vascular remodeling and tumor immune activation[75-77]. In a phase Ib study, lenvatinib and pembrolizumab were investigated. The median PFS, OS, and ORR for this combination were 9.7 mo, 20.4 mo, and 46%, respectively, showing substantial anti-tumor effectiveness. Most of the AEs might be controlled by changing the dosage[78]. The combination of camrelizumab, an anti-PD-1 antibody, and apatinib, an orally active VEGFR-2 inhibitor, was also investigated in a dose-expansion and escalation phase I research. The suggested dosage of camrelizumab 200 mg every 2 wk with apatinib 250 mg daily showed therapeutic advantages with a 50% ORR. This regimen was thus investigated in the phase 2 RESCUE study. For the treatment of naïve uHCC patients or those who had previously failed or were intolerant to TKIs, apatinib 250 mg was administered orally every day combined with camrelizumab 200 mg IV (body weight > 50 kg) or 3 mg/kg (body weight 50 kg) every 2 wk. A total of 70 patients and 120 patients, who were mainly HBV-infected (88.3%) in the first-line and second-line settings, respectively, were included. The median time since the cutoff for the data was 29.1 mo. The 2-year OS was 43.3% and the median OS was 20.1 mo in the first-line setting. The median OS in the second-line condition was 21.8 mo, with a 2-year OS of 44.6%. A phase 3 study is underway to evaluate its effectiveness in the first-line situation compared to that of sorafenib[79].

To assess the effectiveness and tolerability of lenvatinib plus camrelizumab *vs* lenvatinib monotherapy as first-line therapy, Li *et al*[80] performed a multicenter, retrospective cohort investigation of 92 uHCC patients. In contrast, 44 patients received oral lenvatinib 12 mg or 8 mg daily and 48 patients received intravenous camrelizumab 200 mg every 3 wk. The ORR in the combination group was shown to be significantly higher than in the monotherapy group (RECIST 1.1: 37.5% *vs* 13.6%, P = 0.009). The median OS in the monotherapy group was 13.9 mo (95%CI: 13.3-18.3), but not in the combination group (P = 0.015). Lenvatinib with camrelizumab had a 1-year survival rate of 79.2%, compared to lenvatinib monotherapy's 56.8%. Lenvatinib with camrelizumab had a significantly longer median PFS than lenvatinib monotherapy (10.3 mo *vs* 7.5 mo, P = 0.009). Subgroup analysis revealed that combination therapy was associated with a longer OS in males, patients with a Child-Pugh score < 7, patients with three or more tumors, patients with AFP levels greater than 200 ng/mL, HBV-positive patients, patients with vascular invasion, and patients without hypertension. In the lenvatinib plus camrelizumab and lenvatinib monotherapy groups, the AEs that affected more than 20% of patients were HFS (22.9% *vs* 25.0%, P = 0.81), hypertension (33.3% *vs* 38.6%, P = 0.59), diarrhea (31.2% *vs* 31.8%, P = 0.95), and loss of appetite (41.7% *vs* 40.9%, P = 0.90). There were no statistically significant variations in the occurrences of any AEs between the two groups. They concluded that first-line lenvatinib with camrelizumab treatment may benefit patients with uHCC better than lenvatinib alone. There were no new safety signals, and the toxicity and tolerance profiles of the two treatment protocols appeared to be comparable. Further research is necessary before deciding if lenvatinib and camrelizumab treatment together can provide uHCC patients a unique therapeutic alternative.

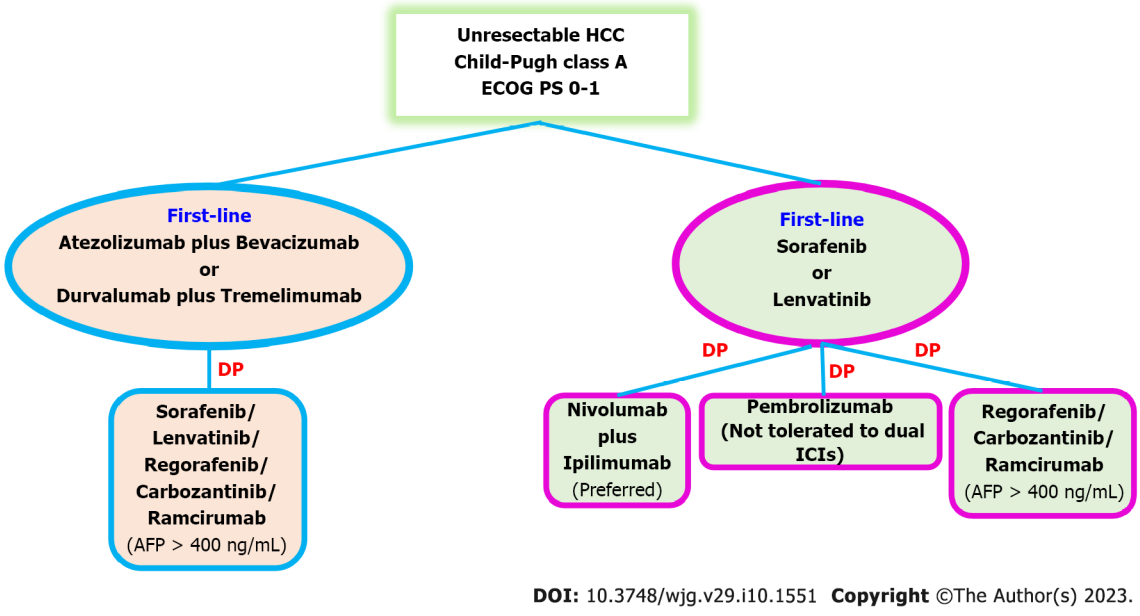

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Figure 2 Second-line systemic therapy in unresectable hepatocellular carcinoma. DP: Disease progress; ECOG PS: Eastern Cooperative Oncology Group Performance Status; ICIs: Immune checkpoint inhibitors; HCC: Hepatocellular carcinoma; AFP: Alpha-fetoprotein.

CONCLUSION

Systemic therapy for HCC has significantly advanced with a major breakthrough since the FDA approved sorafenib in 2007. Although several MTKIs have shown promise in the therapy of uHCC, the discovery of ICIs has completely changed the field, bringing about remarkable ORR and OS benefit. This is demonstrated by the innovative outcomes from the HIMALAYA trial, the IMbrave150 study, and the CHECKMATE-040 trial for the second-line combination of nivolumab-ipilimumab, atezolizumab-bevacizumab, durvalumab-tremelimumab, and atezolizumab-bevacizumab, respectively. Each of the aforementioned regimens has a somewhat distinct toxicity profile, and combination therapy is correlated with greater toxicities. In clinical practice, single-agent therapies are explored for patients who are less fit or have severe medical comorbidities, whereas combination treatments are studied for very fit patients, such as those with a status of performance 0 to 1 and no importance medical comorbidities. Additionally, an esophago-gastro-duodenoscopy must be performed before starting atezolizumab-bevacizumab therapy in order to address any esophageal varices due to the relatively high dose of bevacizumab used-15 mg/kg-which is associated with a higher bleeding risk.

Because of this, it is still unclear which patient groups may benefit from or tolerate a certain combination of therapies better than others. Additionally, there are very few clinical trials investigating the function of future therapy in individuals who respond to ICIs. This topic is being actively investigated by a number of current studies, including a phase II study looking at cabozantinib in the post-ICI situation and other studies comparing the combination of atezolizumab-lenvatinib to sorafenib in the post-atezolizumab-bevacizumab scenario. Recently, anti-PD-1 medication has been controversially used in patients with non-alcoholic steatohepatitis-related HCC (NASH-HCC)[81]. In addition, Pfister *et al* [82] examined anti-PD-1 therapy animal models with NASH in both therapeutic and preventative contexts. They demonstrated that anti-PD-1 therapy accelerated hepatocarcinogenesis and produced hepatic fibrosis in NASH-mice without tumors. Treatment with anti-PD-1 did not result in tumor regression in NASH-mice carrying HCC. Surprisingly, it has, on the contrary, sped up the tumor's growth. They conducted a meta-analysis of 1656 patients from 3 significant studies to determine whether similar outcomes were also observed in human HCC (CHECKMATE-459, IMBrave150, and KEYNOTE-240). In accordance with the underlying etiology of HCC, they also evaluated the survival results of HCC treated with immunotherapy. Individuals with viral HCC had a longer survival time with immunotherapy (HR: 0.64; 95%CI: 0.48-0.94), but those with non-viral HCC did not (HR: 0.92; 95%CI: 0.77-1.11). According to a research, non-viral HCC patients who received anti-PD-1 medication had the same ORR and PFS as patients with viral HCC. Alternative explanations have been developed in response to these seemingly incongruous findings, including the varied population with non-viral HCC and the dearth of knowledge surrounding follow-up therapies[83]. It's also essential to remember that these worse outcomes for immunotherapy-treated non-viral HCC patients were retrospectively determined. As a result, based on the etiology of HCC, this cannot result in a shift in clinical management for uHCC patients. To clarify these concerns, more clinical studies with predetermined stratification should be planned.

Unanswered questions about therapeutic drug resistance and predictive biomarkers still exist. Drug resistance is prevalent and is generally believed to be the leading cause of therapeutic failure. EGFR activation, the existence of cancer stem cells, tumor-initiating cells, and the epithelial-mesenchymal transition (EMT) are examples of potential pathways. Cancer stem cells are created when EMT signals are activated, and these cancer stem cells have the ability to self-renew and differentiate. In human liver cell lines, prolonged sorafenib exposure results in EMT, which is characterized by changes in cell shape, loss of E-cadherin, and elevated vimentin expression[84,85]. In a genetically engineered mouse model of HCC, it was discovered that β-catenin activation encourages immune evasion and resistance to PD-1 inhibitors[86]. Although additional study is required to translate this bench-to-bedside data, these biomarkers have the potential to affect treatment choices. Our knowledge of tumor biology is still lacking, and there is an unmet need for cutting-edge drugs to deal with these resistance mechanisms. To accurately predict prognosis and the therapeutic response to target treatment or immunotherapy, there are yet no meaningful molecular indications. In actuality, imaging methods are used to identify the majority of HCCs, and the available treatments are much the same. It makes sense to look at biomarkers that might inform treatment choices in the era of personalized medicine. The most extensively researched immunotherapy biomarkers, including as tumor mutational burden, microsatellite instability, and PD-L1 expression, are presently employed very seldom in uHCC[87]. Although it has been shown that circulating markers including AFP, tumor necrosis factor-alpha, and IL-6 associated with HCC treatment outcomes, further studies are required to confirm these findings. A gene expression test has also recently been investigated as a possible biomarker for predicting immunotherapy response[88].

Ultimately, more research is necessary to determine any potential indirect drug interactions between antibiotics, proton pump inhibitors, ICIs, or multiple therapies. It is known that administering immunotherapy and antibiotics at the same time has a detrimental impact on the effectiveness of anti-cancer treatment. Numerous studies have shown the close relationship between ICIs and gut flora. Intestinal homeostasis and the reduction of systemic inflammation are crucially dependent on the microbiota. Antibiotics have a deleterious effect by causing dysbiosis, which changes the immune system's systemic anti-tumor response during, or in the 1st few weeks before commencing ICIs[89].

Since sorafenib was the only first-line therapy option for uHCC for 15 years, three alternative first-line therapeutic options have emerged. This represents a significant improvement in the management of uHCC (atezolizumab-bevacizumab, sorafenib, and lenvatinib). Cabozantinib, regorafenib, and ramucirumab are now included in the second-line therapy alternatives (AFP > 400 ng/mL). Nivolumab and pembrolizumab, ICIs, have been suggested as second-line treatments for individuals who are intolerant to MTKIs.

FOOTNOTES

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Country/Territory of origin: Thailand

ORCID number: Wattana Leowattana 0000-0003-4257-2480; Tawithep Leowattana 0000-0003-2316-3585.

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MINIREVIEWS

Systemic treatment for metastatic colorectal cancer

Wattana Leowattana, Pathomthep Leowattana, Tawithep Leowattana

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Wattana Leowattana, Pathomthep Leowattana, Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

Tawithep Leowattana, Department of Medicine, Faculty of Medicine, Srinakharinwirot University, Bangkok 10110, Thailand

Corresponding author: Wattana Leowattana, BMed, MD, MSc, PhD, Full Professor, Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajavithi Road, Bangkok 10400, Thailand. wattana.leo@mahidol.ac.th

Abstract

Significant progress has been achieved in the treatment of metastatic colorectal cancer (mCRC) patients during the last 20 years. There are currently numerous treatments available for the first-line treatment of mCRC. Sophisticated molecular technologies have been developed to reveal novel prognostic and predictive biomarkers for CRC. The development of next-generation sequencing and whole-exome sequencing, which are strong new tools for the discovery of predictive molecular biomarkers to facilitate the delivery of customized treatment, has resulted in tremendous breakthroughs in DNA sequencing technology in recent years. The appropriate adjuvant treatments for mCRC patients are determined by the tumor stage, presence of high-risk pathologic characteristics, microsatellite instability status, patient age, and performance status. Chemotherapy, targeted therapy, and immunotherapy are the main systemic treatments for patients with mCRC. Despite the fact that these novel treatment choices have increased overall survival for mCRC, survival remains optimal for individuals with non-metastatic disease. The molecular technologies currently being used to support our ability to practice personalized medicine; the practical aspects of applying molecular biomarkers to regular clinical practice; and the evolution of chemotherapy, targeted therapy, and immunotherapy strategies for the treatment of mCRC in the front-line setting are all reviewed here.

Key Words: Systemic treatment; Metastatic colorectal cancer; Personalized medicine; Biomarkers; Chemotherapy; Targeted therapy; Immunotherapy

Core Tip: Advances in the molecular profiling of metastatic colorectal cancer allow treatment to be tailored to the biologic characteristics of the tumor for certain patient subgroups. Although cures are still rare, more people can expect to live longer. Genomic profiling enables therapy selection, allowing more individuals to benefit while exposing fewer to the harm of ineffective medicines. An important component in determining treatment results is the choice of an effective first-line therapy, which should consider both clinical considerations and molecular indicators. The systemic treatments used in the first-line regimen determine the second-line regimen. Third-line therapy, which includes epithelial growth factor receptor inhibitors for patients with rat sarcoma virus wild-type, should consider molecular profiling. Patients with high microsatellite instability illnesses may be candidates for immunotherapy with pembrolizumab or nivolumab plus ipilimumab.

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INTRODUCTION

The third most commonly occurring cancer in humans is colorectal cancer (CRC). More than 2 million individuals are diagnosed with this cancer each year worldwide. This year, about one million individuals will die from CRC. The liver is the most common target of CRC hematogenous metastasis, as well as the site most responsible for death from this common malignancy. When patients are diagnosed in the late stage of disease, their prognosis remains poor[1,2]. The majority of individuals who have a CRC diagnosis are over 50, whereas only 12% of all new CRC diagnoses are found in those under 50. Overall, the lifetime risk of developing CRC is about 1 in 23 (4.3%) for men and 1 in 25 (4.0%) for women. According to statistics from the Surveillance, Epidemiology, and End Results Program, at the time of diagnosis, 38% of patients have localized disease, 35% have regional disease, 21% have distant disease, and 6% have no stage[3]. CRC diagnoses have decreased overall since 2000 as a result of increased screening efforts, although it has increased in young people under 50 since the 1990s. Preventive measures, such as routine colonoscopies, remain the most effective way to combat CRC. With a rising interest in non-invasive biomarkers, many additional approaches have been developed. However, nearly 50% of patients are still diagnosed at an advanced stage. Metastatic CRC (mCRC) has a poor prognosis with a 5-year survival rate of 14%, and this number has remained constant over the past 5 years. Until the late twentieth century, mCRC was thought to always be fatal. The discovery that metastatic cancer is mostly localized to the liver in postmortem investigations and radiologic examinations utilizing computer-assisted tomography scanners led early pioneers to resect liver metastasis. To establish liver resections for metastatic carcinoma as an acceptable treatment, pioneers conducted and published data[4]. Only one standard regimen to manage CRC has been shown to be inefficient, resulting in high rates of treatment failure and disease resistance. Recently, the clinical outcomes of patients with mCRC have improved dramatically as a result of the discovery of prognostic and predictive molecular biomarkers and the subsequent individualization of treatment options. High genetic heterogeneity, including but not limited to chromosomal instability (CIN), microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP), has been identified by molecular profiling of CRC. Different CRC subtypes have varying prognoses and therapeutic outcomes. Promising improvements for the use of systemic therapy and precision medicine in mCRC have resulted from recent advancements in our understanding of the molecular signaling networks that control intestinal regeneration and homeostasis[5].

The discovery of the key molecular drivers in CRC pathogenesis, coupled with the ability to screen tissue for measurable mutations crucial to disease progression, led to the development of an innovative treatment model for patients with advanced CRC. Despite substantial breakthroughs in tumor biology understanding, these have not entirely translated into proven novel therapies for all patients, since the chemotherapeutic strategy is still built around combination cytotoxic regimens targeted at proliferative epithelium. Although there have been some achievements with targeted therapy, such as the synergistic effect of protein kinase B-Raf (BRAF) inhibitors and epithelial growth factor receptor (EGFR) inhibitors in *BRAF*-mutant CRC, certain medications have not been able to offer clinically meaningful improvements for other pathways. As evidenced by the effect of immune checkpoint inhibition in microsatellite unstable CRC, therapeutic exploitation of intercompartmental signaling in the malignant epithelium may represent an important new drug paradigm in CRC. This is because we are becoming more aware of the signaling crosstalk that controls intestinal cell fate in health and disease, as well as the function of the tumor microenvironment (TME)[6,7].

We have split these important signaling pathways into those that control the destiny of cancer cells or intestinal epithelium directly and those that function indirectly by leveraging the TME in this review. Additionally, we explore how signaling affects each cancer cell's destiny and comment on some possible treatment prospects that result from effective pathway modulation for each.

RISK FACTORS FOR CRC

Behaviors, diets, and lifestyle

Only a small proportion of CRCs are associated with germline mutations or discovered in the presence of a strong family history; the majority of CRCs are random[8,9]. The significance of environmental exposure has been further demonstrated by the variation in CRC risks throughout the world and the discovery that younger generations are at a higher risk of CRC in westernized nations. Numerous studies have been conducted in an effort to identify and quantify the environmental and dietary risk factors for CRC. Global studies have shown a 45-fold variation in the age-standardized incidence of CRC worldwide[10]. Gambia and other non-industrialized nations have the lowest rates of CRC, whereas westernized nations have the highest rates. The prevalence of CRC has been seen to rise over time when a nation industrializes and starts to follow a westernized lifestyle and diets low in fiber[11,12]. A westernized diet, or one that is low in fiber, fruits, and vegetables and heavy in processed meats, sugary drinks, and refined grains, is linked to greater risks of CRC. It has been challenging to pinpoint everything that increases the risk of CRC in a westernized diet. This diet's many components are probably a factor in the greater prevalence of CRC. Studies have repeatedly shown that diets rich in processed foods and red meat are linked to higher risks of CRC. For every 100 g of red and processed meat consumed, CRC incidence increases by 12%, according to a recent meta-analysis of 111 studies involving 400 individuals[13] (Table 1). Smoking increases the likelihood of both serrated polyps and colorectal adenomas[14]. According to large observational studies, more pack-years result in higher CRC rates[15]. Recent research suggests that smoking is marginally related to MSI-high (MSI-H) tumors, increases the rates of rectal and proximal CRC, and is correlated with *BRAF*-mutant malignancies. Similar to smoking, drinking alcohol is a recognized risk factor for CRC, and recent pooled studies have demonstrated that even occasional drinking increases the risk of CRC[16].

Numerous studies have shown that an increased risk of CRC is related to obesity and decreased physical activity. CRC has repeatedly proven to be correlated with excess body fat, which is most typically quantified using body mass index and waist circumference. Sedentary activity, such as extended sitting or TV viewing, is linked to a higher risk of CRC. In populations over 50, the majority of research verifying obesity and a lack of physical exercise as risk factors for CRC has been demonstrated. Attempts to quantify the impact of obesity and physical activity on rates of early-onset CRC have rekindled attention in light of the rising burden of young-onset CRC in developed nations[17,18]. These and other investigations support the advice from the American Cancer Society and other cancer organizations that maintaining a healthy weight and engaging in more physical exercise are crucial for lowering the risk of CRC.

Genetic factors

A family history of cancer without a specific condition is thought to be the cause of 25% of CRCs, while hereditary cancer syndromes are thought to be the cause of 5% of CRCs. The hereditary component of CRC is predicted to be 35%-40%. Hereditary non-polyposis CRC (Lynch syndrome), familial adenomatous polyposis (FAP), and MUTYH-associated polyposis (MAP) are the most prevalent hereditary cancer syndromes. About 2%-4% of all CRCs are caused by Lynch syndrome, the most prevalent hereditary CRC condition. Patients with Lynch syndrome are susceptible to endometrial, ovarian, stomach, small intestine, hepatobiliary tract, pancreatic, ureter, and renal pelvis malignancies. Lynch syndrome is caused by mutations in the DNA mismatch repair genes *hMLH1*, *hMSH2*, *hMSH6*, and *hPMS2*[19,20]. The lifetime risk of CRC for these people is between 5% and 85%. FAP makes up roughly 1% of CRC cases and is the second most prevalent hereditary CRC syndrome. A germ line mutation in the adenomatous polyposis coli (*APC*) gene that causes a shortened APC protein is the secondary cause of FAP. With a 90% inheritance, it is inherited in an autosomal dominant manner. For these people, thousands of polyps form in the gastrointestinal system, the majority of which are in the colon. By the third or fourth decade, CRC will manifest in all FAP-affected individuals without preventative colectomy[21]. The lifetime risk of CRC for these MAP individuals is expected to be 43%-100%. Serrated polyposis syndrome, Peutz-Jeghers syndrome, and juvenile polyposis syndrome are further polyposis syndromes associated with a higher risk of CRC. These are all uncommon disorders that together only contribute to 1% of CRC incidence. Race, age, and sex are other unmodifiable risk factors for CRC in addition to family history. Male patients are generally at a higher risk of developing CRC than female patients; ideas explaining this include the fact that women typically have less visceral fat and benefit from estrogen's general preventive properties against CRC. Men are also less likely to pursue screening, and they may be more exposed to environmental risks, including drinking, smoking, and unhealthy diets[22].

Table 1 Modifiable and non-modifiable risk factors and preventive factors for colorectal cancer

	Modifiable	Non-modifiable
Risk factors	Excess body fat	Family history of CRC
	Sedentary life-style	Advanced polyps
	Westernized diet	Polyposis syndrome (FAP, MAP)
	Processed meats	Lynch syndrome (HNPCC)
	Red meats	Black people
		Older age
		Male
Preventive factors	High fiber diet	Female
	Whole grain diet	
	No alcohol	
	No smoking	

CRC: Colorectal cancer; FAP: Familial adenomatous polyposis; HNPCC: Hereditary non-polyposis colorectal cancer; MAP: MUTYH-associated polyposis.

Polyps

Most CRCs develop from a harmless precursor polyp. Therefore, people who have a considerable personal or family history of high-risk polyps are at a higher risk of developing CRC. There are many different forms of polyps, some of which are non-neoplastic, including hyperplastic polyps, mucosal polyps, inflammatory polyps, and harmartomatous polyps. The remaining polyps, including adenomatous and serrated polyps, have cancerous potential. Adenomatous polyps are where the vast majority of sporadic CRCs originate. By the time they are 50, roughly one-third of individuals are predicted to develop polyps. However, the majority of these will not progress to CRC. Villous histology, high-grade dysplasia, and polyps larger than 1 cm all enhance the likelihood that they may develop into CRC. Serrated polyps are believed to be antecedents for up to 10%-15% of sporadic CRCs, albeit less frequent[23,24].

COLORECTAL CARCINOGENESIS

Three molecular pathways have been hypothesized for colorectal carcinogensis, two of which center on the growth of polyps into cancerous tumors. The traditional pathway depicts the long-term evolution of normal cells to adenomas and finally to carcinomas (the adenocarcinoma sequence). This causes 85%-90% of all sporadic CRCs and is mostly related to the growth of tumors that are CIN. It is frequently accompanied by an early *APC* gene mutation, activation of the growth-promoting oncogenes Kristin rat sarcoma virus (*KRAS*) or *BRAF*, and additional mutations that cause cancer to proceed. About 10%-15% of sporadic CRCs are caused by the CIMP/serrated pathway[25,26]. Due to these changes in the methylation of gene promoter regions and general hypomethylation, many genes are silenced. Early BRAFV600E mutations are frequently seen in these tumors, which activate the mitogen-activated protein kinase (MAPK) pathway and cause the growth of hyperplastic polyps[27]. The MSI-H pathway is the third route. More than 95% of the malignancies related to Lynch syndrome are caused by this important pathway. Outside of Lynch syndrome, MSI is a rare condition that is caused by a lack of DNA mismatch repair genes, which eventually results in altered DNA sequences (Figure 1).

KRAS

A proto-oncogene called *KRAS*, which produces a GTPase protein, is essential for intracellular signal transduction downstream of membrane-bound receptors like EGFR. Uncontrolled cell growth, proliferation, survival, migration, and invasion ensue from a mutation in *KRAS* because it causes constitutive activation of the MAPK pathway, regardless of independent activation of the upstream EGFR receptor. Activating *KRAS* mutations have been reported in a variety of cancers and have been found between 30% and 50% of CRC patients[28,29]. *KRAS* is a crucial biomarker for prognosis and prediction in the management of mCRC. *KRAS*-mutated tumors in mCRC have a poorer prognosis and are more likely to exhibit aggressive biology, spread metastatically, recur, and result in mortality. *KRAS* mutation is independently linked to poorer progression-free survival (PFS) and overall survival (OS) in individuals who underwent hepatic resection for mCRC, according to data from those patients. *KRAS* codon 12 has been linked to lower recurrence-free survival in variations of *KRAS* mutations throughout all stages of

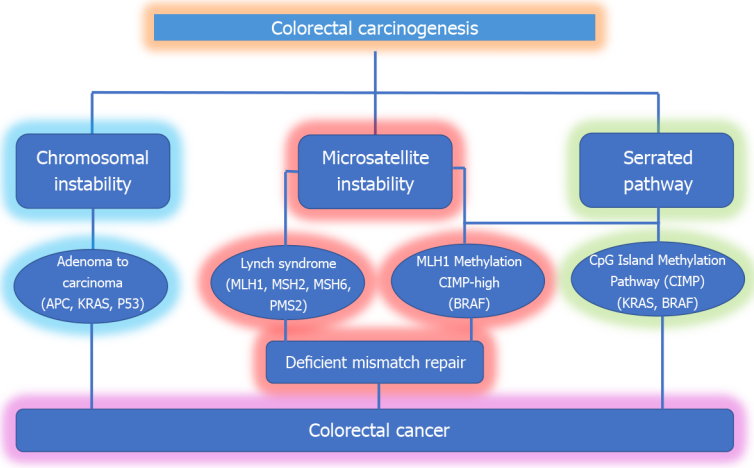

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Figure 1 Three molecular pathways can lead to colorectal cancer. For colorectal carcinogenesis, three molecular pathways have been proposed: the chromosomal instable or classic pathway, the microsatellite instability pathway, and the serrated pathway. APC: Adenomatous polyposis coli; BRAF: Protein kinase B-Raf; CIMP: CpG island methylator phenotype; KRAS: Kristin rat sarcoma virus.

mCRC[30]. Data suggest that curative resection may not be advantageous in the metastatic scenario in *KRAS* mutant patients who also have other poor clinical prognostic characteristics, such as node-positive disease or extensive metastases, due to the prognostic consequences of *KRAS* mutations[31]. Additionally, the existence of a *KRAS* mutation acts as a biomarker for the therapeutic efficacy of some therapies, such as EGFR inhibitor therapy. Anti-EGFR treatment has been shown to be effective in treating KRAS wild-type (WT) cancers in several clinical studies. Due to the independent constitutive activation of KRAS downstream of EGFR, which persistently encourages cell growth and division, anti-EGFR treatment is not helpful in patients with *KRAS* mutations[32]. Although EGFR inhibition is effective in treating the majority of KRAS WT tumors, some patients continue to have resistance, necessitating more research. Mutations in other RAS family oncogenes, such as neuroblastoma RAS (NRAS) and Harvey RAS, identify tumors that are resistant to anti-EGFR treatment. Other phosphorylation pathway genes that work downstream of EGFR, such as phosphoinositide 3-kinases, phosphatase and tensin homolog, MAPK, and mitogen-activated protein kinase kinase (MEK), have not been demonstrated to be accurate predictors of the EGFR response for mCRC, and research into the causes of anti-EGFR resistance in this patient group is still underway[33].

BRAF

BRAF, a serine/threonine-protein kinase, is an essential component of the MAPK signaling cascade downstream of KRAS. In less than 10% of CRC patients, *BRAF* mutations have been found. The same MAPK pathway that KRAS uses for BRAF signaling also uses it, and functional mutations in either of these genes have identical effects on phenotypic and treatment implications. As a predictive biomarker for mCRC, the *BRAF* mutation is linked to worse outcomes, shorter survival, and a greater incidence of peritoneal and distant lymph node involvement. In patients with mCRC undergoing curative-purpose hepatectomy, the *BRAF* mutation is associated with poorer survival compared to both *BRAF* WT and *KRAS* mutated tumors[34,35]. BRAF also functions as a prognostic marker. Vemurafenib, a direct BRAF inhibitor, was first discovered through early attempts at targeted medication treatment for melanoma. Studies have been conducted to determine if BRAF inhibition has comparable effects on CRC. BRAF inhibition and EGFR inhibitors together produced an OS improvement. In patients with *BRAF* mutant mCRC, encorafenib in conjunction with EGFR inhibition is a potent form of newer generation BRAF inhibition therapy[36].

Human epidermal growth factor 2

A further promising target for mCRC is human epidermal growth factor 2 (HER2), which is essential for intracellular signal transduction. HER2 plays a role in the development of breast cancer, and trastuzumab and other HER2 inhibitors can be used to specifically treat the disease. Because there are so many targeted treatments available, many researchers have concentrated on HER2 mutations in the mCRC population, despite the fact that only a small percentage of patients (10%) overexpress HER2. Trastuzumab with pertuzumab, trastuzumab with lapatinib, and fam-trastuzumab deruxtecannxki are currently available as HER2 inhibitors. Even though it only accounts for a small proportion of all mCRC patients, the HER2 amplified condition serves as a predictive biomarker for HER2 targeted therapy with the potential for a therapeutic response[37-39].

SYSTEMIC TREATMENT FOR MCRC

A surgeon may be able to completely remove a few metastatic foci of mCRC, which are often located in the liver or lung. When the main tumor and all metastases can be completely removed surgically, mCRC is said to be resectable. However, nodal infiltration and covert micrometastatic spread are frequent in these individuals. Less than 20% of individuals with mCRC who undergo resection are permanently cured. Oncologists from surgical and medical branches should work together to develop treatment strategies when mCRC may be resectable. If the main tumor is in the rectum, radiation oncologists should be consulted. The main therapy for mCRC is systemic chemotherapy. The United States Food and Drug Administration (FDA) has authorized many medications for the treatment of mCRC. However, for the majority of patients, mCRC is still incurable. Despite the rarity of a cure for mCRC, recent major clinical trials with patients who could tolerate chemotherapy have demonstrated that patients can live for 2 to 3 years with intense treatment and numerous systemic medicines. Survival is influenced by the molecular subtype, which provides information about the prognosis by describing the natural history of a tumor and the therapies that are and are not likely to be successful. The median OS for the 50% of patients with KRAS/NRAS/BRAF WT mCRC is about 30 mo with survival rates of 80% at 1 year, 40% at 3 years, and 20% at 5 years after the start of first-line chemotherapy (Table 2).

SYSTEMIC TREATMENT FOR LIVER METASTASIS CRC

Nearly half of individuals with initial CRC develop liver metastatic diseases from CRC or colorectal liver metastasis (CLM). The resectability of CLM determines how to manage it, and interdisciplinary approaches are frequently used. Conversion treatment (CT), a kind of systemic treatment, is used for liver metastases that are initially incurable. Both the number (4 *vs* > 4) and size (diameter < 6 cm *vs* ≥ 6 cm) of CLMs are independent variables linked to successful CT[40,41].

Targeted treatment

FOLFOX (5-fluorouracil [5-FU], leucovorin, and oxaliplatin) and FOLFIRI (5-FU, leucovorin, and irinotecan) are the mainstays of systemic chemotherapy used to treat mCRC. EGFR inhibitors (EGFRis) for RAS WT tumors (cetuximab [Cet] and panitumumab [Pan]) and anti-vascular endothelial growth factor (VEGF) (bevacizumab [Bev]) are the two main groups of medications now added to these chemotherapy regimens.

Resectable CLM with no extrahepatic metastasis

There is debate regarding the benefits of adding a targeted treatment, however, the addition of Bev or Cet to FOLFOX or FOLFIRI is tolerable in resectable liver metastases. However, a single-arm phase 2 study found that the addition of Bev to the capecitabine and oxaliplatin combination (CAPOX) (six cycles with no Bev on the final cycle), before surgery in high-risk CRC, had a remarkable objective response rate (ORR) of 73%[42]. In 2020, Bridgewater *et al*[43] conducted a multicenter, open-label, randomized, controlled, phase 3 trial to investigate the effects of Cet plus chemotherapy compared with those given chemotherapy alone in 257 adult patients (aged ≥ 18 years) with KRAS WT (codons 12, 13, and 61) resectable or suboptimally resectable. At a median follow-up of 66 mo after the last patient was recruited, this analysis was conducted. In the chemotherapy alone group, the median PFS was 22.2 mo, whereas in the chemotherapy plus Cet group, it was 15.5 mo (P = 0.304). In the chemotherapy alone group, the median OS was 81.0 mo, but in the chemotherapy plus Cet group, it was 55.4 mo (P = 0.036). The status of pathological resection or the preoperative response were secondary outcomes that did not significantly differ between groups. High-risk CRC in this study included those with synchronous liver metastases, metastatic disease discovered within a year of initial resection, primary tumors with positive lymph nodes, CLMs > 1 or > 5 cm, and positive carcinoembryonic antigen (CEA) levels. They concluded that Cet had no effect when used with perioperative treatment. As a result, targeted therapy is deemed ineffective in CRC patients with resectable CLM, whereas Bev may be beneficial in high-risk patients. FOLFOX adjuvant treatment is advised following resection.

Unresectable CLM with potential for resection and no extrahepatic metastasis

In high-risk CRC (> 4 metastases, diameter > 5 cm, poor viable liver function if undertaking upfront resection, or inability to maintain liver vascular supply), neoadjuvant treatment (NAT) with CAPOX plus Bev achieved a 78% ORR[44]. Of the 46 patients included, 40% of the individuals with unresectable disease upon diagnosis got a resection. Four patients responded so well that they were kept under surveillance without having surgery. In comparison to FOLFOX, Bev with FOLFOXIRI (combination of 5-FU/leucovorin (LV), oxaliplatin, and irinotecan) had a greater resection rate (61% *vs* 49%), R0 resection rate (49% *vs* 29%), ORR (81% *vs* 62%), and mean PFS (18.6 m *vs* 11.5 m)[45]. After a hepatic artery infusion chemotherapy pump has been installed, adding Bev to the chemotherapy has no survival benefit. On the other hand, it worsens liver toxicity (hyperbilirubinemia > 3 mg/dL) and is not advised[46]. In the POCHER study, 60% (25/43) of the 43% of patients with unresectable CLM who

Table 2 Chemotherapy and targeted therapies for metastatic colorectal cancer patients

Ref.	Country	Drug(s)	Number of patients	Study phase	ORR, %	Mean OS in mo	Mean PFS in mo	Results
Resectable CLM with no extra-hepatic metastasis								
Gruenberger *et al*[42]	Austria	Capecitabine, oxaliplatin plus bevacizumab	56	2	73.2	-	-	Bevacizumab can be safely administered until 5 wk before liver resection in patients with metastatic CRC without increasing the rate of surgical or wound healing complications or the severity of bleeding
Bridgewater *et al*[43]	United Kingdom	Oxaliplatin, L-folinic acid, fluorouracil or capecitabine, oxaliplatin plus cetuximab	257	3	-	81.0/55.4 (Chemo/Chemo+)	22.2/15.5 (Chemo/Chemo+)	In the perioperative setting, patients with operable diseases are at a disadvantage in terms of OS; hence, cetuximab should not be used in this setting
Unresectable CLM with potential for resection and no extra-hepatic metastasis								
Wong *et al* [44]	United Kingdom	Capecitabine, oxaliplatin plus bevacizumab	46	-	78.0	-	-	A high response rate for patients with CLMs with poor-risk features not selected for upfront resection and converted 40% of patients to resectability
Gruenberger *et al*[45]	United Kingdom, Austria, France, and Spain	Bevacizumab plus FOLFOX-6 (5-fluorouracil folinic acid oxaliplatin) or FOLFOXIRI (5-fluorouracil folinic acid oxaliplatin irinotecan)	80	2	81/62 (BF/BF6)	-	18.6/11.5 (BF/BF6)	In patients with CLM that were originally unresectable, bevacizumab-FOLFOXIRI was correlated with better response and resection rates as well as a longer PFS than bevacizumab-mFOLFOX-6
Garufi *et al* [47]	Italy	Cetuximab plus chrono-IFLO (chrono-irinotecan 5-fluorouracil leucovorin oxaliplatin)	43	2	-	37	-	Cetuximab in combination with chrono-IFLO resulted in 60% full resectability of CLM patients
Folprecht *et al*[48]	Germany	Cetuximab plus FOLFOX (5-fluorouracil folinic acid oxaliplatin) or FOLFOXIRI (5-fluorouracil folinic acid oxaliplatin irinotecan)	56/55 (CFX/CFI)	-	-	35.7/29.0 (CFX/CFI)	10.8/10.5 (CFX/CFI)	Both FOLFOX/FOLFIRI with cetuximab appear to be effective conversion therapy regimens in patients with KRAS codon 12/13/61 wild-type tumors. Thus, liver surgery can be deemed curative or as a second line of therapy in people who are not cured
Unresectable CLM								
Rivera *et al* [50]	Spain, Germany, United States, Belgium, Switzerland, and Italy	mFOLFOX6 plus panitumumab or bevacizumab	170/156 (RAS WT/RAS WT/BRAF WT)	-	RAS WT 65/60, RAS WT/BRAF WT 65/62 (FXP/FXB)	RAS WT 36.9/28.9, RAS WT/BRAF WT 46.3/28.9 (FXP/FXB)	RAS WT 12.8/10.1, RAS WT/BRAF WT 13.1/10.1 (FXP/FXB)	Panitumumab in combination with mFOLFOX6 is a successful first-line therapy for individuals with RAS WT and RAS

								WT/BRAF WT mCRC
Stintzing *et al*[53]	Germany	FOLFIRI (5-fluorouracil leucovorin irinotecan) plus cetuximab or bevacizumab	400	3	65.3/58.7 (FIC/FIB)	33.1/25.0 (FIC/FIB)	10.3/10.2 (FIC/FIB)	In the first-line therapy of patients with RAS wild-type metastatic colorectal cancer, FOLFIRI with cetuximab may be preferable than FOLFIRI plus bevacizumab
Pfeiffer *et al* [58]	Denmark	Trifluridine plus tipiracil hydrochloride (TAS-102) or TAS-102 plus bevacizumab	47/46 (T/TB)	2	51/67 (T/TB)	6.7/9.4 (T/TB)	2.6/4.6 (T/TB)	In terms of efficacy and safety, bevacizumab may be an effective companion for TAS-102 in patients with chemorefractory metastatic colorectal cancer
Cremolini *et al*[59]	Italy	Cetuximab plus irinotecan	13/12 (RWT/RMT)	2	-	12.5/5.2 (RWT/RMT)	4.0/1.9 (RWT/RMT)	In patients with RAS and BRAF wild-type mCRC who have acquired resistance to first-line irinotecan and cetuximab-based treatment, a re-challenge approach with cetuximab and irinotecan may be effective. The examination of RAS mutational status on cDNA may aid in the selection of potential patients
Sartore-Bianchi *et al* [60]	Italy	Panitumumab (re-challenge)	25	-	30	13.7	4	Interventional liquid biopsies can be used efficiently and safely to guide anti-EGFR re-challenge treatment with panitumumab in patients with mCRC
Kopetz *et al* [63]	United States	Irinotecan cetuximab or irinotecan cetuximab plus vemurafenib	50/50 (IC/ICV)	-	-	12/12 (IC/ICV)	2.0/4.2 (IC/ICV)	Vemurafenib in combination with cetuximab and irinotecan is an active combination that increases PFS. This is a well-planned research based on a solid grasp of the mechanisms of adaptive resistance in mCRC
Tabernero *et al*[64]	United States, France, Italy, Spain, United Kingdom, Germany, Australia, Hong Kong, Norway, Switzerland, Japan, South Korea, and the Netherlands	Encorafenib cetuximab binimetinib or encorafenib cetuximab or FOLFIRI cetuximab	224/220/221 (3D/2D/CD)	3	26.8/19.5/1.8 (3D/2D/CD)	9.3/9.3/5.9 (3D/2D/CD)	-	When compared to conventional chemotherapy, encorafenib plus cetuximab improved OS, ORR, and progression-free survival in previously treated patients with metastatic disease. Encorafenib with cetuximab is a new standard-of-care regimen for previously treated patients with BRAF V600E mCRC, according to main and revised studies

Siena *et al* [68]	United States, Italy, United Kingdom, Spain, and Japan	Trastuzumab deruxtecan (DS-8201)	53/7/18 (A/B/C)	2	45.3 (A)	5.4 (A)	6.9 (A)	Trastuzumab deruxtecan shown promising and sustained effect in HER2-positive mCRC that was resistant to conventional therapy, as well as a favorable safety profile
Mayer *et al* [71]	Belgium, Italy, Japan, Spain, Australia, and United States	Trifluridine plus tipiracil hydrochloride (TAS-102) or placebo	800 (2:1)	-	1.6/0.4 (TAS/placebo)	7.1/5.3 (TAS/placebo)	2.0/1.7 (TAS/placebo)	A significant number of Japanese and Western patients with mCRC who had had a lot of prior treatment, including those whose condition was resistant to fluorouracil, were shown to respond clinically to TAS-102

2D: Encorafenib plus cetuximab; 3D: Encorafenib plus cetuximab plus binimetinib; A: Cohort A; B: Cohort B; BF: Bevacizumab plus FOLFOXIRI; BF6: Bevacizumab plus FOLFOX-6; BRAF: Protein kinase B-Raf; C: Cohort C; CD: FOLFIRI plus cetuximab; CFI: Cetuximab plus FOLFOXIRI; CFX: Cetuximab plus FOLFOX; CRC: Colorectal cancer; EGFR: Epidermal growth factor receptor; FIB: FOLFIRI plus bevacizumab; FIC: FOLFIRI plus cetuximab; FOLFIRI: Folinic acid, fluorouracil, and irinotecan hydrochloride; FXB: mFOLFOX6 plus bevacizumab; FXP: mFOLFOX6 plus panitumumab; HER2: Human epidermal growth factor receptor 2; IC: Irinotecan plus cetuximab; ICV: Irinotecan plus cetuximab plus vemurafenib; mCRC: Metastatic colorectal cancer; ORR: Objective response rate; OS: Overall survival; PFS: Progression-free survival; RAS: Rat sarcoma virus; RMT: Rat sarcoma virus mutate-type; RWT: Rat sarcoma virus wild-type; T: Trifluridine plus tipiracil hydrochloride; TB: Trifluridine plus tipiracil hydrochloride plus bevacizumab; WT: Wild-type.

received chronomodulated irinotecan (Iri) and 5-FU/LV on days 2-6 every 2 wk as NAT experienced full resections[47]. Twenty-nine participants with more than four lesions and nine patients with more than 5 cm in diameter made up the study population. The 2-year survival rate was 68% for the whole population. In people who have had surgery, it may reach 80%. As a result, research was conducted to evaluate the role of EGFRi in NAT or POT. When Cet was given to FOLFOX or FOLFIRI in patients with unresectable CLM at diagnosis who were eligible (with KRAS WT codons 12, 13, and 61), it improved long-term survival[48].

Unresectable CLM

First-line plus chemotherapy: Bev is advised as first-line treatment for RAS-mutated tumors in mCRC with CLM and without surgical consideration. A meta-analysis of two randomized control trials and three observational studies revealed that Cet had a higher OS, ORR, and complete response rate than Bev in RAS-WT malignancies (with chemotherapy as the backbone). The same trial found no appreciable differences between the two medications in PFS, disease control rate, or partial response rate[49]. In the PEAK study, Pan outperformed Bev in terms of PFS while maintaining the same OS[50]. In the past 5 years, a lot of research has been done on the function of the main tumor side (right *vs* left). Regardless of the CLM status, two meta-analyses have conclusively demonstrated that left-sided cancers react better to EGFRi than Bev[51,52]. However, there was no statistically significant difference in OS or PFS between Bev and EGFRi in tumors on the right side. In the PEAK study and the FIRE-3 trial, EGFRi with chemotherapy produced deeper responses in RAS-WT tumors than Bev plus chemotherapy. Tolerability is a crucial consideration when choosing a medication. Patients with a history of thromboembolic illness, uncontrolled hypertension, proteinuria, significant bleeding risk, or gastrointestinal perforation are not advised to get anti-VEGF medication. As a result, EGFRi (Cet or Pan) is preferable over Bev plus chemotherapy in left-sided tumors in RAS-WT mCRC with CLM, although either of them can be administered in right-sided tumors[53,54]. There is not any conclusive proof that Pan or Cet is superior to the other. Patients who experience adverse reactions to Cet, a mouse-based monoclonal antibody (mab), frequently prefer Pan since it is a humanized mab.

Second-line plus chemotherapy: The continuation of Bev in the second line after first-line treatment improved PFS and OS without a worsening in side events. There is insufficient information to definitively conclude that changing eligible patients from Bev to Cet or Pan following clinical progression would be beneficial[55,56]. There is no advantage to switching Bev to another VEGF medication. Patients who progress on oxaliplatin-based therapy (FOLIRI-naive) benefit from ziv-aflibercept or ramucirumab when used in conjunction with FOLIRI[57]. Bev may also be used in the third or fourth line of TAS-102 (trifluridine and tipiracil hydrochloride)[58]. Continuing EGFRi has no advantage for OS if Cet or Pan are used as the first-line treatments[59]. Circulating tumor DNA can be used to identify acquired resistance, which may manifest in a small number of patients. It is anticipated that this resistance would fade with time. As a result, switching to Bev is advised[60,61]. After the

disease progressed, switching from Cet to Pan or vice versa is not useful. If not used in the first-line setting, EGFRi may be administered either alone (monotherapy) or with Iri, FOLFIRI, or FOLFOX but not with CAPEOX, or to patients who cannot handle chemotherapy[62]. In ongoing studies, they are being used with additional medications such as immune checkpoint inhibitor (ICI), BRAF, and tyrosine kinase inhibitors.

Patients with resistant mCRC who have *BRAF* mutations, notably BRAF V600E and KRAS WT, may benefit from a combination therapy that combines BRAF inhibitors with Cet or Pan[63]. In the interim analysis of the BEACON trial, triple treatment with the MEK inhibitor (binimetinib) demonstrated a survival benefit over the control group (Cet plus Iri or FOLFIRI) and combination therapy. A more recent investigation, however, found no benefit of triplet treatment over doublet treatment compared to the control group[64]. In the initial studies with Pan, other BRAF inhibitors, dabrafenib and vemurafenib, with/without MEK inhibitors (trametinib), showed favorable outcomes[65]. In approximately 6% of CRCs, HER2 is amplified or overexpressed[66]. Trastuzumab, a HER2 inhibitor, in combination with pertuzumab (an mAb that prevents dimerization of HER2 and HER3) or lapatinib (inhibitor that binds to the cytoplasmic ATP-binding site inhibitor EGFR/HER1 and HER2 receptors), is well tolerated in refractory mCRC patients with HER2 amplification and RAS/BRAF WT[67]. HER2-amplified, a humanized anti-HER2 Ab combined with a topoisomerase I inhibitor, known as trastuzumab deruxtecan (T-dx), demonstrated an excellent ORR (45.3%) after a median follow-up of 27.1 wk in refractory mCRC[68]. Patients who were resistant to HER2 inhibitors also showed activity when given T-dx. Neurotropic tyrosine receptor kinase (*NTRK*) genes code for the tyrosine kinase receptors that control cell growth, and rearrangements result in unregulated cell proliferation. It was first discovered in CRC and occurs in just 0.3% of solid tumors[69]. There was 7% CRC in the studies that administered NTRK inhibitors to solid tumors, such as entrectanib and larotectinib. Patients with *NTRK* mutations may choose this as a treatment option[70].

A multinational, randomized, placebo-controlled, phase 3 trial of a multikinase tyrosine kinase inhibitor is called the CORRECT trial. The regorafenib therapy group outlived the placebo group by 1.4 years (mOS for regorafenib is 6.4 years *vs* 5 years for placebo; $P = 0.005$). The use of a regulator was associated with higher medication toxicity (93% in the regorafenib *vs* 61% in the placebo). Hand-foot skin reactions were the most common adverse event (AE) (83%) observed, followed by tiredness (48%) and hypertension (36%). ICIs including ipilimumab/nivolumab, pembrolizumab, and EGFRi are now being explored in conjunction with it. Its usage is frequently contrasted with that of the TAS-102, which had an OS improvement of 1.8 m above placebo. Combinations of ICI and EGFRi are being investigated [71] (Figure 2).

IMMUNOTHERAPY

The goal of immunotherapy is to use the immune system to fight cancer. For patients with mCRC that is mismatch-repair-deficient (dMMR) or MSI-H (dMMR/MSI-H mCRC), ICIs have emerged as a very effective treatment. ICIs modify the interaction of T cells, antigen-presenting cells, and tumor cells to help unleash suppressed immune responses. The FDA approved pembrolizumab and nivolumab (with or without Ipilimumab) for the treatment of these patients due to their effective, stable, and long-lasting responses. The fundamental difficulty is to offer the advantage of immunotherapy to the great majority of mCRC patients who are mismatch-repair-proficient (pMMR), microsatellite-stable (MSS), or have low MSI (MSI-L), since mCRC is characterized by an inadequate number of mutant tumor antigens[72].

ICIs-based immunotherapy

Use of ICIs in DMMR/MSS mCRC: To preserve DNA integrity, MMR is essential. CRC can be classified as dMMR or pMMR CRC based on the detection of the MMR proteins MLH1, MSH2, MSH6, or PMS2 utilizing immunohistochemical staining. Moreover, insertions and deletions can cause MSI, which can be precisely identified by PCR or next-generation sequencing, resulting in a change in microsatellite length. Major histocompatibility complex (MHC) class I peptide complexes, including mutant peptides that might be identified as neoantigens and subsequently increase immune cell priming and infiltration, are present on the surface of tumor cells in dMMR-MSI-H malignancies. T helper 1 CD4+ T cells, macrophages, and CD8+ tumor-infiltrating lymphocytes (TILs) enter the TME and produce interferon gamma. Programmed cell death ligand-1, cluster of differentiation 80 (CD80), and CD86 of the B7 family are examples of T cell inhibitory ligands that dMMR-MSI-H tumor cells persistently upregulate to support immune escape[73-75]. The percentage of dMMR-MSI-H CRCs, which accounts for about 15% of all CRCs, is correlated with tumor stage. dMMR-MSI-H cancers make up about 5%-20% of stage 2 and 11% of stage 3, but only 5% of stage 4. Additionally, dMMR-MSI-H is a predictive biomarker for individuals at various phases of their condition. Patients with dMMR-MSI-H tumors have a much better prognosis than those with pMMR-MSI-L cancers in stages 2 and 3. Surprisingly, individuals with stage 4 dMMR-MSI-H have a poor prognosis yet respond well to immune checkpoint inhibition[76,77].

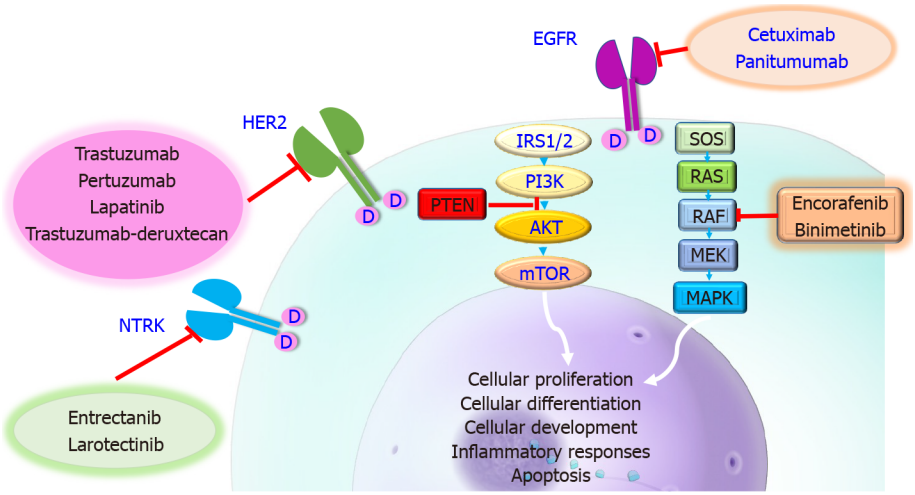

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Figure 2 Targeted therapy for metastatic colorectal cancer. AKT: Ak strain transforming; D: Down regulation pathway; EGFR: Epidermal growth factor receptor; HER2: Human epidermal growth factor receptor 2; IRS1/2: Insulin receptor substrates 1 or 2; MAPK: Mitogen activated protein kinase; mTOR: Mammalian target of rapamycin; MEK: Mitogen-activated protein kinase kinase; NTRK: Neurotrophic tyrosine receptor kinase; PTEN: Phosphatase and tensin homolog; PI3K: Phosphoinositide 3-kinases; RAF: Rapidly accelerated fibrosarcoma; RAS: Rat sarcoma virus; SOS: Son of sevenless.

Le *et al*[78] conducted a phase 2 trial in 41 patients with progressive mCRC with or without dMMR to investigate the clinical efficacy of pembrolizumab, an anti- Programmed cell death protein 1 (PD-1) ICI, in 2015. Pembrolizumab was given intravenously every 14 d at a dosage of 10 mg/kg body weight to patients with dMMR CRC, pMMR, and patients with dMMR who were not colorectal. The immune-related ORR and PFS for dMMR CRC were 40% and 78%, respectively, and 0% and 11% for pMMR CRC. In the group with dMMR CRC, the median PFS and OS were not attained, but in the cohort with pMMR CRC, they were 2.2 and 5.0 mo, respectively (hazard ratio [HR] for PFS and death = 0.10 and 0.22). Responses in patients with dMMR non-CRC were comparable to those in individuals with dMMR CRC. High somatic mutation loads were related to longer PFS (P = 0.02), and whole-exome sequencing found that dMMR tumors had an average of 1782 somatic mutations per tumor, compared to 73 somatic mutations in pMMR tumors (P = 0.007). They concluded that MMR status predicted the therapeutic benefit of immune checkpoint inhibition with pembrolizumab (Figure 3).

In 2020, the KEYNOTE-164 study analyzed pembrolizumab's effectiveness in 124 patients with dMMR/MSI-H mCRC who had undergone treatment. The ORR was 32% among the 63 patients examined, and the median PFS was 4.1 mo. The overall median survival rate has not yet been reached. The percentages of OS and PFS at 1 year were 41% and 76%, respectively. A single ICI therapy for individuals with dMMR/MSI-H mCRC demonstrated sustained anticancer efficacy[79]. In a phase 3, open-label study, 307 mCRC patients with dMMR/MSI-H who had not previously received treatment were enrolled to assess the effectiveness of PD-1 blockers or chemotherapy as first-line treatments. They were given a 1:1 random assignment to undergo chemotherapy (5-FU-based treatment with or without bevacizumab or cetuximab) every 2 wk, or pembrolizumab at a dosage of 200 mg every 3 wk. After advancement of the condition, patients taking chemotherapy could switch to pembrolizumab therapy. Of the 307 patients enrolled, 153 received single-agent pembrolizumab and 154 received chemotherapy. The median PFS time was 16.5 mo in the pembrolizumab group and 8.2 mo in the chemotherapy group at a median follow-up of 32.4 mo (HR = 0.60; P < 0.001). Significant differences were seen between the pembrolizumab group's 12- and 24-mo PFS values, which were 55% and 48%, respectively, *vs* 37% and 19% in the chemotherapy group. These data show that, compared to chemotherapy, pembrolizumab demonstrates more stable anticancer activity and fewer treatment-related AEs[80]. Based on the compelling evidence from this trial, the FDA approved pembrolizumab for the first-line treatment of patients with dMMR/MSI-H or advanced, unresectable, or metastatic CRC in June 2020.

In certain clinical studies, the use of nivolumab, another PD-1 inhibitor that targets dMMR/MSIH CRC, is also being investigated. Nivolumab's effectiveness was examined in the phase 2 study CheckMate 142 on 74 patients with dMMR/MSI-H mCRC. The ORR was 31%, and 69% of patients had disease control for 12 wk or longer. The median duration of response was not attained. The PFS and OS rates during the past 12 mo were 50% and 73%, respectively. Nivolumab's safety profile in this cohort study was consistent with that previously reported in other solid tumor trials, and there were no additional AEs noted[81]. Nivolumab was approved by the FDA in August 2017 for the treatment of dMMR/MSI-H mCRC in adults and children older than 12-years-old on the basis of these research findings[82]. Nivolumab's administration in conjunction with the cytotoxic T-lymphocyte-associated protein 4 mAb ipilimumab was also investigated in the CheckMate142 trial. Nivolumab 3 mg/kg in combination with ipilimumab 1 mg/kg was given once every 3 wk (four doses) to a total of 119 patients

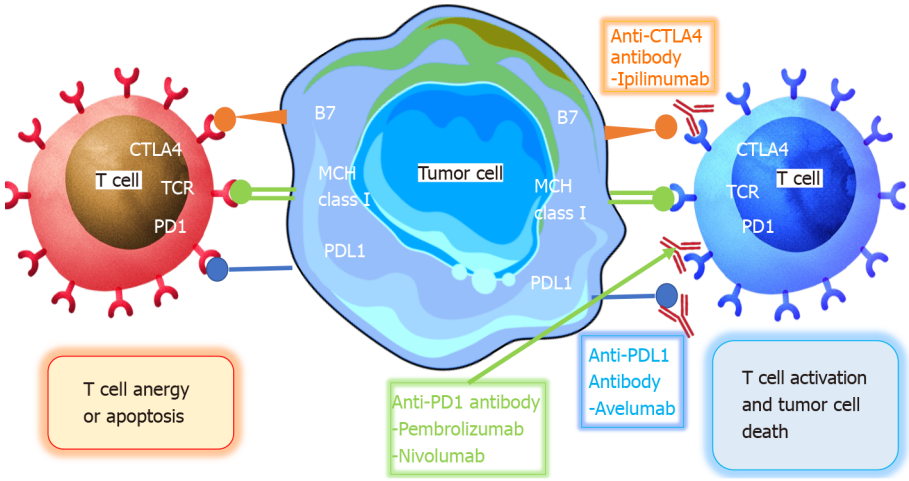

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Figure 3 Immune check point inhibitors treatment for metastatic colorectal cancer. CTLA4: Cytotoxic T-lymphocyte-associated protein 4; MHC: Major histocompatibility complex; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand; TCR: T cell receptor.

with dMMR/MSI-H mCRC who had not responded to conventional therapy. Nivolumab 3 mg/kg was then given once every 2 wk. The ORR was 55% at a median follow-up of 13.4 mo. In 80% of patients, the disease was under control for at least 12 wk; however, the median PFS was not met. The PFS rates for the 9th and 12th mo were 76% and 71%, respectively. The 9- and 12-mo OS rates were 87% and 85%, respectively, but the median OS was not met[83]. The FDA expedited the approval of nivolumab in combination with ipilimumab for the treatment of patients with dMMR/MSI-H mCRC in July 2018 based on the findings of this trial. Furthermore, the most recent information from the 2-year follow-up was used to update the study outcomes. The ORR and disease control rates determined by the study were 69% and 84%, respectively, over a median follow-up of 29 mo. The 2-year PFS and OS rates were 74% and 79%, respectively, but the median PFS and OS was not attained[84].

After first-line chemotherapy failed, Kim *et al*[85] conducted a prospective, open-label, multicenter phase 2 trial in 2020 to assess the effectiveness and safety of avelumab in 30 mCRC patients who had dMMR)/MSI-H and 3 mCRC patients who had polymerase-epsilon (POLE) mutations. All of the respondents were dMMR/MSI-H, and the ORR was 24.2%. At a median follow-up time of 16.3 mo, the median PFS and OS for all patients was 3.9 and 13.2 mo, respectively. They concluded that in patients with previously treated mCRC carrying dMMR/MSI-H, avelumab demonstrated anticancer efficacy with controllable toxicity. To assess the effectiveness and safety of cetuximab re-challenge treatment combined with avelumab in 71 MSS, 3 MSI-H, and 3 patients with uncertain microsatellite status with mCRC, Martinelli *et al*[86] conducted a single-arm, multicenter phase 2 study in 2021. The patients were given cetuximab (400 mg/m^2, then 250 mg/m^2 weekly) and avelumab (10 mg/kg every 2 wk) until the disease progressed or the side effects became intolerable. With a median OS of 11.6 mo and a median PFS of 3.6 mo, the trial accomplished its primary aim; 4% of grade 3 AEs were diarrhea, and 14% of them were skin eruptions. There were 48 people with WT illnesses and 19 people with mutations. Those with RAS/BRAF WT cDNA had a median OS of 17.3 mo, opposed to patients with mutations, who had a median OS of 10.4 mo. In contrast to patients with mutant cDNA, those with RAS/BRAF WT had a median PFS of 4.1 mo opposed to 3.0 mo. They concluded that an active, well-tolerated challenge treatment for RAS WT mCRC is cetuximab plus avelumab.

Use of ICIs in PMMR/MSS mCRC: pMMR/MSS CRC, which makes up about 95% of all mCRCs, is referred to as a "cold tumor." Single-agent ICI had no effect on pMMR/MSS CRCs in contrast to inflammatory tumors of dMMR/MSI-H. The results of recent investigations on the use of combination ICIs have raised the prospect of enhancing immunotherapy efficacy in this population. ICIs in conjunction with systemic chemotherapy were proven to dramatically increase tumor treatment response in refractory mCRC treated with conventional chemotherapy and immunotherapy, especially in pMMR/MSS CRCs. Existing data show that immunogenic chemotherapy might improve the efficacy of ICIs by increasing tumor infiltration of CD4+ and CD8+ T cells and interrupting the function of immunosuppressive cells such as regulatory T cells and myeloid-derived suppressor cells[87,88]. To investigate ways to make cold tumors heated to boost sensitivity to immunotherapy, a number of ICI-based combination treatment studies tested ICIs in conjunction with chemotherapy, targeted therapy, ICI therapy, and radiation.

Pembrolizumab combined with modified FOLFOX6 was tested in a single-arm, multicenter phase 1b study by Herting *et al*[89] for the treatment of mCRC. In this study, 87% of the participants had pMMR/MSS mCRC. At a median follow-up of 19.9 mo among the 30 patients, the investigators noted an ORR of 57% and the mean time it took for the responding patients to respond was 37.57 wk. The median PFS

time was 8.8 mo, and the median OS was not reached. Recently, two phase 2 trials, AtezoTRIBE and MAYA, evaluating combinations of ICIs with chemotherapy, revived optimism for the use of immunotherapy in pMMR/MSS mCRC patients, indicating a significant breakthrough and a potential basis for future research in this scenario.

AtezoTRIBE trial: Regardless of microsatellite status, 218 patients with unresectable and chemo-naive mCRC were randomized in a 1:2 ratio to receive FOLFOXIRI. The control group received first-line FOLFOXIRI (intravenous 165 mg/m^2 irinotecan, 85 mg/m^2 oxaliplatin, 200 mg/m^2 leucovorin, and 3200 mg/m^2 fluorouracil as a 48-h infusion) plus bevacizumab (5 mg/kg intravenously), and the atezolizumab group received the same regimen plus atezolizumab (840 mg intravenously) in the AtezoTRIBE phase 2 multicenter, open-label, comparative study. According to the randomized arm, both treatments were given for up to eight cycles, then 5-FU with bevacizumab, with or without atezolizumab, was given until the condition progressed, there were unacceptable side effects, or the patient withdrew their consent. PFS was the main endpoint, with a one-sided alpha error of 0.10 and an 85% power. A median follow-up of 19.9 mo was being used. In the atezolizumab group, the median PFS was 13.1 mo, compared to 11.5 mo in the control group (HR = 0.69; P = 0 012). Neutropenia (42% of 142 patients in the atezolizumab group *vs* 36% of 72 individuals in the control group), diarrhea (15% *vs* 13%), and febrile neutropenia (10% *vs* 10%) were the most common all-cause grade 3-4 AEs. A total of 39 patients (27%) in the atezolizumab group and 19 patients (26%) in the control group suffered serious AEs. Acute myocardial infarction and bronchopulmonary hemorrhage caused two (1%) treatment-related fatalities in the atezolizumab group; none were recorded in the control group. They concluded that first-line FOLFOXIRI plus bevacizumab with atezolizumab added was safe and enhanced PFS in patients with mCRC who had not previously received treatment[90].

MAYA trial: MAYA is a prospective single-arm phase 2 trial that included patients with chemo-resistant mCRC who had centrally confirmed MSS status, O6-methylguanine-methyltransferase (MGMT) silence determined by promoter methylation of the MGMT gene, and total immunohistochemical loss of MGMT protein. Only in situations where disease control is obtained during phase 1 are participants to receive two cycles of temozolomide (TMZ) (phase 1), followed by the addition of nivolumab and low-dose ipilimumab (phase 2). The 8-mo PFS rate in patients included in phase 2 of the trial served as the study's main objective. The MAYA trial's design is supported by an intriguing biological theory. In short, the MGMT gene plays a role in repairing DNA damage brought on by alkylating drugs like TMZ[91]. Sensitivity to TMZ is increased when MGMT is inactivated by hypermethylation of its promoter. As demonstrated in studies evaluating the efficacy of TMZ alone or in combination with other chemotherapeutic agents, such as capecitabine and irinotecan, retrospective data revealed that sensitivity to TMZ was primarily restricted to pMMR/MSS tumors with complete MGMT protein loss detected with immunohistochemistry[92]. After the first disease response, MGMT re-expression, the selection of sub-clones that express MGMT, or a hypermutated condition resulting from acquired mutations in MMR genes that might make mCRC sensitive to ICIs can all lead to secondary resistance to TMZ[93]. In the MAYA study, 204 of 703 assessed patients (29%) were found to be molecularly suitable. Overall, 142 of 703 (19%) patients were enrolled in phase 1, with just 33 (5% of the initial 703 screened patients) progressing to phase 2. The 8-mo PFS rate was 36% after a median follow-up of 23.1 mo. The median PFS and OS were 7.0 and 18.4 mo, respectively, with a 45% response rate. Skin rash (6%), colitis (3%), and hypophysitis (3%), were all immune-related side effects of grade 3-4 severity. There were no unanticipated AEs or treatment-related fatalities recorded. They concluded that TMZ priming followed by a combination of low-dose ipilimumab and nivolumab might result in long-term therapeutic benefit in MSS and MGMT-silenced mCRC. These findings should be considered with caution due to the lack of a control arm testing the effectiveness of TMZ monotherapy, which prevented the investigators from distinguishing the effect of immunotherapy addition *vs* TMZ alone. Given that not all patients who are initially susceptible to TMZ develop a hypermutated phenotype, only a subset of individuals may benefit from immunotherapy. Future analyses separating the ORR observed during phase 1 (with TMZ) from the ORR reported during phase 2 (with TMZ plus ipilimumab plus nivolumab), as well as current translational investigations, might reduce this issue.

Despite the need for more mature follow-up data, the good findings from the AtezoTRIBE and MAYA trials bring an end to a long period of stagnation and dismal outcomes in the landscape of immunotherapy in pMMR/MSS mCRC. In the first-line scenario, chemotherapy escalation and TMZ delivery in MGMT-silenced chemo-refractory patients are capable of sensitizing immune-deficient or cold mCRCs to immunotherapy, perhaps rewiring an inflamed/hot TME, then unleashed against the tumor by ICIs. Larger confirmatory and translational trials, however, are required to identify people who benefited the most from these therapies[94].

ADOPTIVE CELL THERAPY

Adoptive cell therapy (ACT), a crucial component of tumor immunotherapy, entails the introduction of immunologically active cells that have been grown and altered *in vitro* to have direct anticancer action

against the cancer-stricken host. Chimeric antigen receptor T (CAR-T) cells, TILs, and T cell receptor-engineered T cells are the three ACT types currently being researched for the treatment of cancer. CAR-T cell therapy entails modifying T cells *in vitro* in an MHC-independent manner so that they can target tumor antigens and produce an anticancer immune response. With its tremendous effectiveness in treating leukemia, multiple myeloma, some forms of lymphoma, and mCRC, CAR-T cell therapy has a lot of room to grow[95]. Numerous clinical investigations on the safety and efficacy of CAR-T cell treatment are now being conducted in order to determine its therapeutic potential in the field of CRC. One of the first human trials using CAR-T cells to treat metastatic CRC was published by Hege *et al*[96]. It consisted of two phase 1 experiments with the same CART72 cells (C9701 and C9702). As first-generation CAR-T cells with a CD3-zeta intracellular signaling domain that specifically targeted the tumor-associated glycoprotein-72, CART72 cells were created. The way CART72 was administered in the two studies was different. In trial C9702, patients with CLM received direct hepatic artery infusions, whereas in trial C9701, CART72 was administered intravenously in increasing dosages. Despite a brief blood persistence and modest trafficking to tumor tissue, the data indicated a good safety profile. In addition, rapid clearance following CAR-T cell infusion was linked to CART72 immunogenicity.

Guanylylcyclase2C (GUCY2C) was mentioned by Magee *et al*[97,98] as a potential CAR-T cell target. In a mouse model lacking autoimmunity, they demonstrated that GUCY2C CAR-T cells may cure parenchymal CRC metastases. Additionally, they showed that GUCY2C targeted CAR-T cell treatment works well against metastatic cancers in mouse models and in human CRC xenograft models. Zhang *et al*[99] conducted a phase 1 study using CEA-positive CRC patients to create and assess CEA CAR-T cell treatment in 10 resistant and relapsed CRC patients with metastases. CAR-T cells were administered at five increasing dosage levels (1×10^5 to 1×10^8/CAR+/kg cells) to these individuals. The findings demonstrated that there were no significant side effects of CAR-T treatment. Seven of the ten patients—those with progressing illness throughout prior therapies—had stable disease following CAR-T cells therapy. Two patients had tumors removed, and two others had stable illnesses for more than 30 wk. They concluded that CEA CAR-T cell treatment, even at large dosages, was well tolerated in CEA+ CRC patients and that the majority of the treated patients showed some effectiveness.

Several ongoing trials are investigating the use of CAR-T cell in the treatment of CRC[100]. These included the safety, cellular kinetics, and efficacy of CYAD-101, an allogenic CAR-T cell therapy targeting ligands of NKG2D that was administered concurrently with FOLFOX, the efficiency and safety of NKR-2 CAR-T cells, EGFR and EGFR IL 12 CAR-T cell safety and feasibility, the use of anti-carcinoembryonic antigen targeted CAR-T cells, and investigating the efficacy and safety of HER2 chimeric antigen receptor-modified adenovirus-specific cytotoxic T lymphocytes administered in association with intratumoral injection of CAdVEC in patients with unresectable mCRC.

CONCLUSION

With the discovery and comprehension of several molecular and anatomical indicators, the landscape of systemic therapy for mCRC has significantly changed. To find the most effective treatment solution for mCRC patients, a baseline, thorough molecular study is now required. The effect of RAS, BRAF, HER2, POLE, MMR, MSS, and MSI status on therapy choice is summarized in this article. The selection of an efficient first-line therapy, which should consider both clinical factors and molecular signs, is crucial for deciding treatment outcomes. The second-line regimen is chosen based on the systemic therapies utilized in the first. For patients with RAS WT, third-line treatment, which includes EGFR inhibitors, should consider molecular profiling. Immunotherapy with pembrolizumab or nivolumab plus ipilimumab may be an option for patients with high microsatellite instability diseases.

The TME has been identified as a crucial player in CRC tumor growth and metastasis. This process involves all of the components from both bacteria and the host. Although each component has a unique function in CRC growth and metastasis, the majority of them act as a double-edged sword, promoting or inhibiting tumor expansion depending on the setting. TME-modulating treatment methods are showing promise. Many researches have confirmed that altering the TME can result in greater anti-tumor actions. Clinical investigations have indicated that TME remodeling has a high potential for improving medication therapeutic efficacy. Furthermore, because tumors tend to acquire resistance, monotherapy is frequently insufficient. Combining TME remodeling techniques with other potential therapies, such as targeted treatment and immunotherapy, is another component we need to investigate in the near future to reduce treatment resistance.

FOOTNOTES

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Country/Territory of origin: Thailand

ORCID number: Wattana Leowattana 0000-0003-4257-2480; Tawithep Leowattana 0000-0003-2316-3585.

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MINIREVIEWS

Gastrointestinal microbiome and cholelithiasis: Current status and perspectives

Wan-Yue Dan, Yun-Sheng Yang, Li-Hua Peng, Gang Sun, Zi-Kai Wang

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Wan-Yue Dan, Yun-Sheng Yang, Li-Hua Peng, Gang Sun, Zi-Kai Wang, Microbiota Division, Department of Gastroenterology and Hepatology, The First Medical Center, Chinese PLA General Hospital, Beijing 100853, China

Wan-Yue Dan, Medical School, Nankai University, Tianjin 300071, China

Corresponding author: Zi-Kai Wang, MD, Associate Chief Physician, Microbiota Division, Department of Gastroenterology and Hepatology, The First Medical Center, Chinese PLA General Hospital, No. 28 Fuxing Road, Beijing 100853, China. wangzikai301@126.com

Abstract

Cholelithiasis is a common digestive disease affecting 10% to 15% of adults. It imposes significant global health and financial burdens. However, the pathogenesis of cholelithiasis involves several factors and is incompletely elucidated. In addition to genetic predisposition and hepatic hypersecretion, the pathogenesis of cholelithiasis might involve the gastrointestinal (GI) microbiome, consisting of microorganisms and their metabolites. High-throughput sequencing studies have elucidated the role of bile, gallstones, and the fecal microbiome in cholelithiasis, associating microbiota dysbiosis with gallstone formation. The GI microbiome may drive cholelithogenesis by regulating bile acid metabolism and related signaling pathways. This review examines the literature implicating the GI microbiome in cholelithiasis, specifically gallbladder stones, choledocholithiasis, and asymptomatic gallstones. We also discuss alterations of the GI microbiome and its influence on cholelithogenesis.

Key Words: Gallstone; Cholesterol gallstone; Common bile duct stone; Bile acid; Bile microbiome; Gastrointestinal microbiome

Core Tip: Cholelithiasis is a common digestive disease that imposes significant global health and financial burdens. High-throughput screening demonstrated the relationship between bile, gallstones, and the fecal microbiome in cholelithiasis and provided evidence that gastrointestinal (GI) microbiota dysbiosis is associated with gallstone formation. We summarize the current literature, pool the available cholelithiasis-related studies about the GI microbiome, discuss the underlying mechanisms by which the GI microbiome modulates cholelithiasis, and suggest potential microbiome-targeting therapeutics for cholelithiasis prevention.

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INTRODUCTION

Cholelithiasis or gallstones is a common digestive disease with a high incidence and relatively low mortality. With an overall prevalence of 11.0% in China[1], cholelithiasis is an important public health problem. Gallstone disease's prevalence ranges from 0.60 to 1.39% per year in European population surveys[2]. In the United States, 20 to 25 million adults have cholelithiasis, costing more than $6 billion annually[3,4]. Although many cholelithiasis patients are asymptomatic, approximately one-third develop biliary-pancreatic diseases, including acute or chronic cholecystitis, cholangitis, pancreatitis, and even biliopancreatic cancerous lesions. These diseases impose significant global health and financial burdens.

The study of the human microbiome, particularly the gastrointestinal (GI) microbiome, has rapidly evolved in recent decades, primarily due to the new generation of sequencing technology. The composition, diversity, and richness of microbial communities in the GI tract change during disease states. In addition to definite intestinal dysbiosis-related digestive diseases, the GI microbiome is altered in many biliary disorders, which are rarely traditionally considered microbial in etiology. A predictive model including the genera *Burkholderia*, *Caballeronia*, and *Paraburkholderia* was better able to predict cholangiocarcinoma than the tumor marker carbohydrate antigen 19-9[5]. The proportion of *Streptococcus* is proportionate to the severity of primary sclerosing cholangitis[6].

Investigations of the GI microbiome have extended to cholelithiasis. In the early 20th century, studies supported the existence of interactions between gallstones and bacteria such as *Helicobacter*. Although it has been recognized that specific bacteria contribute to gallstone formation, studies have recently shown that a complex GI microbiome rich in *Desulfovibrionales* promotes gallstone formation by regulating bile metabolism. This review examines the literature implicating the role of the GI microbiome in cholelithiasis, including the biliary, gallstone, and fecal microbiomes (Table 1). We will summarize the literature and discuss mechanisms by which the GI microbiome modulates cholelithiasis; finally, we discuss advances in microbiome-based therapeutics.

THE GI MICROBIOME RELATED TO CHOLELITHIASIS

The biliary microbiome

Studies suggest that the healthy biliary tract is a sterile environment. With the development of sequencing technology, studies revealed the existence of a biliary microbiome. Stewart *et al*[7] detected bacteria in bile samples in nearly a third of gallstone patients. The first study to identify the existence of human biliary microbiota in the gallbladder described the composition of human biliary microbiota using 16S ribosomal RNA (rRNA) gene sequencing. The healthy biliary microbiota is dominated by *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia* at the phylum level[8]. In addition to bacteria, *Cyanobacteria* and *Spirochaetes* were also present in low amounts.

There are some resemblances and dissimilarities between the biliary and GI microbiomes. The comparative metagenomic analysis demonstrated no significant differences between the GI tract and bile in the predominant phylum *Firmicutes* and the rare phylum *Fusobacteria*. However, the microbial diversity in the biliary tract is more diverse than in the GI tract[9]. The bile duct and duodenum share the core microbiota with the genus *Escherichia–Shigella*, *Fusobacterium*, and *Enterococcus*[10]. Given that the bile duct is anatomically connected to the GI tract *via* the duodenal papilla, it was hypothesized that the biliary microbiota originates from intestinal bacteria and migrates retrograde into the biliary tract. Consistent with the hypothesis, studies demonstrated that the biliary microbiota shared a compositional similarity to duodenal microbiota, and all bacteria in bile were detected in the upper GI using 16S

Table 1 Studies of the gastrointestinal microbiome in cholelithiasis

Microbial changes	Samples	Disease types *vs* control	Methods	Ref.
↑*Desulfovibrionales*	Feces	Cholesterol gallstone *vs* gallstone-free	16S sequencing	Hu *et al*[51]
↑*Megamonas*, *Comamonas*, *Ruminococcaceae*_UCG-014, *Coprobacillus*, *Adlercreutzia*, unclassified_*p*_*Firmicutes*, *Morganella*, CHKCI002, and *Tyzzerella*_4; ↓*Ruminococcaceae*_UCG-008, *Sutterella*, GCA-900066755, *Butyricicoccus*, unclassified_*o*_*Lactobacillales*, and *Lachnospiraceae*_ND3007_group	Feces	Asymptomatic gallstone *vs* gallstone-free	16S sequencing	Song *et al*[33]
↓*Akkermansia muciniphila*, *Prevotella spp.*, *Bifidobacterium adolescentis*, *Alistipes spp.*, *Bacteroides spp.*, *Dorea spp.*, *Methanobacteria*, *Methanobrevibacter smithii*, *Ruminococcus spp.*, and *Faecalibacterium prausnitzii*	Feces	Cholesterol gallstone *vs* pigment gallstone	16S sequencing	Georgescu *et al*[37]
↑7α-dehydroxylating bacteria	Feces	Gallstone *vs* gallstone-free	Culture	Wells *et al*[49]
↑*Proteobacteria*; ↓*Faecalibacterium*, *Lachnospira*, and *Roseburia*	Feces	Gallstone *vs* controls	16S sequencing	Wu *et al*[9]
↑*Aeromonas*, *Enterococcus*, Unclassified_*Enterobacteriaceae*, and *Citrobacter*↓*Prevotella*, *Alloprevotella*, *Nesterenkonia*, and *Pyramidobacter*	Bile	Recurrent CBD stone *vs* new-onset CBD stone	16S sequencing	Chen *et al*[19]
↑*Synergistetes*; ↓*Bacteroidetes*, and *Actinobacteria*	Bile	Recurrent CBD stone *vs* primary CBD stone	16S sequencing	Tan *et al*[20]
↑*Bacteroidaceae*, *Prevotellaceae*, *Porphyromonadaceae*, and *Veillonellaceae*	Bile	Gallstone *vs* controls	16S sequencing	Molinero *et al*[8]
↑*Brevundimonas* and *Prevotella*_1; ↓*Actinomyces*, *Proteus*, *Clostridiumsensu_stricto*, *Klebsiella*, *Actinobacillus*, *Lachnospiraceae*_UCG-008, *Butyrivibrio*, *Roseburia*, *Porphyromonas*, *Streptococcus*, *Helicobacter*, *Enterobacter*	Bile	Primary CBD stone *vs* controls	16S sequencing	Lyu *et al*[12]
↑*Alcaligenaceae*	Bile	Primary bile duct stone *vs* secondary bile duct stone	16S sequencing	Feng *et al*[18]

CBD: Common bile duct.

sequencing[11,12]. It is noteworthy that the intestinal microbiome contributes to the heterogeneity of the biliary microbiome in cholelithiasis patients, despite the high prevalence of oral cavity and respiratory tract inhabitants to intestinal inhabitants[13].

Because of interactions between the biliary microbiome and metabolic disease, investigators analyzed the bacterial composition associated with cholelithiasis. A Colombian study demonstrated a predominance of *Pseudomonas spp*. in gallbladder tissue and bile[14]. To compare the difference in bile microbiome between gallbladder stone patients and healthy individuals, Molinero *et al*[8] measured bile samples using 16S rRNA sequencing. They demonstrated that the relative abundance of the family *Propionibacteriaceae* in patients with gallbladder stones was lower than in healthy controls. In contrast, the relative abundance of the family *Bacteroidaceae*, *Prevotellaceae*, *Porphyromonadaceae*, and *Veillonellaceae* was higher.

Short-chain fatty acids (SCFAs) consist of butyrate, propionate, and acetate; these are associated with inflammatory diseases and cancers and may serve as nutritional and therapeutic agents in diseases[15,16]. Evidence suggests that low levels of expression of bacteria-producing SCFAs harm the microbial balance of the biliary tract, affecting gallstone formation. There is a significantly decreased abundance of *Clostridiumsensu_stricto*, *Lachnospiraceae* _UCG-008, *Butyrivibrio*, and *Roseburia* (which produce SCFAs) at the genera level in patients with primary choledocholithiasis. Lyu *et al*[12] found that specific bacteria-producing SCFAs might participate in generating common bile duct stones.

In recent years, several groups interrogated the human biliary microbiome in recurrent bile duct stones patients, albeit in different disease states. The biliary microbiome in the recurrence of choledocholithiasis showed a significantly low diversity[17]. The composition of biliary bacteria differed significantly between primary bile duct stone patients and those with secondary bile duct stones. By contrast, several families, such as *Propionibacteriaceae*, *Sphingomonadaceae*, and *Lactobacillaceae*, were enriched in the recurrent cholelithiasis group[18]. Chen *et al*[19] analyzed the biliary microbiota of 16 patients with recurrent choledocholithiasis and 44 patients with primary choledocholithiasis. The 16S rRNA sequencing revealed a prevalence of the phyla *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* (all of which are intestinal bacteria) in the primary choledocholithiasis group. These data suggest that the biliary microbiota might originate from gut microbiota and access the biliary tract through the duodenal papilla. Furthermore, there was substantial enrichment of the genera *Prevotella*, *Alloprevotella*, *Nesterenkonia*, and *Pyramidobacter* (without significant alterations in microbial diversity)

compared with recurrent choledocholithiasis patients[19]. One study described the biliary microbiome in recurrent cholelithiasis[20]. Investigators compared the biliary microbiomes of patients with primary choledocholithiasis with those with recurrent choledocholithiasis. Consistently, the phyla *Proteobacteria* and *Firmicutes* predominated in both groups. Patients with recurrent choledocholithiasis showed less microbial biliary diversity (with reduced *Bacteroidetes* and *Actinobacteria* and enrichment of *Synergistetes* at the phylum level) than controls. A study considered 11 adults with recurrent choledocholithiasis and nine post-endoscopic removal patients to assess the characteristics of the biliary microbiome. The richness and diversity decreased in the recurrence group. The recurrence group had a higher relative abundance of phylum *Actinobacteria* and *Firmicutes* and a lower relative abundance of *Bacteroidetes* than the non-recurrence group[21].

These association studies demonstrate a correlation between the biliary microbiome and cholelithiasis; however, these studies sampled the microbiome from bile but not the bile duct epithelium.

The gallstone microbiome

Awareness of the gallstone microbiome arose *via* a circuitous route. The first microbial study of gallstone formation dates back decades; the authors found a lower prevalence of gallstones in germ-free mice[22]. In the 1990s, bacterial DNA was detected using PCR in all common bile duct stones, mixed cholesterol stones, and brown pigment stones[23], demonstrating the existence of bacteria in gallstone formation. In the 21st century, researchers found that 42% of patients had bacteria gallstone samples[7], and over 80% of the stone cores contained bacteria, primarily from the intestine[24]. These results were vital for assessing the connections between the microbiota and cholelithiasis.

According to scanning electron microscopy studies, there are bacterial biofilms on gallstone surfaces [25]. Bacterial biofilms are microbial communities attached to the surface or embedded in the matrix [26]. The typical surface-related biofilm originates from single planktonic cells attached to the surface. These cells divide and produce extracellular polymeric substances. Microbial communities then form the three-dimensional structure attached to the surface. Biofilms are environmental reservoirs for pathogens that correlate with infectious kidney stones, bacterial endocarditis, and airway infections in cystic fibrosis patients[27]. Studies found that biofilm formed by *Salmonellae* aggregates on the surface of human gallstones[28]. The appearance of gallstones led to a 4.5-fold increase in hyperbiofilm isolates. *Salmonella spp.* increased in persistence in bile *via* genetic alterations[29].

The gut microbiome

The gut microbiome in human health and diseases has received considerable attention. The intestinal microbiota is dominated by *Bacteroidetes*, followed by *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia*[30]. Investigations of the microbial composition of cholelithiasis patients using 16S rRNA profiling identified a predominance of *Firmicutes* and, to a lesser extent, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* at the phylum level[31-33]. These findings suggest a shift in the gut microbiome from healthy individuals to cholelithiasis patients, suggesting an association with cholelithiasis pathophysiology.

The gut microbiome of cholelithiasis patients is maladjusted. Most studies showed reduced gut microbiota diversity in cholelithiasis patients[5,32]. There are alterations of the gut microbiome in cholelithiasis patients of various statuses. Song and colleagues demonstrated that *Klebsiella*, *Roseburia*, *Collinsella*, *Dialister*, and *Enterobacerin* at the genus level were significantly decreased, whereas *Streptococcus*, *Lactobacillus*, *Dorea*, *Romboutsia*, *Fusobacterium*, and *Megamonas* were over-represented in asymptomatic gallstone patients relative to controls[33]. Wu *et al*[9] compared the gut microbiota of twenty-nine patients with gallbladder stones and thirty-eight healthy controls. They found increased abundances of *Proteobacteria* and decreased abundances of *Faecalibacterium*, *Lachnospira*, and *Roseburia*; this was the first study to characterize gut microbiota dysbiosis in gallstone patients. A genome-wide search suggested an underlying link between the biliary tract core microbiome and the formation of cholesterol gallstones.

There have been attempts to utilize dysbiosis as a predictive and diagnostic tool or biomarker. Keren *et al*[34] described a significantly increased abundance of *Oscillospira* and a reduced abundance of *Roseburia* at the genus level in cholelithiasis patients compared with healthy controls. They suggested that the genera *Oscillospira* and *Roseburia* may be used in the early prediction or diagnosis of cholelithiasis as microbial biomarkers[34]. The relative abundance of the genus *Eubacterium*, which metabolizes and removes cholesterol, was lower in cholelithiasis patients. Line discriminant analysis effect size analysis showed that *Ruminococcus gnavus* predicted cholelithiasis[32]. These findings suggest that the gut microbiome is altered during cholelithiasis and that these changes may be valuable for diagnosis.

CHARACTERISTIC MICROBIOME BASED ON GALLSTONES COMPONENTS

Cholesterol gallstones

Gallstones are organic matrixes of cholesterol crystals, calcium bilirubinate, mucin, and proteins in the gallbladder or biliary tract[35]. Based on the major constituents, gallstones are classified as cholesterol gallstones (> 90%) or pigment gallstones (< 10%)[36]. Cholesterol gallstones form mixed or pure cholesterol gallstones. More than 80% of gallbladder stones are composed of pure cholesterol. Cholecystolithiasis originates primarily from the gallbladder and is composed of cholesterol or mixed gallstones, primarily cholesterol or black pigment stones. Several groups independently analyzed the human microbiome of cholelithiasis patients with different components. Bacterial diversity and several functional bacterial species of cholesterol-rich gallstones significantly decreased compared to those of pigment gallstones. These species include *Akkermansia muciniphila*, *Prevotella spp.*, *Bifidobacterium adolescentis*, *Alistipes spp.*, *Bacteroides spp.*, *Dorea spp.*, *Methanobacteria*, *Methanobrevibacter smithii*, *Ruminococcus spp.*, and *Faecalibacterium prausnitzii*[37]. There were various compositions of the gut microbiome in the mice in which a lithogenic diet-induced cholesterol gallstones. There was reduced richness, α diversity, the proportion of *Firmicutes*, and the ratio of *Firmicutes* to *Bacteroidetes* in the lithogenic diet group[38]. These findings suggest that alterations in the gut microbiome may play an essential role in forming cholesterol gallstones.

Helicobacter spp. were reported to participate in the formation of murine cholesterol gallstones. Mice fed a lithogenic diet and infected with various enterohepatic *Helicobacter spp.* showed a significantly higher prevalence of cholesterol gallstones than uninfected controls[39]. In humans, a retrospective cohort study demonstrated that patients with gallstone disease were at increased risk of *Helicobacter pylori* (*H. pylori*) infection[40].

Pigment gallstones

Pigment gallstones include brown pigment stones or black pigment stones. Brown pigment stones are associated with biliary tract infections. By contrast, black pigment stones are common in patients with hemolytic anemia, cirrhosis, and cardiac valve replacements. These findings were supported by human and animal research, which found changes in human microbiota in cholesterol gallstones. Nevertheless, there has been little attention paid to pigment gallstones. Notably, a pilot study indicated that the gallstone microbiome might participate in developing pigment stones[41]. The genera *Klebsiella* and *Enterococcus* (involved in bacterial biofilm formation) were dominant in pigment stones. These results suggest the participation of a gallstone microbiome in cholelithiasis. Kim and colleagues compared the biliary microbiota in patients with pigment common bile duct stones to other causes of biliary obstruction; there was enrichment of the genus *Enterococcus* in patients with common pigment bile duct stones[42]. These findings suggest a possible association between *Enterococcus* and pigment stone formation.

THE POTENTIAL MICROBIOTA-RELATED TRIGGERS IN CHOLELITHIASIS

The GI microbiome induces gallstone formation by regulating bile acid (BA) metabolism

Gallstones are formed when there is an imbalance of biliary cholesterol homeostasis. The primary pathophysiological defect in gallstones is the supersaturation of cholesterol in bile. Other factors include genetic factors (particularly lithogenic gene 1 and mitochondrial DNA variant), hepatic hypersecretion, gallbladder motility function obstacle, cholesteric phase transition, excessive secretion and accumulation of mucin, and excessive cholesterol[43,44]. Neutrophil extracellular DNA traps are involved in human gallstone formation and growth[45]. This evidence suggests the involvement of the GI microbiome in cholelithiasis. The alteration of the host GI microbiome modulates gallbladder motility and inflammation (especially mucin content), inhibiting cholesterol cholelithogenesis. The prevalence of gallstones in germ-free mice was higher than in specific pathogen-free mice[46], suggesting an underlying role of the GI microbiome in cholesterol cholelithogenesis.

Like gastric secretions and hydrochloric acid, bile is a bactericidal agent in the GI system[47]. The human gut microbiome is highly capable of transforming BAs. The oxidation of 3α-, 7α-, or 12α-hydroxyl groups on the steroid core, catalyzed by hydroxysteroid dehydrogenases, were the most prevalent BAs transformations[48]. A study found that 43 isolates of 41 species can modify human unconjugated BAs *in vitro*[48]. The gut microbiome chemically modifies primary BAs (*e.g.*, dehydroxylation) to affect physiology. The modified BAs are considered "secondary" BAs. The 7α-dehydroxylation of primary BAs into secondary BAs is responsible for the GI microbiota.

The genus *Clostridium* appears to be active in 7α-dehydroxylation. A study identified other strains, including *Bacteroides vulgatus*, *Bifidobacterium adolescentis*, and *Roseburia intestinalis* at the phylum level [48]. The abundance of 7α-dehydroxylating bacteria significantly increased[32] and was 42-fold higher in gallstone subjects than in gallstone-free contrlos[49]. Antibiotic treatment significantly reduced 7α-dehydroxylation activity and cholesterol saturation[50], suggesting a possible pathogenic role for 7α-

dehydroxylating bacteria. Hu *et al*[51] found that patients with cholesterol gallstone disease showed significant enrichment of *Desulfovibrionales* at the order level compared with gallstone-free controls. Following fecal transplantation from gallstone patients into mice, the mice showed a higher prevalence of gallstones; this study suggested *Desulfovibrionales* as a microbial trigger contributing to gallstone formation.

These studies elucidated the underlying mechanisms of the biliary microbiome on gallstone formation. *Desulfovibrionales* were enriched in cholelithiasis patients. The bacterial overgrowth shifts the biliary microbiome to a cholelithogenesis phenotype. The GI microbiome, rich in *Desulfovibrionales*, induces the formation of cholesterol gallstones by regulating hepatic BA metabolism in several ways [51]. First, the secondary BAs in the cecum increase with the number of 7α-dehydroxylating bacteria. Second, the biliary microbiome regulates the expression of hepatic farnesoid X receptor (FXR)-CYP7A, inhibiting synthesis. Third, a specific microbiome promotes intestinal cholesterol absorption and secretion of canalicular cholesterol into bile[51].

Lipopolysaccharide upregulates mucins via several pathways

Mucin is a glycoprotein with high molecular weight. It protects and lubricates the ducts and lumens *in vivo*. Mucins are classified according to their structural characteristics as secreted gel-forming mucins, soluble mucins, and trans-membrane mucins. The gel-forming mucin known as MUC5AC and the trans-membrane mucin known as the multifunctional protein MUC4 participate in cell signaling due to differential expression in normal and pathophysiological conditions. For example, the abnormal expression of MUC4 and MUC5AC was detected in biliary tract cancer, whereas it is rarely detected in healthy biliary tract[52]. Yoo *et al*[53] reported that concentrations and gene expression of MUC3 and MUC5B were significantly overexpressed in a cholesterol stone group than in normal controls. Patients with gallbladder stones were subdivided according to the density of gallstones into an isopycnic group and a calcified group. The enriched expression of MUC4 was detected, and the proportion of bacteria (especially gram-positive bacteria) was positively associated with the expression of MUC4 in the calcification group[54]. Because of the function of MUC4 in adhesion, a high concentration of MUC4 may be beneficial for bacterial growth and subsequently modulate gallstone formation and calcification.

Mucin hypersecretion should be considered a requirement in gallstone formation[43]. Studies found that MUC5AC plays an essential role in hepatolithiasis formation and recurrence; there was increased expression of MUC5AC and MUC2 in hepatolithiasis patients. In another investigation, the effect of MUC5AC on hepatolithiasis formation was elucidated. MUC5AC was upregulated through several pathways. Lipopolysaccharide (LPS), a major surface component of the gram-negative bacteria, upregulated MUC5AC expression in biliary epithelial cells. LPS significantly upregulated the expression of prostaglandin E_2 (PGE_2). The expression of MUC5AC and MUC2 mRNA was induced by exogenous PGE_2. The agonist of EP4, a G-protein coupled receptor, significantly increased MUC2 and MUC5AC expression. $P38^{MAPK}$ mediates PGE_2/EP4-induced MUC2 and MUC5AC upregulation. PGE_2 induces MUC2 and MUC5AC expression through the EP4/p38MAPK pathway[55]. LPS promoted epidermal growth factor receptor activation by increasing the secretion of transforming growth factor-α, resulting in overexpression of MUC5AC. By contrast, the LPS-induced MUC5AC overexpression was abolished by inhibiting tumor necrosis factor-α converting enzyme activity[56]. Mucin hypersecretion in bile may result from the upregulation of MUC genes and result in a higher bile viscosity, retaining cholesterol crystals in the biliary tract[53].

β-glucuronidase and phospholipase accelerate the precipitation of calcium bilirubinate

Bacterial enzymes, including β-glucuronidase (GUS) and phospholipase (PL), which are related to bacterial proliferation and severe infections[7], contribute to cholelithogenesis. Some bacteria produce exogenous GUS, including *Escherichia coli* and *Salmonella enterica*. GUS induces the hydrolysis of bilirubin diglucuronides to produce unconjugated bilirubin, resulting in the precipitation of calcium bilirubinate[57]. PL hydrolyzes lecithin to water-insoluble free fatty acids and lysophospholipids, enhancing the precipitation of calcium salts and mucin secretion from the biliary epithelium[58]. Nearly one-third of the cultured strains of cholesterol gallstone could secrete GUS and PLA_2[59]. GUS and PLA_2 levels in *Pseudomonas aeruginosa* (*P. aeruginosa*) strains were highest in culturable strains, suggesting that *P. aeruginosa* was involved in gallstone pathogenesis.

Helicobacter species induce gallstone formation by precipitating calcium

In addition to Desulfovibrionales, *Helicobacter spp.* are essential mediators during gallstone formation. *Helicobacter* infections (especially with *H. pylori*) positively correlate with the prevalence of chronic cholecystitis and cholelithiasis[60]. *H. pylori* is the primary pathogenic agent of chronic gastritis, gastric ulcer, and gastric cancer.

Helicobacter spp. play a unique role in the formation of murine cholesterol gallstones. Belzer *et al*[61] identified an underlying mechanism of gallstones by testing the ability of different *Helicobacter spp.* to precipitate calcium. Urease-positive *Helicobacter spp.* precipitate calcium, while urease-negative *Helicobacter* species cannot[61]. This finding suggests that gallstone formation may be induced by *Helicobacter spp.*, which precipitate calcium directly *via* urease activity. Nevertheless, there is scant evidence to

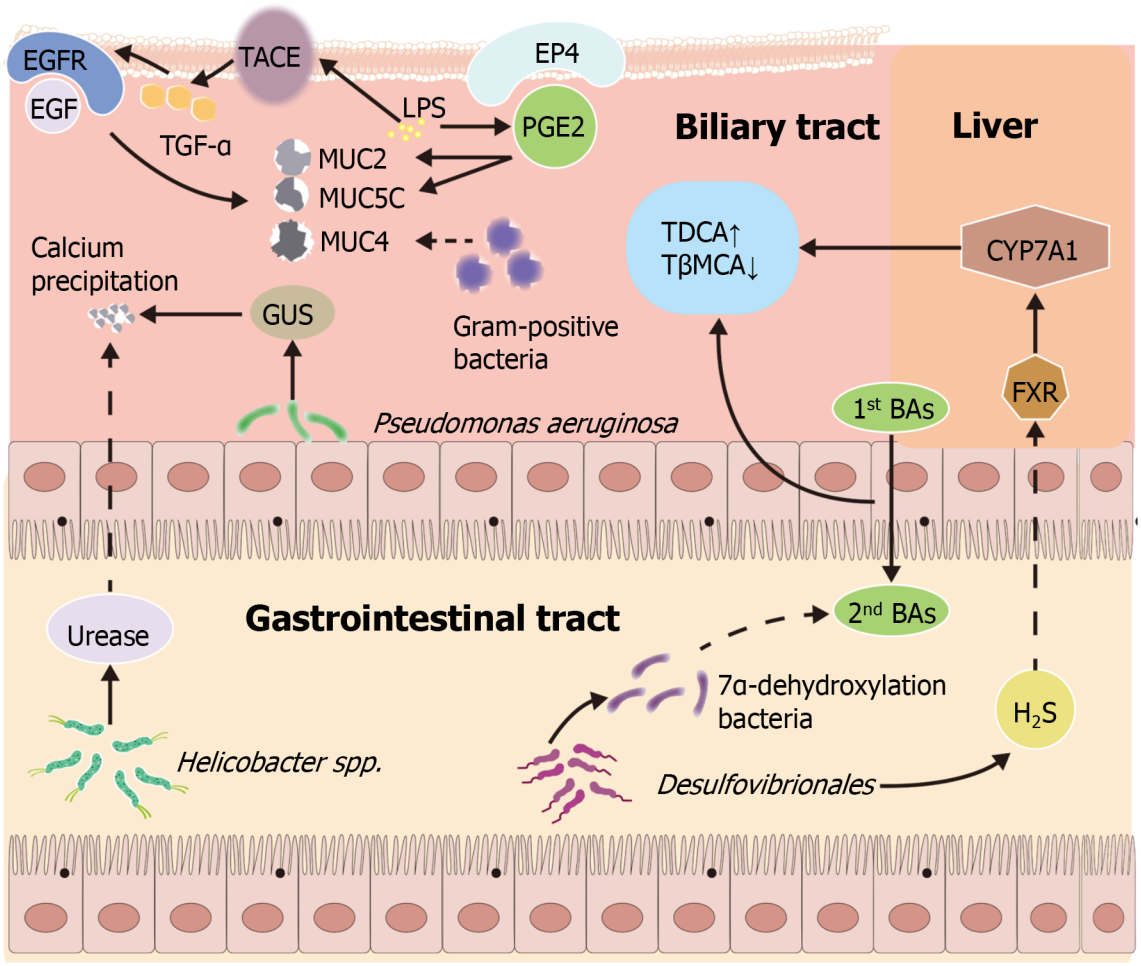

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Figure 1 The underlying microbial mechanisms of gallstone formation. The gastrointestinal microbiome may drive cholelithogenesis by: (1) The fecal microbiome enriched in *Desulfovibrionales* led to an increase of 7α-dehydroxylation bacteria, thus converting primary bile acids (Bas) to secondary BAs. Attributed to the regulation of the FXR-CYP7A1 pathway, *Desulfovibrionales* increased taurodeoxycholic acid and decreased Tauro-β-muricholic acid, which was induced by the production of H_2S; (2) Lipopolysaccharide upregulated mucins *via* TACE/TGF-α/EGFR pathway and EP4/p38MAPK pathway; (3) Gram-positive bacteria contributed to the enriched expression of mucin 4 and subsequently modulated calcification; (4) *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa* produced exogenous GUS to induce the hydrolysis of bilirubin diglucuronides, thus accelerating precipitation of calcium bilirubinate; and (5) *Helicobacter spp.* precipitated calcium *via* the urease activity. BA: Bile acids; CYP7A1: Cholesterol 7α-hydroxylase; EGFR: Epidermal growth factor receptor; EP4: E-prostanoid receptor 4; FXR: Farnesoid X receptor; GUS: β-glucuronidase; LPS: Lipopolysaccharide; MUC: Mucin; PGE2: Prostaglandin E2; PL: Phospholipase; TACE: Tumor necrosis factor-α converting enzyme; TβMCA: Tauro-β-muricholic acid; TDCA: Taurodeoxycholic acid; TGF: Transforming growth factor.

support causality in mechanisms for the GI microbiome contributing to cholelithogenesis as described above (Figure 1), and the mechanistic links between pathobionts and cholelithiasis formation require further exploration.

INFLUENCE OF HOST FACTORS ON THE MICROBIOME ASSOCIATED WITH CHOLELITHIASIS

Diet and lifestyle

The interactions and mutual influences between diet and GI microbiota are well known. Nevertheless, there is little information about the interactions between diet and biliary microbiota. The relationship between diet, biliary microbiota, and cholelithiasis is intricate. A case-control study compared the diet and biliary microbiota of patients and healthy people with cholelithiasis to identify potential associations. The authors found that the intake of dairy products was inversely associated with the relative abundance of the phylum *Bacteroidetes*, the family *Bacteroidaceae*, and the genus *Bacteroides* in bile. In contrast, seafood and meats were positively associated with the relative abundance of the family *Pasteurellaceae*[62].

Age

Epidemiological studies indicated that age, gender, pregnancy, rapid weight loss, excessive obesity, and diabetes are the primary risk factors for gallstones[63]. The prevalence of gallstone disease progressively increases with age[35]. Age impacts the composition of the human microbiome, potentially *via* the influence of health conditions, medication use, and lifestyle factors[64]. A retrospective study investigated positive bile samples from patients with biliopancreatic system diseases and found that age was positively associated with gram-negative bacterial infections and negatively related to gram-positive bacterial infections in bile[65].

History of cholecystectomy

Guidelines recommend endoscopic sphincterotomy (EST) and stone extraction for treating bile duct stones patients and cholecystectomy as the first-line treatment of symptomatic cholelithiasis[66,67]. Cholecystectomy alters the communication between the bile and intestine, altering the BA metabolism pathway and the intestinal microbiota. Several studies explored the effect of cholecystectomy on the GI microbiota. One study compared the composition of gut microbiota before and after cholecystectomy. Post-cholecystectomy patients showed significant enrichment in the phylum *Bacteroidetes*[34] and a reduction in the genus *Faecalibacterium*[68]. Other studies compared the gut microbiota of volunteers with and without cholecystectomy. Compared to controls, post-cholecystectomy patients had a lower relative abundance of the genera *Prevotella*, *Desulfovibrio*, *Barnesiella*, *Paludibacter*, and *Alistipes*[69] and a higher relative abundance of the *Blautia obeum* and *Veillonella parvula*, which are members of the phylum *Firmicutes*[70]. Reductions of *Candida albicans* and enrichments of *Candida glabrata* and *Aspergillus unassigned* were also reported[71]. These findings suggest that cholecystectomy affects the GI microbiota.

History of EST

EST is an invasive procedure recommended for treating bile duct stones. When comparing the biliary microbial composition of choledocholithiasis patients with or without a history of EST, significant differences were found between these groups; the genus *Pyramidobacter* showed positive associations with previous EST[72]. After EST, sphincter of Oddi laxity (SOL) resulted in duodenal content flow into the bile duct, altering the biliary microbiome. In two case-control studies, cholelithiasis patients with and without SOL significantly differed in the biliary microbiome[73,74]. Compared with those without SOL, cholangiolithiasis patients with SOL had increased phylotypes of family, including *Desulfovibrionaceae* and *Shewanellaceae*, and a larger abundance of *Bilophila* and *Shewanella algae*[73]. Compared with those without SOL, there was an enrichment of *Rhizobiaceae* in choledocholithiasis patients with SOL [74]. These findings suggest that SOL after EST plays a pivotal role in the bile duct microenvironment of cholelithiasis patients.

THE POTENTIAL MICROBIOME PREVENTIVE TARGETS IN CHOLELITHIASIS

Management of cholelithiasis depends on the gallstone location. Patients with gallbladder stones are usually treated with cholecystectomy and medical dissolution, whereas patients with extrahepatic bile duct stones are usually treated with EST or endoscopic papillary balloon dilation. Despite many strategies for cholelithiasis, efficacious methods of prevention are still needed. Oral administration of ursodeoxycholic acid, statins, and ezetimibe can prevent gallstones[75]. Given the cost-benefit ratios, oral administration of ursodeoxycholic acid or statins is not recommended for cholelithiasis prevention [36]. In recent years, the GI microbiome has emerged as one of the critical regulators of gallstone formation. Therefore, regulation of the host GI microbiome might be a method to prevent cholelithiasis. Some prevention strategies might include *Lactobacilli*, nanoscale iron sulfide (nFeS), and Astragalus polysaccharide.

Lactobacillus

Lactobacillus species are among the most common probiotics and are associated with health benefits, including antimicrobial activity and tumor suppression[76]. Indeed, many intervention studies using *Lactobacillus* showed promising results[77], including on cholesterol, triglyceride, and low-density lipoprotein levels[78]. Investigators demonstrated the preventive effects of *Lactobacillus* on gallstone formation in a murine model based on the probiotic's hypocholesterolemic properties. The mechanism by which *Lactobacillus* targets the GI microbiome and attenuates cholesterol gallstones was elucidated. Oh and colleagues found that *Lactobacillus acidophilus* ATCC 43121 had a hypocholesterolemic effect by decreasing the expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in the liver, which in turn reduces the expression of MUC5AC and MUC5B in the gallbladder[79]. Ye and colleagues found that *Lactobacillus reuteri* CGMCC 17942 and *Lactobacillus plantarum* CGMCC 14407 contribute to BA redistribution through the activation of the FXR pathway[80]. These findings suggest that supplementation with *Lactobacilli* might prevent cholesterol gallstone formation. However, further clinical

trials are needed to develop this approach as a probiotic supplement to prevent human cholelithiasis.

nFeS

Antimicrobial clinical management with bacteria or bacterial biofilms may benefit patients with gallbladder stones; nFeS is a nanomaterial with high levels of antibacterial efficacy. The evidence suggests that oral administration of nFeS supernatants significantly reduces bacterial activity and disrupts biofilm structure, inhibiting gallstone formation[81]. In a mouse model of cholelithiasis, oral administration of nFeS supernatants resulted in approximately twice the antibacterial efficacy of oral ciprofloxacin. Moreover, nFeS significantly cleared gallbladder stones compared with controls.

Astragalus polysaccharide

Astragalus polysaccharide is a natural macromolecule extracted from a standard traditional Chinese medicine known as the "Astragalus," which has immunomodulatory, anti-inflammatory, and anti-cancer effects in several diseases, including kidney stones[82], ulcerative colitis[83], constipation[84], and lung adenocarcinoma[85]. A recent study reported that Astragalus polysaccharide had beneficial effects on ameliorating the formation of cholesterol gallstones and reversing GI dysbiosis in mice[84]. Gallstone mice with Astragalus polysaccharide supplementation had a higher relative abundance of *Bacteroidota* and a lower relative abundance of *Vemucomicrobiota* at the phylum level compared to controls. These results suggest that Astragalus inhibits gallstone formation by improving intestinal microbial diversity.

CONCLUSION

These findings suggest a significant alteration of the GI microbiome in cholelithiasis patients. The GI microbiome is involved in the pathogenesis of cholelithiasis through several pathways: Biliary microbiome induces gallstone formation by regulating BA metabolism; *Helicobacter* species induce gallstone formation by precipitating calcium; LPS upregulates mucins *via* the tumor necrosis factor-α converting enzyme/transforming growth factor-α/epidermal growth factor receptor pathway and the EP4/p38MAPK pathway; GUS and PL accelerate precipitation of calcium bilirubinate. Nevertheless, the mechanisms for the GI microbiome contributing to cholelithogenesis lack evidence to support causality. The composition of the GI microbiome could be regulated in individuals with cholelithiasis by surgery, SOL, age, diet, and lifestyle. These modifiable factors for cholelithiasis may be crucial to prevent cholelithiasis. Given the regulability of the GI microbiota, studies should explore microbiome-targeting interventions for preventing cholelithiasis, including *Lactobacilli*, nFeS, and Astragalus polysaccharide.

Studies on cholelithiasis in the GI microbiome are mostly single-omics types, whereas multi-omics studies, including genome, epigenome, transcriptome, proteomics, and metabolomics, are limited. The field of the human microbiome in cholelithiasis is relatively young and limited to the bacterial microbiome (*i.e.*, there is no study of the mycobiome and virome). Existing studies are heterogeneous, possibly due to the influence of disease states, disease types, sample types and sites, gallstone components, and medical intervention. They fail accurately to characterize the structural, functional, and metabolic features of the GI microbiomes in cholelithiasis, and most lack validation cohorts. Moreover, these results do not test the specific strains, functional genes, and metabolites identified by screening validation *in vitro*, preventing in-depth studies on the pathogenesis of cholelithiasis. Consequently, longitudinal human intervention and in-depth analysis of the mechanism are needed to address the critical question of causality. If host-microbiome interactions are to be targeted as pathogenesis of cholelithiasis, well-designed mechanistic studies of the interactions between the GI microbiome and host are required. Such studies might identify causality between the GI microbiome and gallstone formation.

FOOTNOTES

Author contributions: Wang ZK established the design and conception of the paper; Dan WY and Wang ZK searched the literature and drafted the first manuscript; Wang ZK, Peng LH, and Sun G checked the manuscript and critically revised the important intellectual content in this manuscript; Yang YS contributed to manuscript revision; all authors have read and agreed to the published version of the manuscript.

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Country/Territory of origin: China

ORCID number: Wan-Yue Dan 0000-0001-9705-2373; Yun-Sheng Yang 0000-0001-7895-7708; Li-Hua Peng 0000-0002-8549-6994; Gang Sun 0000-0001-8594-557X; Zi-Kai Wang 0000-0002-6293-7179.

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ORIGINAL ARTICLE

Retrospective Study

Quantitative parameters in novel spectral computed tomography: Assessment of Ki-67 expression in patients with gastric adenocarcinoma

Li-Ting Mao, Wei-Cui Chen, Jian-Ye Lu, Han-Liang Zhang, Yong-Song Ye, Yu Zhang, Bo Liu, Wei-Wei Deng, Xian Liu

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Li-Ting Mao, Wei-Cui Chen, Jian-Ye Lu, Han-Liang Zhang, Yong-Song Ye, Bo Liu, Xian Liu, Department of Radiology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, Guangdong Province, China

Yu Zhang, Department of Pathology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, Guangdong Province, China

Wei-Wei Deng, Department of Scientific Research, Clinical & Technical Support, Philips Healthcare China, Shanghai 200040, China

Corresponding author: Xian Liu, PhD, Professor, Department of Radiology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, No. 111 Dade Road, Yuexiu District, Guangzhou 510120, Guangdong Province, China. liuxian74@hotmail.com

Abstract

BACKGROUND

The level of Ki-67 expression has served as a prognostic factor in gastric cancer. The quantitative parameters based on the novel dual-layer spectral detector computed tomography (DLSDCT) in discriminating the Ki-67 expression status are unclear.

AIM

To investigate the diagnostic ability of DLSDCT-derived parameters for Ki-67 expression status in gastric carcinoma (GC).

METHODS

Dual-phase enhanced abdominal DLSDCT was performed preoperatively in 108 patients with gastric adenocarcinoma. Primary tumor monoenergetic CT attenuation value at 40-100 kilo electron volt (kev), the slope of the spectral curve (λ_{HU}), iodine concentration (IC), normalized IC (nIC), effective atomic number (Z^{eff}) and normalized Z^{eff} (nZ^{eff}) in the arterial phase (AP) and venous phase (VP) were retrospectively compared between patients with low and high Ki-67 expression in gastric adenocarcinoma. Spearman's correlation coefficient was used to analyze the association between the above parameters and Ki-67 expression status. Receiver operating characteristic (ROC) curve analysis was performed to compare

the diagnostic efficacy of the statistically significant parameters between two groups.

RESULTS

Thirty-seven and 71 patients were classified as having low and high Ki-67 expression, respectively. $CT_{40\ kev\text{-}VP}$, $CT_{70\ kev\text{-}VP}$, $CT_{100\ kev\text{-}VP}$, and Z^{eff}-related parameters were significantly higher, but IC-related parameters were lower in the group with low Ki-67 expression status than the group with high Ki-67 expression status, and other analyzed parameters showed no statistical difference between the two groups. Spearman's correlation analysis showed that $CT_{40\ kev\text{-}VP}$, $CT_{70\ kev\text{-}VP}$, $CT_{100\ kev\text{-}VP}$, Z^{eff}, and n Z^{eff} exhibited a negative correlation with Ki-67 status, whereas IC and nIC had positive correlation with Ki-67 status. The ROC analysis demonstrated that the multi-variable model of spectral parameters performed well in identifying the Ki-67 status [area under the curve (AUC) = 0.967; sensitivity 95.77%; specificity 91.89%)]. Nevertheless, the differentiating capabilities of single-variable model were moderate (AUC value 0.630 - 0.835). In addition, the nZ^{eff}_{VP} and nIC_{VP} (AUC 0.835 and 0.805) showed better performance than $CT_{40\ kev\text{-}VP}$, $CT_{70\ kev\text{-}VP}$ and $CT_{100\ kev\text{-}VP}$ (AUC 0.630, 0.631 and 0.662) in discriminating the Ki-67 status.

CONCLUSION

Quantitative spectral parameters are feasible to distinguish low and high Ki-67 expression in gastric adenocarcinoma. Z^{eff} and IC may be useful parameters for evaluating the Ki-67 expression.

Key Words: Spectral computer tomography; Quantitative parameters; Gastric carcinoma; Iodine concentration; Effective atomic number; Ki-67 expression

Core Tip: This is a retrospective study to preoperatively distinguish the expression of Ki-67 index based on the parameters of spectral computer tomography (CT) in patients with gastric adenocarcinoma. The CT attenuation of virtual monoenergetic images in venous phase and effective atomic number were negatively related to the expression of Ki-67, while iodine concentration exhibited positive associations with it. Multi-variable model of spectral parameters exhibited a better diagnostic efficiency than other single-variable model of spectral parameter in discriminating low and high Ki-67 expression in gastric adenocarcinoma.

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INTRODUCTION

Gastric carcinoma (GC) is the fifth commonest malignancy and the fourth most predominant cause of cancer-related mortality worldwide according to the Global Cancer Statistics 2020[1]. Although the global age-standardized rates of incidence and mortality presented a slight decrease from 1990 to 2019, China had a high incidence-mortality ratio (0.845) and five-year prevalence (27.6/100 000)[2]. A large number of GC cases are found at the advanced stage and have a relatively poor prognosis, with an overall survival rate of 25% worldwide[3]. The high proportion of tumor metastasis, intratumor heterogeneity and chemotherapeutic resistance leads to unfavorable survival outcomes in patients with GC. Conventionally, the tumor-node-metastasis (TNM) stage, histologic classification and differentiation are the major prognostic indicators for GC[4]. In addition, some oncogenic protein markers, such as antigen Ki-67, have been associated with the prognosis of GC patients[5].

Cell proliferation is a distinguishing feature of cancer. The Ki-67 protein, a nucleus-associated antigen, which is a convenient and reproducible biomarker in this process, is expressed during the cell proliferation cycle including G1, S, G2, and mitosis phases[6]. Some studies have demonstrated that Ki-67 proliferation index could be a potential indicator to predict the prognosis and identify high-risk GC cases, and was relevant to TNM stage, tumor differentiation grade, invasion depth and distant metastasis[5,7,8]. Additionally, Ki-67 index was associated with chemotherapy efficacy in the advanced GC, because cytotoxic chemotherapeutic drugs are effective against tumor cells that have entered the cell division cycle. Thus, identifying the Ki-67 status noninvasively would be beneficial for predicting

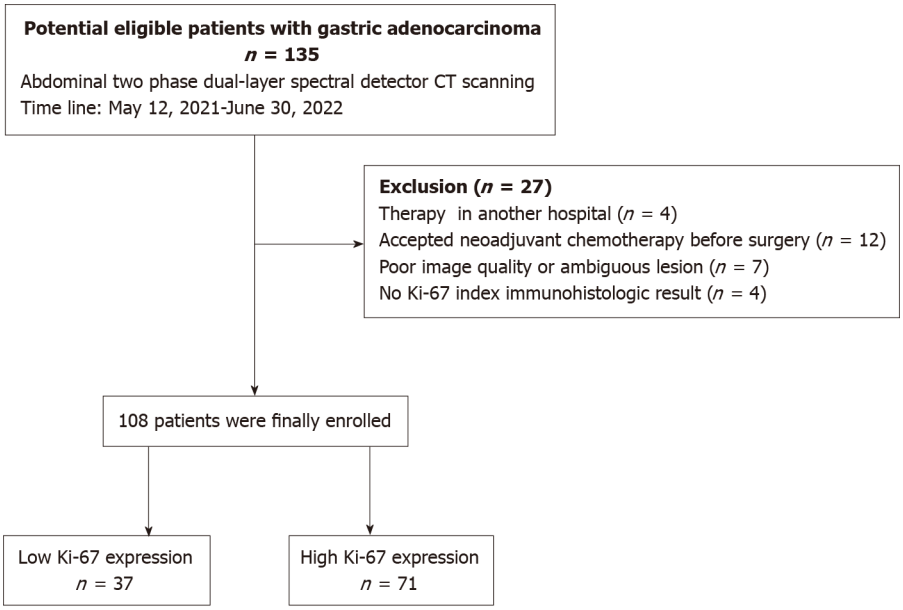

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Figure 1 The flow chart of the process of patient selection. CT: Computed tomography.

the prognosis and chemotherapeutic response in the patients with GC.

Recently, a novel dual-layer spectral detector CT (DLSDCT), which utilizes a detector-based dual-energy separation technology to acquire low- and high-energy data synchronously with two layers of detectors, makes for beam-hardening rectification, material decomposition, and image de-noising[9,10]. This system applies projection-space decomposition and generates various spectral basis images (SBIs) except for the conventional polyenergetic images, including material-specific images [iodine concentration (IC) images, virtual non-contrast images, effective atomic number (Z^{eff}) images] and energy-specific images [virtual monoenergetic images (VMIs)]. These spectral images are widely used for enhancing image contrast, improving lesion detection, characterizing materials, reducing artifact, and lowering radiation dosage[9-12]. Some studies have investigated the gastric lesions based on the IC derived from dual-energy spectral CT, such as distinguishing between malignant and benign gastric mucosal lesions[13], diagnosing GC and its histological type[14], and detecting serosal invasion of GC [15]. The latest study demonstrated that virtual monochromatic CT values were related to proliferative activity of tumor cells[16]. The Z^{eff} was correlated with Ki-67 expression in laryngeal squamous cell carcinoma[17], and could predict the vascular density of affected lesions[18]. However, only a few studies have applied the quantitative parameters [IC and normalized IC (nIC)] from spectral CT to evaluate the Ki-67 expression status in patients with gastric adenocarcinoma, but not including the CT attenuation value and Z^{eff} derived from the novel DLSDCT. Thus, the aim of this study is to explore the clinical usefulness of the quantitative parameters of novel DLSDCT in assessing the Ki-67 expression status in patients with gastric adenocarcinoma.

MATERIALS AND METHODS

Patients

This retrospective study was approved by Institutional Review Board of our hospital, with a waiver for written informed consent. From May 2021 to June 2022, 135 patients diagnosed with gastric adenocarcinoma through endoscopic biopsy underwent non-contrast and contrast-enhanced CT scans on a DLSDCT scanner (IQon Spectral CT, Philips Healthcare, Best, The Netherlands). Twenty-eight cases were excluded for the following factors: (1) Transferred to another hospital for further treatment (n = 4); (2) Accepted or required neo-adjuvant chemotherapy prior to surgery (n = 12); (3) Poor image quality or the lesion is ambiguous on the image (n = 7); or (4) Lack of the Ki-67 index immunohistologic result (n = 4). Finally, 108 patients were analyzed in this study. The detailed procedure of patient selection is shown in Figure 1.

Spectral CT imaging protocol

The interval between the DLSDCT examination and surgery was less than one week. Before CT examination, patients were required to be fast for 6-8 h and drank 800-1000 mL of water. The scan ranged from the diaphragm to the symphysis pubis in a supine position and cranio-caudal direction.

Nonionic contrast agent (Ultravist, Byer HealthCare) (370 mg/mL, 80 mL) was injected intravenously at a flow rate of 2.5 mL/s, with an automated injector (Stellant, Medrad, Byer HealthCare), following 30 mL of normal saline flushing at the same flow rate. Using bolus-tracking technique, the arterial phase (AP) scan was triggered at a threshold of 200 hounsfield unit (HU) and an additional delay of 6 s. The venous phase (VP) images were respectively collected at 35 s after injecting the contrast agent.

CT scan parameters were as follows: Tube voltage 120 kVp, automatic tube current 37-84 mAs, detector collimation 64 mm × 0.625 mm, reconstruction matrix 512 × 512. Conventional and SBI were reconstructed using the iDose 4 algorithm (Philips Healthcare). All CT images were reconstructed with a slice thickness of 1 mm and an increment of 1 mm, using a standard kernel. All image data were transferred to a workstation (IntelliSpace Portal, version 10.1, Philips Healthcare) for post-processing and analysis.

Image analysis

The image analysis was conducted by two gastrointestinal radiologists (reader 1 and reader 2, with 10 and 26 years of experience, respectively), who were blind to pathological results. The two-dimensional region-of-interest (ROI) was drawn on lesion manually, according to the following principles: (1) Polygon ROIs covered the enhanced areas of the lesions as much as possible; (2) Be careful to avoid the areas of necrosis, calcification, and vessels; (3) All lesions were measured on three consecutive axial layers by the same evaluator, and average values were calculated; and (4) The size, form, and position of the ROIs were maintained consistent between two phases images, by applying the copy-and-paste function of the workstation. A circular ROI was placed in the abdominal aorta parallel with lesion. The intraclass correlation coefficient (ICC) between the two radiologists was calculated. The final results of all ROIs were measured by the radiologist with 10 years' experience.

The following quantitative spectral parameters were automatically calculated using the post-processing software: The CT attenuation values of monochromatic images [40 kilo electron volt (kev), 70 kev and 100 kev], IC, and the Z^{eff}. Additionally, three related parameters were measured: (1) The nIC was computed as Nic = IC_{lesion}/IC_{aorta}, where IC_{lesion} and IC_{aorta} are the ICs of the lesions and abdominal aorta, which help to minimize individual variation; (2) The normalized Z^{eff} (nZ^{eff}) was counted similarly to nIC, $nZ^{eff} = Z^{eff}_{lesion}/Z^{eff}_{aorta}$; and (3) The slope of the spectral curve (λ_{HU}) was calculated as the CT attenuation values, $\lambda_{HU} = (CT_{40\,kev} - CT_{70\,kev})/30$, where $CT_{40\,kev}$ and $CT_{70\,kev}$ are the attenuation of the tumors at 40 kev and 70 kev monochromatic images, respectively[19]. Two phases (AP and VP) of CT attenuation value, IC, nIC, Z^{eff}, nZ^{eff}, and λ_{HU} were measured.

Histopathology evaluation

A pathologist with 22 years of experience (Yu Zhang) conducted the pathologic analysis. The TNM staging was determined on the basis of the American Joint Committee on Cancer (AJCC) 8th manual of gastric cancer[20]. The Ki-67 proliferation index was evaluated according to the normal immunohistochemistry process and evaluated by the pathologist blindly. The Ki-67 polyclonal antibody used (Roche #) was produced by Shanghai Rebiosci Biotech Co., Ltd. The specimens were analyzed in a high power field (× 400). The pathologist selected five fields of view randomly and each region was observed. Then 100 cells were selected in each field and the number and intensity of positively stained cells were counted. Finally, an average number of five fields were recorded. The Ki-67 indexes were categorized as low expression (< 50% positive cells) or high expression (≥ 50% positive cells), according to relevant reports[7,21].

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software version 19.4.1 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2020). Continuous variables were presented as the mean ± SD, and categorical variables as proportions. ICC analyses were performed with the data of 20 patients to evaluate the reliability of spectral parameters measurement. An ICC > 0.75 was considered good.

The Shapiro-Wilk test was used to test the normality of data distributions. The Student's *t*-test was employed to analyze the differences in clinical demographics and imaging parameters between low and high Ki-67 expressing status. Spearman's correlation coefficient was used to assess the correlation between the quantitative imaging parameters and the Ki-67 expression status.

Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic efficacy in classifying Ki-67 statuses. Area under the curve (AUC) and their 95% confidence intervals (CIs) were determined using unstratified bootstrap replicates 10 000 times. The optimal cutoff value was determined according to the Youden index. The difference between ROC curves were evaluated by pairwise comparison test. A two-sided *P* value of less than 0.05 was regarded statistically significant.

Table 1 Inter-reader agreement of spectral parameters measurement

Parameters	ICC (95%CI)	
	AP	VP
Z^{eff}_{lesion}	0.88 (0.71, 0.95)	0.86 (0.69, 0.94)
Z^{eff}_{aorta}	0.94 (0.85, 0.97)	0.95 (0.88, 0.98)
IC_{lesion} (mg/mL)	0.86 (0.56, 0.95)	0.87 (0.73, 0.95)
IC_{aorta} (mg/mL)	0.98 (0.94, 0.99)	0.95 (0.86, 0.98)
$CT_{40\ kev}$ (HU)	0.95 (0.88, 0.98)	0.96 (0.79, 0.99)
$CT_{70\ kev}$ (HU)	0.92 (0.78, 0.97)	0.91 (0.50, 0.98)
$CT_{100\ kev}$ (HU)	0.86 (0.69, 0.94)	0.89 (0.70, 0.96)

ICC: Intraclass correlation coefficient; Z^{eff}_{lesion} and Z^{eff}_{aorta}: The effective atomic number of lesion and aorta; $CT_{40\ kev}$, $CT_{70\ kev}$ and $CT_{100\ kev}$: The computed tomography attenuation value of 40 kilo electron volt (kev), 70 kev and 100 kev monochromatic images; IC_{lesion} and IC_{aorta}: The iodine concentration of lesion and aorta; HU: Hounsfield unit; AP: Arterial phase; VP: Venous phase; 95%CI: 95% confidence interval.

RESULTS

Patient demographics and histopathological findings

A total of 108 patients (mean age 61.9 years, range 34-85 years) were analyzed, consisting of 46 females and 62 males. The gastric tumors were located in the antrum in 48 cases (44.4%), in the corpus in 24 cases (22.2%), in the fundus in 11 cases (10.1%), in the antrum and corpus in 9 cases (8.3%), in the gastric angle in 9 cases (8.3%), and in the cardia in 7 case (6.4%).

Referring to the pathologic TNM staging of GC (AJCC 8th edition), 27 patients had gastric cancer of less than stage pT2, 32 patients had stage pT3 gastric cancer, and 49 patients had stage T4 gastric cancer. Twenty-six patients had no regional lymph node metastasis, whereas 24 patients had less than three lymph nodes invaded (pN1), 30 patients had 3-6 regional lymph nodes invaded (pN2), and 28 patients had seven or more regional lymph nodes invaded (pN3). The immunohistochemical staining results revealed that 37 cases were categorized as having low expression of the Ki-67, while 71 cases were categorized as having high expression. According to the World Health Organization grading criteria, 29 (26.8%) and 79 (73.1%) tumors were respectively classified as moderately and poorly differentiated adenocarcinoma.

Reliability of measurements

The ICC values for Z^{eff}_{lesion}, Z^{eff}_{aorta}, IC_{lesion}, IC_{aorta}, $CT_{40\ kev}$, $CT_{70\ kev}$, $CT_{100\ kev}$ in AP and VP were all more than 0.85, and the specific values are shown in Table 1.

Correlation between spectral parameters and Ki-67 status

Z^{eff} and nZ^{eff} in AP and VP presented a moderate negative correlation with Ki-67 status ($P < 0.001$), whereas the IC and nIC in AP and VP were moderately positive-correlated with Ki-67 status ($P < 0.001$). The $CT_{40\ kev\text{-}VP}$, $CT_{70\ kev\text{-}VP}$ and $CT_{100\ kev\text{-}VP}$ were weakly negative-correlated with Ki-67 status (all $P < 0.05$). However, the $CT_{40\ kev\text{-}AP}$, $CT_{70\ kev\text{-}AP}$, $CT_{100\ kev\text{-}AP}$ and $\lambda_{HU\text{-}AP}$ and $\lambda_{HU\text{-}VP}$ were not correlated with Ki-67 expression (all $P > 0.05$). The correlation coefficients and 95%CIs are shown in Table 2.

Comparison of spectral parameters between different Ki-67 status

Table 3 and Figure 2 show the results of quantitative analysis. Compared with high Ki-67 status, the low Ki-67 status had higher Z^{eff}_{AP}, Z^{eff}_{VP}, nZ^{eff}_{AP}, nZ^{eff}_{VP}, $CT_{40\ kev\text{-}VP}$, $CT_{70\ kev\text{-}VP}$ and $CT_{100\ kev\text{-}VP}$, and had lower IC_{AP}, IC_{VP}, nIC_{AP} and nIC_{VP}. Although the $CT_{40\ kev\text{-}AP}$, $CT_{70\ kev\text{-}AP}$ and $CT_{100\ kev\text{-}AP}$ of low Ki-67 status were slightly higher than that of high Ki-67 status, it was not statistically significant. Similarly, no significant differences were found in the λ_{HU} of AP and VP between the two groups. Figures 3 and 4 show the spectral parameter images of two cases with high and low Ki-67 expression, respectively.

Diagnostic performance

Table 4 summarizes the results of ROC analysis for evaluating the diagnostic performance of 12 significant spectral parameters in discriminating the Ki-67 status. For diagnosing the high Ki-67 labeling index, the Z^{eff}, nZ^{eff}, IC and nIC in AP and VP performed moderate efficiency (AUC value, ranged from 0.747 to 0.835), and there were no significant differences among the AUC values of these parameters. Nevertheless, the $CT_{40\ kev\text{-}VP}$, $CT_{70\ kev\text{-}VP}$, and $CT_{100\ kev\text{-}VP}$ showed general differentiating capabilities (AUC value 0.630, 0.631, 0.662, respectively) in differentiating low from high Ki-67 expression in GC, and the

Table 2 Correlation between spectral parameters and Ki-67 status

Parameters		Ki-67 status	
		r (95%CI)	*P* value
AP	$CT_{40\ kev}$	-0.162 (-0.362, 0.043)	0.093
	$CT_{70\ kev}$	-0.097 (-0.290, 0.102)	0.316
	$CT_{100\ kev}$	-0.074 (-0.277, 0.117)	0.449
	λ_{HU}	-0.137 (-0.328, 0.056)	0.156
	Z^{eff}	-0.427 (-0.606, -0.208)	< 0.001
	nZ^{eff}	-0.487 (-0.649, -0.318)	< 0.001
	IC	0.409 (0.228, 0.567)	< 0.001
	nIC	0.449 (0.288, 0.598)	< 0.001
VP	$CT_{40\ kev}$	-0.213 (-0.397, -0.005)	0.027
	$CT_{70\ kev}$	-0.216 (-0.407, -0.004)	0.025
	$CT_{100\ kev}$	-0.266 (-0.438, -0.096)	0.005
	λ_{HU}	-0.090 (-0.278, 0.093)	0.354
	Z^{eff}	-0.455 (-0.647, -0.215)	< 0.001
	nZ^{eff}	-0.555 (-0.692, -0.403)	< 0.001
	IC	0.405 (0.223, 0.571)	< 0.001
	nIC	0.502 (0.355, 0.651)	< 0.001

Z^{eff}_{AP} and Z^{eff}_{VP}: The effective atomic number in the arterial and venous phase; nZ^{eff}_{AP} and nZ^{eff}_{VP}: The normalized effective atomic number in the arterial and venous phase; IC_{AP} and IC_{VP}: The iodine concentration in the arterial and venous phase; nIC_{AP} and nIC_{AP}: The normalized iodine concentration in the arterial and venous phase; $CT_{40\ kev}$, $CT_{70\ kev}$ and $CT_{100\ kev}$: The computed tomography attenuation value of 40 kilo electron volt (kev), 70 kev and 100 kev monochromatic images; λ_{HU}: The slope of the spectral curve; HU: Hounsfield unit; AP: Arterial phase; VP: Venous phase; 95%CI: 95% confidence interval.

AUC values of these parameters had no statistical differences. Comparing the AUC values of CT attenuation with that of the Z^{eff}- and IC-related parameters, the AUC value of nZ^{eff}_{AP} was higher than $CT_{40\ kev-VP}$ (0.796 *vs* 0.630, $P = 0.047$), and the AUC values of nZ^{eff}_{VP} and nIC_{VP} were higher than $CT_{40\ kev-VP}$, $CT_{70\ kev-VP}$ and $CT_{100\ kev-VP}$ ($P = 0.02$, 0.01, 0.009 and 0.03, 0.01, 0.02, respectively). In addition, the multivariable model ($CT_{70\ kev-VP}$, nZ^{eff}_{AP}, nZ^{eff}_{VP}, nIC_{AP}, nIC_{VP}) was selected for most powerful parameters by multivariate logistic regression, and the model demonstrated excellent efficiency (AUC = 0.967; sensitivity 95.77%; specificity 91.89%) in discriminating high expression of Ki-67 in GC.

DISCUSSION

The recently developed DLSDCT could quantitatively map the IC and Z^{eff} of the tissue in enhanced images, and offer CT attenuation values on a wide range of VMIs. In this study, we explored the association between quantitative parameters derived from DLSDCT and the Ki-67 labeling index of gastric adenocarcinoma. Our results revealed that the $CT_{40\ kev-VP}$, $CT_{70\ kev-VP}$, $CT_{100\ kev-VP}$, and Z^{eff}-related parameters were significantly higher, but IC-related parameters were lower in the group with low Ki-67 status. Additionally, the $CT_{40\ kev-VP}$, $CT_{70\ kev-VP}$, $CT_{100\ kev-VP}$, and Z^{eff}-related parameters exhibited negative correlations with Ki-67 status, whereas IC-related parameters positively correlated with it. These results were partially in agreement with previous reports that used different spectral CT systems, which demonstrated that IC and nIC were positively associated with Ki-67 status, and the values of nIC were higher in poorly differentiated gastric adenocarcinomas significantly[22,23].

We found a negative correlation between Z^{eff} and Ki-67 labeling index, which is seemingly at variance with a previous study that Z^{eff} was positively correlated with Ki-67 expression in the laryngeal squamous cell carcinoma[17] and invasive breast cancer[24]. Z^{eff} reflects the total atomic numbers of complex or mixture of materials, and has a close relationship with fundamental properties of the elements[25]. Previous research indicated that the evaluation of Z^{eff} could be able to distinguish the different tissues showing similar attenuative properties at given energy[26]. On account of the concentrations of elements (Cl, K, Ca, Ti, Mn, Fe, Co, Cu, and Zn) are lower in the stomach cancerous tissue than normal tissue[25], gastric cancer tissue exhibits a lower Z^{eff} than its healthy counterpart[27]. It is

Table 3 Comparison of dual-layer spectral detector computed tomography-derived parameters between low and high Ki-67 expression status

	Parameters	Low Ki-67 status	High Ki-67 status	*t* value	*P* value
AP	$CT_{40\ kev}$	177.3 ± 43.9	162.3 ± 37.8	1.85	0.068
	$CT_{70\ kev}$	90.9 ± 20.1	85.5 ± 16.6	1.48	0.141
	$CT_{100\ kev}$	63.8 ± 12.7	61.7 ± 12.6	0.81	0.422
	λ_{HU}	2.88 ± 1.04	2.56 ± 0.98	1.58	0.118
	Z^{eff}	8.30 ± 0.19	8.21 ± 0.10	2.87	0.006
	nZ^{eff}	0.78 ± 0.04	0.74 ± 0.03	5.33	< 0.001
	IC (mg/mL)	1.61 ± 0.15	1.73 ± 0.19	-3.53	0.001
	nIC	0.15 ± 0.02	0.19 ± 0.04	-5.88	< 0.001
VP	$CT_{40\ kev}$	265.9 ± 61.4	237.3 ± 45.8	2.50	0.016
	$CT_{70\ kev}$	126.5 ± 33.4	108.0 ± 20.9	3.07	0.003
	$CT_{100\ kev}$	84.3 ± 18.7	73.9 ± 17.2	3.71	0.005
	λ_{HU}	4.65 ± 1.43	4.31 ± 1.15	1.33	0.185
	Z^{eff}	8.75 ± 0.24	8.62 ± 0.15	3.04	0.004
	nZ^{eff}	0.87 ± 0.04	0.83 ± 0.03	6.59	< 0.001
	IC (mg/mL)	2.62 ± 0.17	2.75 ± 0.21	-3.40	0.001
	nIC	0.41 ± 0.04	0.46 ± 0.06	-5.87	< 0.001

$CT_{40\ kev}$, $CT_{70\ kev}$ and $CT_{100\ kev}$: The computed tomography attenuation values of at 40 kilo electron volt (kev), 70 kev and 100 kev respectively; λ_{HU}: The slope of the spectral curve; Z^{eff}: Effective atomic number; nZ^{eff}: The normalized effective atomic number; IC: Iodine concentration; nIC: Normalized iodine concentration; AP: Arterial phase; VP: Venous phase.

known that deficiency or excess of certain essential trace metals is relevant to carcinogenesis of the specific organs[27,28]. The abnormal levels of these elements lead to the discrepancy of toxicity and proliferation activity of cancer cells. We hypothesize that Z^{eff} difference between high and low Ki-67 expression status is more significant due to abnormal metal concentration than tumor heterogeneity and angiogenesis, especially in case of using nZ^{eff}, which eliminate individual differences in hemodynamics.

The blood vessels of tumors are supplied by tumor angiogenesis and invasion of vessels around the tumor. The degree of tumor angiogenesis is strongly linked to tumor growth, progression, and metastasis[29,30]. Wang *et al*[31] found that the degree of CT enhancement is correlated with tumor angiogenesis and the malignancy of the tumor. Compared to CT contrast enhancement, IC can quantitatively indicate the degree of tumor neovascularization and reflect the deposition of iodine in the tissue objectively[32]. In this study, we detected significantly higher IC and nIC values in the AP and VP of the high-expression Ki-67 group, indicating a richer blood supply in these tumors. Compared to IC, the normalized parameter nIC minimized hemodynamic variations between individuals, which could be more comparable among different groups. These findings are consistent with the fact that the high proliferative activity is accompanied by abundant angiogenesis.

In our study, we found no statistical difference in the CT attenuation values at 40-100 kev (at 30 kev interval) in AP between low- and high-expression Ki-67 groups. Likewise, the λ_{HU} of AP and VP between the two groups showed no significant differences, which was different from the result reported by Cheng *et al*[22], who found that the λ_{HU} values were significantly different among the low, medium and high level Ki-67 groups in both VP and delayed phase, and had positive correlation with Ki-67 level. We deemed that the discrepancy might be attributable to the grouping method of Ki-67 index and the constituent ratio of differentiation degree of the analyzed cases was distinct from our study. It is necessary to further explore the usefulness of λ_{HU} values.

When evaluating the diagnostic performance of spectral parameters in discriminating the Ki-67 status, the results from ROC analysis demonstrated that the multi-variable model of spectral parameters performed excellent capacity, with high sensitivity and specificity. In contrast, the single-variable model of Z^{eff}- and IC-related parameters demonstrated moderate efficiency, and 40-100 kev (at 30 kev interval) in VP showed general efficiency. Therefore, the parameters derived from DLSDCT were feasible for the prediction of Ki-67 expression in the gastric adenocarcinoma, which is of great significance in predicting prognosis and guiding treatment for the patients with GC.

Table 4 Receiver operator characteristic analysis of spectral parameters in differentiating Ki-67 low expression status from high expression status

		AUC (95%CI)	TV	YI	Sen (%)	Spe (%)	PPV (%)	NPV (%)	Acc (%)
AP	Z^{eff}	0.759 (0.668, 0.836)	≤ 8.27	0.67	85.92	81.08	92.96	37.84	71.30
	nZ^{eff}	0.796 (0.707, 0.867)	≤ 0.74	0.67	80.28	86.49	85.92	54.05	75.00
	IC	0.752 (0.660, 0.830)	> 1.63	0.60	78.87	81.08	88.73	21.62	65.74
	nIC	0.773 (0.683, 0.848)	> 0.16	0.61	74.65	86.49	81.69	48.65	70.37
VP	$CT_{40\ kev}$	0.630 (0.531, 0.721)	≤ 271.2	0.26	80.28	45.95	95.77	24.32	71.30
	$CT_{70\ kev}$	0.631 (0.533, 0.722)	≤ 138.8	0.31	92.96	37.84	94.37	32.43	73.15
	$CT_{100\ kev}$	0.662 (0.565, 0.750)	≤ 89.3	0.28	81.69	45.95	87.32	32.43	68.52
	Z^{eff}	0.777 (0.687, 0.851)	≤ 8.75	0.73	88.73	83.78	92.96	29.73	74.07
	nZ^{eff}	0.835 (0.751, 0.899)	≤ 0.83	0.68	78.87	89.19	85.92	51.35	74.07
	IC	0.747 (0.654, 0.826)	> 2.65	0.63	84.51	78.38	85.92	27.03	65.74
	nIC	0.805 (0.718, 0.875)	> 0.43	0.71	78.87	91.89	85.92	37.84	69.44
Multi-parameters		0.967 (0.913, 0.992)	-	0.88	95.77	91.89	97.18	86.49	93.52

AUC: Area under the curve; TV: Threshold value; YI: Youden index; Sen: Sensitivity; Spe: Specificity; NPV: Negative predictive value; PPV: Positive predictive value; Acc: Accuracy; Z^{eff}_{AP} and Z^{eff}_{VP}: The effective atomic number in the arterial and venous phase; nZ^{eff}_{AP} and nZ^{eff}_{VP}: The normalized effective atomic number in the arterial and venus phase; IC_{AP} and IC_{VP}: The iodine concentration in the arterial and venous phase; nIC_{AP} and nIC_{AP}: The normalized iodine concentration in the arterial and venous phase; $CT_{40\ kev}$, $CT_{70\ kev}$ and $CT_{100\ kev}$: The computed tomography attenuation values of at 40 kilo electron volt (kev), 70 kev and 100 kev respectively.

There were several limitations in our study. First, this is a retrospective study in which case grouping is not random, hence, an unconscious selection bias may exist. Second, a relatively small number of patients might overstate the consequence of association. Third, the number of cases with different degrees of differentiation was disproportionate, resulting in significance hard to achieve for partially analyzed variables. Fourth, our uniform standard of enhanced scanning protocol with DLSDCT may be different from other centers, thus, the results acquired from other vendors or different scanning parameters may not be directly concluded from our results. It demands further studies to confirm and outspread our preliminary results.

CONCLUSION

Quantitative spectral parameters are feasible to distinguish low and high Ki-67 expression in gastric adenocarcinoma. Multi-variable model of spectral parameters exhibited a better diagnostic efficiency than single-variable model of spectral parameter in discriminating low and high Ki-67 expression in gastric adenocarcinoma. Z^{eff} and IC derived from DLSDCT may be useful parameters for evaluating the Ki-67 proliferation index for gastric adenocarcinoma.

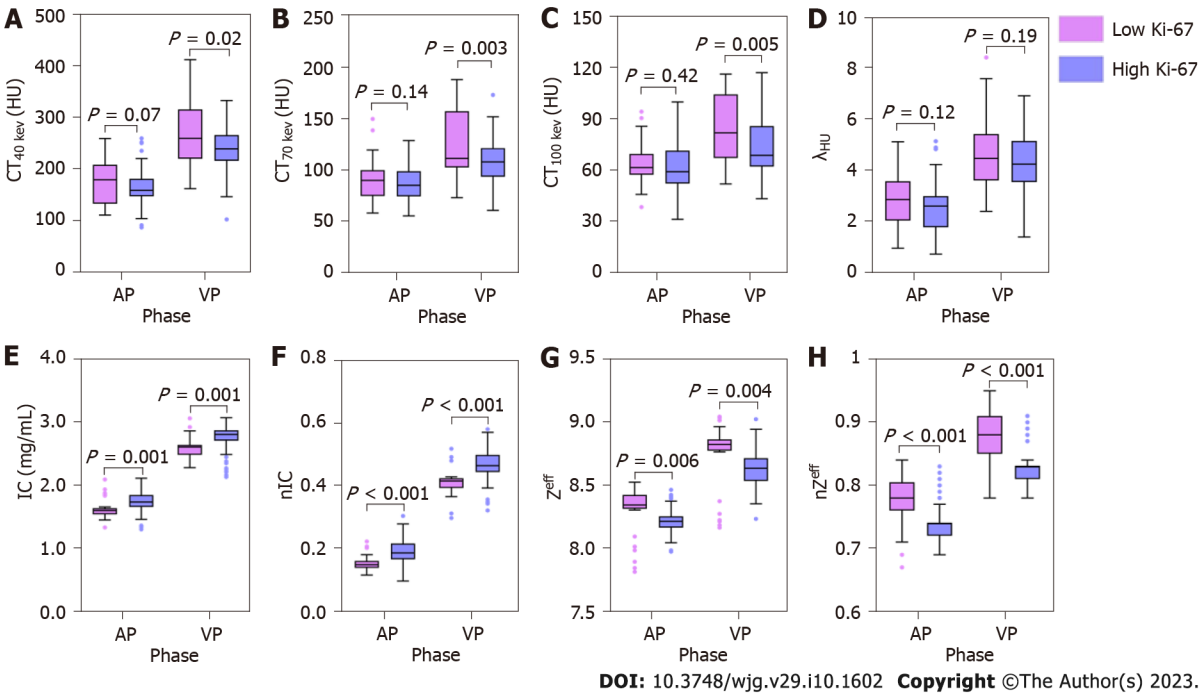

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Figure 2 Boxplots showing a comparison between low and high Ki-67 expression status demonstrated by computed tomography attenuation value of 40 kilo electron volt, 70 kilo electron volt, 100 kilo electron volt, slope of the spectral curve, iodine concentration, the normalized iodine concentration, effective atomic number, and normalized effective atomic number in both arterial phase and venous phase. A: Computed tomography attenuation value of 40 kev; B: Computed tomography attenuation value of 70; C: Computed tomography attenuation value of 100; D: The slope of the spectral curve; E: Iodine concentration; F: The normalized iodine concentration; G: Effective atomic number; H: Normalized effective atomic number. HU: Hounsfield unit; AP: Arterial phase; VP: Venous phase; IC: Iodine concentration; nIC: The normalized iodine concentration; $CT_{40\ kev}$, $CT_{70\ kev}$ and $CT_{100\ kev}$: The computed tomography attenuation value of 40 kev, 70 kev and 100 kev monochromatic images; Z^{eff}: Effective atomic number; nZ^{eff}: The normalized effective atomic number.

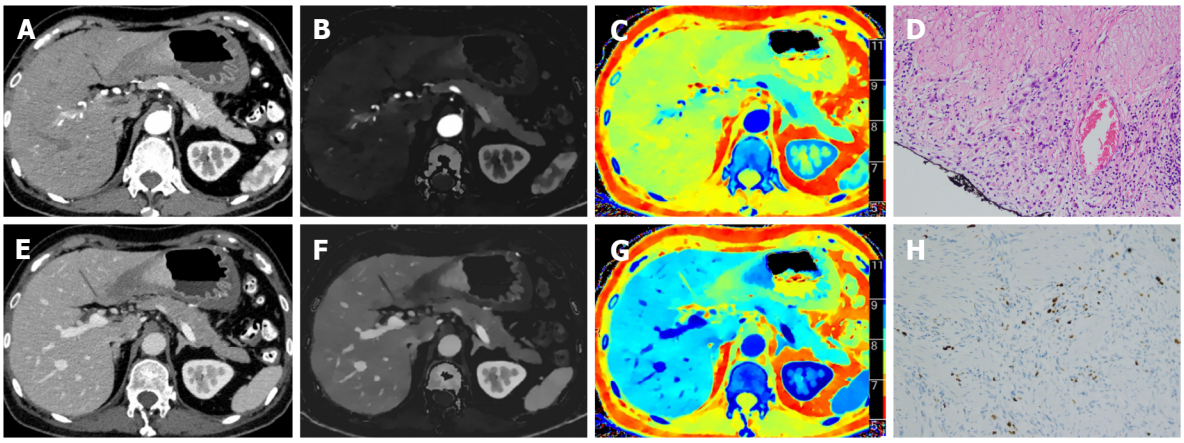

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Figure 3 A 57-year-old man with gastric adenocarcinoma in the antrum. A: The 70 kilo electron volt (kev) image in arterial phase (AP) show that the computed tomography attenuation value of 70 kev ($CT_{70\ kev}$) value of solid mass is 128.8 HU; B: The iodine map in AP show that the iodine concentration (IC) value of solid mass is 3.44 mg/mL; C: The effective atomic map in AP show that the effective atomic number (Z^{eff}) value of solid mass is 8.25; D: Histologic specimen shows a gastric adenocarcinoma of T4 staging in solid with HE staining (magnification, × 200); E: A 70 kev image in venous phase (VP) show that the CT_{70kev} value of solid mass is 146.9 HU; F: An iodine map in VP show that the IC value of solid mass is 3.32 mg/mL; G: An effective atomic map in VP show that the Z^{eff} value of solid mass is 8.65; H: Ki-67 immunohistochemical staining demonstrated that approximately 80% of cells were positive for nuclear staining (magnification, × 400).

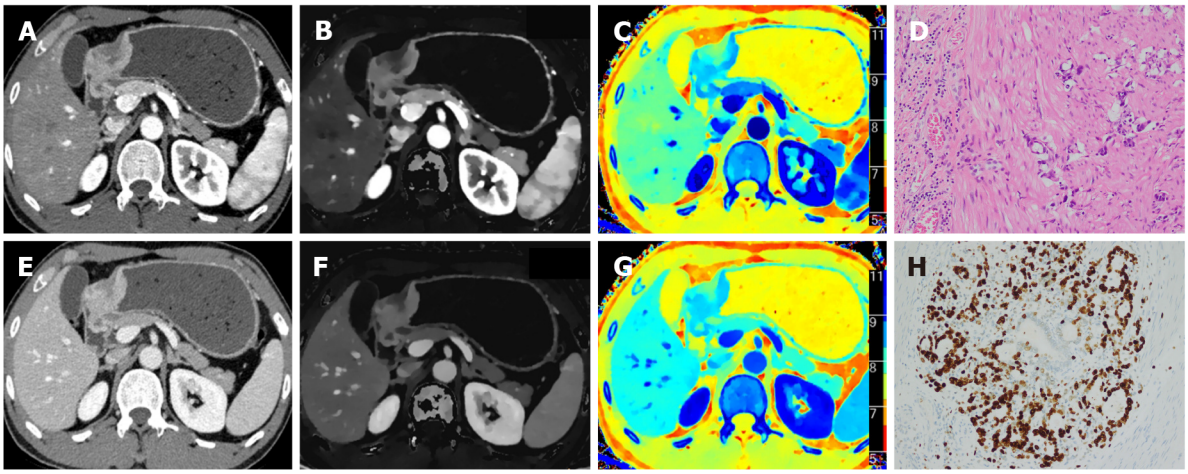

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Figure 4 A 65-year-old woman with gastric adenocarcinoma in the gastric angle. A: The 70 kilo electron volt (kev) image in arterial phase (AP) show that the computed tomography attenuation value of 70 kev ($CT_{70\ kev}$) value of solid mass is 79.6 HU; B: The iodine map in AP show that the iodine concentration (IC) value of solid mass is 1.38 mg/mL; C: The effective atomic map in AP show that the effective atomic number (Z^{eff}) value of solid mass is 8.36; D: Histologic specimen shows a gastric adenocarcinoma of T4 staging in solid with HE staining (magnification, × 200); E: A 70 kev image in venous phase (VP) show that the $CT_{70\ kev}$ value of solid mass is 158.3 HU; F: An iodine map in VP show that the IC value of solid mass is 2.52 mg/mL; G: An effective atomic map in VP show that the Z^{eff} value of solid mass is 9.07; H: Ki-67 immunohistochemical staining demonstrated that approximately 40% of cells were positive for nuclear staining (magnification, × 400).

ARTICLE HIGHLIGHTS

Research background

The level of Ki-67 expression is a valuable prognostic factor in gastric cancer. However, the quantitative parameters based on the novel dual-layer spectral detector computed tomography (DLSDCT) in discriminating the Ki-67 expression status are unclear.

Research motivation

The relationship between the Ki-67 expression in gastric carcinoma (GC) and part spectral parameters (including the effective atomic number (Z^{eff}) and the monoenergetic CT attenuation) is unclear.

Research objectives

This study aimed to investigate the diagnostic ability of DLSDCT-derived parameters for Ki-67 expression status in GC.

Research methods

Dual-phase enhanced abdominal CT was performed preoperatively in 108 patients with GC. The monoenergetic CT attenuation value at 40-100 kilo electron volt (kev), the slope of the spectral curve (λ_{HU}), iodine concentration (IC), normalized IC (nIC), Z^{eff} and normalized Z^{eff} (nZ^{eff}) in the arterial phase (AP) and venous phase (VP) were retrospectively compared between the groups of low and high Ki-67 expression status. The relationship between the spectral parameters and Ki-67 expression status were analyzed, and the diagnostic efficacy of the statistically significant parameters between the two groups was compared.

Research results

The low and high Ki-67 expression groups consisted of 37 and 71 patients respectively. $CT_{40\ kev\text{-}VP}$, $CT_{70\ kev\text{-}VP}$, $CT_{100\ kev\text{-}VP}$, and Z^{eff}-related parameters were significantly higher, but IC-related parameters were lower in the low Ki-67 expression group than in the high Ki-67 expression group. $CT_{40\ kev\text{-}VP}$, $CT_{70\ kev\text{-}VP}$, $CT_{100\ kev\text{-}VP}$, Z^{eff}, and nZ^{eff} exhibited negative correlations with Ki-67 status, whereas IC and nIC positively correlated with it. The results of receiver operating characteristic analysis showed that the multi-variable model of spectral parameters performed well in identifying the Ki-67 status [area under the curve (AUC) = 0.967; sensitivity 95.77%; specificity 91.89%]. Nevertheless, the differentiating capabilities of single-variable model were moderate (AUC value from 0.630 to 0.835).

Research conclusions

Z^{eff} and IC may be useful parameters for evaluating the Ki-67 expression in GC.

Research perspectives

The spectral CT images are prospective to provide the pathological information of Ki-67 expression of GC in the future.

FOOTNOTES

Author contributions: Mao LT, Chen WC and Liu B designed the research; Lu JY, Zhang HL, Ye YS, and Deng WW collected the data; Mao LT and Liu X analyzed the data and wrote the manuscript; and all authors have read and approved the final manuscript.

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ORCID number: Li-Ting Mao 0000-0003-2749-1850; Yong-Song Ye 0000-0003-1808-7418; Xian Liu 0000-0002-1662-477X.

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ORIGINAL ARTICLE

Retrospective Study

Clinical outcomes of lenvatinib plus transarterial chemoembolization with or without programmed death receptor-1 inhibitors in unresectable hepatocellular carcinoma

Yan-Yu Wang, Xu Yang, Yun-Chao Wang, Jun-Yu Long, Hui-Shan Sun, Yi-Ran Li, Zi-Yu Xun, Nan Zhang, Jing-Nan Xue, Cong Ning, Jun-Wei Zhang, Cheng-Pei Zhu, Long-Hao Zhang, Xiao-Bo Yang, Hai-Tao Zhao

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Yan-Yu Wang, Xu Yang, Yun-Chao Wang, Jun-Yu Long, Hui-Shan Sun, Yi-Ran Li, Zi-Yu Xun, Nan Zhang, Cong Ning, Jun-Wei Zhang, Cheng-Pei Zhu, Xiao-Bo Yang, Hai-Tao Zhao, Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China

Jing-Nan Xue, Long-Hao Zhang, Department of General Surgery, Digestive Disease Hospital, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou Province, China

Corresponding author: Hai-Tao Zhao, MD, PhD, Professor, Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 1, Shuaifuyuan, Dongcheng District, Beijing 100730, China. zhaoht@pumch.cn

Abstract

BACKGROUND

Programmed death receptor-1 (PD-1) inhibitors have been approved as second-line treatment regimen in hepatocellular carcinoma (HCC), but it is still worth studying whether patients can benefit from PD-1 inhibitors as first-line drugs combined with targeted drugs and locoregional therapy.

AIM

To estimate the clinical outcome of transarterial chemoembolization (TACE) and lenvatinib plus PD-1 inhibitors for patients with unresectable HCC (uHCC).

METHODS

We carried out retrospective research of 65 patients with uHCC who were treated at Peking Union Medical College Hospital from September 2017 to February 2022. 45 patients received the PD-1 inhibitors, lenvatinib, TACE (PD-1-Lenv-T) therapy, and 20 received the lenvatinib, TACE (Lenv-T) therapy. In terms of the dose of lenvatinib, 8 mg was given orally for patients weighing less than 60 kg and 12 mg for those weighing more than 60 kg. Of the patients in the PD-1 inhibitor combination group, 15 received Toripalimab, 14 received Toripalimab, 14 received Camrelizumab, 4 received Pembrolizumab, 9 received Sintilimab, and 2 received Nivolumab, 1 with Tislelizumab. According to the investigators' assessment,

TACE was performed every 4-6 wk when the patient had good hepatic function (Child-Pugh class A or B) until disease progression occurred. We evaluated the efficacy by the modified Response Evaluation Criteria in Solid Tumors (mRECIST criteria). We accessd the safety by the National Cancer Institute Common Terminology Criteria for Adverse Events, v 5.0. The key adverse events (AEs) after the initiation of combination therapy were observed.

RESULTS

Patients with uHCC who received PD-1-Lenv-T therapy (n = 45) had a clearly longer overall survival than those who underwent Lenv-T therapy (n = 20, 26.8 *vs* 14.0 mo; P = 0.027). The median progression-free survival time between the two treatment regimens was also measured {11.7 mo [95% confidence interval (CI): 7.7-15.7] in the PD-1-Lenv-T group *vs* 8.5 mo (95%CI: 3.0-13.9) in the Lenv-T group (P = 0.028)}. The objective response rates of the PD-1-Lenv-T group and Lenv-T group were 44.4% and 20% (P = 0.059) according to the mRECIST criteria, meanwhile the disease control rates were 93.3% and 64.0% (P = 0.003), respectively. The type and frequency of AEs showed little distinction between patients received the two treatment regimens.

CONCLUSION

Our results suggest that the early combination of PD-1 inhibitors has manageable toxicity and hopeful efficacy in patients with uHCC.

Key Words: Lenvatinib; Programmed death receptor-1 inhibitor; Immunotherapy; Hepatocellular carcinoma; Transarterial chemoembolization; Combination therapy

Core Tip: This retrospective research was designed to evaluate the treatment outcome and safety of transarterial chemoembolization (TACE) and lenvatinib plus programmed death receptor-1 (PD-1) inhibitors in the treatment of patients with unresectable hepatocellular carcinoma (uHCC). Patients with uHCC who underwent PD-1 inhibitors, lenvatinib, TACE therapy (n = 45) had a evidently longer overall survival than those who underrwent lenvatinib, TACE therapy (n = 20, 26.8 *vs* 14.0 mo; P = 0.027). Early combination of PD-1 inhibitors has manageable toxicity and hopeful efficacy in patients with uHCC.

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INTRODUCTION

Primary liver carcinoma is the sixth most common cancer type worldwide and leads to the third most cancer-related deaths[1]. The proportion of hepatocellular carcinoma (HCC) is approximately 80% in primary liver cancers[2]. Due to the powerful compensatory power of the liver, most patients with liver carcinoma are already in the advanced stage when they develop symptoms. Therefore, the systemic therapies of advanced HCC have attracted much attention.

Lenvatinib is an oral small molecule inhibitor of receptor tyrosine kinases [platelet-derived growth factor receptor (PDGFR), KIT, rearranged in transfection, fibroblast growth factor receptor (FGFR) 1-4 and vascular endothelial growth factor receptor (VEGFR) 1-3] that was applied for the first-line therapy of patients with unresectable HCC on the basis of the clinical outcomes from a randomized, multinational, open-label, noninferiority phase III trial[3]. In this clinical trial, lenvatinib demonstrated a better treatment effect than sorafenib. The overall survival (OS) with lenvatinib was 5 mo longer than that with sorafenib and the objective response rate (ORR) was approximately 2 times higher than that of sorafenib in hepatitis B virus (HBV) background subgroup analysis[3].

The main nutrients for liver cancer growth are supplied by the hepatic artery, which can lead to tumor ischemic necrosis by embolization[4]. Therefore, transarterial chemoembolization (TACE) is proposed as the standard therapeutic regimen for stage B HCC as classified by the Barcelona Clinic Liver Cancer (BCLC) staging system[5,6]. However, TACE also aggravates tumor hypoxia, resulting in the accumulation of hypoxia-inducible factor 1α, upregulating the expression of PDGF and VEGF,

which facilitates angiogenesis-induced collateral vessel formation, and contributes to tumor revascularization and locoregional recurrence[7-9]. Therefore, several clinical trials have made an attempt to associate TACE with systemic antiangiogenic treatment[10-12]. A randomized, multicenter prospective trial comparing TACE plus sorafenib and TACE alone in patients with liver cancer confirmed that in patients with unresectable HCC (uHCC), TACE plus sorafenib clearly improved progression-free survival (PFS) compared with TACE alone[12]. As another antiangiogenic drug, lenvatinib has significantly improved clinical efficacy in the remedy of uHCC with TACE plus lenvatinib compared with TACE monotherapy in previous studies[13,14].

Immunotherapy, as a systemic therapy that has attracted much attention in recent years, has also made significant progress in liver cancer[15]. Programmed death receptor-1 (PD-1) expresses on the surface of T cells, and is a key immunosuppressive transmembrane protein[16]. In the tumor microenvironment, cancer cells can express PD ligand 1 (PD-L1), which can bond to PD-1 to inhibit the function of T cells and reduce their killing effect on tumor cells[17]. Studies have shown that lenvatinib has a synergistic effect with immune checkpoint inhibitors (ICIs)[18]. VEGF is a highly expressed angiogenic factor in the tumor microenvironment of HCC that supports tumor growth and promotes immune rejection and is a key mediator in the immunosuppressive microenvironment[19]. Previous researches have suggested that lenvatinib can alleviate immunosuppression in the tumor microenvironment by inhibiting VEGF and can also increase T lymphocyte infiltration in the immunosuppressive microenvironment, providing an effective immunotherapeutic microenvironment for ICIs to function[20].

In conclusion, the combination of systemic and local treatment for liver cancer can synergistically kill tumors and prolong the survival of patients in various ways. Currently, PD-1 inhibitors have been applied as second-line remedy in HCC[21], and it is still worth studying whether patients can obtain clinical benefit from PD-1 inhibitors as first-line drugs combined with targeted drugs and local therapy. This retrospective research was designed to evaluate the clinical outcome and safety of TACE and lenvatinib plus PD-1 inhibitors in the treatment of uHCC and to determine whether the early combination of PD-1 inhibitors could benefit uHCC patients.

MATERIALS AND METHODS

Patients and study design

In this retrospective research, the clinical information and image data of HCC patients were collected from Peking Union Medical College Hospital from September 2017 to February 2022. uHCC was confirmed by more than two clinical experts according to the National Comprehensive Cancer Network guidelines. The main inclusion criteria were as the following: (1) Histologically or clinically confirmed HCC; (2) 1 or more measurable lesion by the modified Response Evaluation Criteria in Solid Tumors (mRECIST criteria); (3) BCLC stage A, B or C HCC; (4) Child-Pugh class scored as A (score 5-6) or B (score 7); (5) Eastern Cooperative Oncology Group performance status (ECOG-PS) score of 0-1; and (6) Prior resection or ablation was allowed. The exclusion criteria were as the following: (1) Secondary malignant tumor of liver; (2) Child-Pugh class C; and (3) Any contraindication to TACE, lenvatinib or PD-1 inhibitors.

Treatment

In this study, patients weighing less than 60 kg received a dose of 8 mg of lenvatinib, and those weighing more than 60 kg received a dose of 12 mg of lenvatinib orally once a day. Of the patients in the PD-1 inhibitor combination group, 15 received Toripalimab, 14 received Toripalimab, 14 received Camrelizumab, 4 received Pembrolizumab, 9 received Sintilimab, and 2 received Nivolumab, 1 with Tislelizumab. Camrelizumab, Sintilimab, Pembrolizumab and Tislelizumab were all given 200 mg every 3 wk. The dose of Toripalimab was 240 mg once every 3 wk. The dose of Nivolumab was 240 mg once every 2 wk.

TACE was performed under local anesthesia. After successful femoral artery puncture, 5-fluorouracil perfusion was performed through the celiac trunk. The tumor-supplying artery was superselected by a microcatheter, and chemoembolization was performed with a mixture of lipiodol and pirarubicin (25-40 mg/m^2). According to the investigators' assessment, TACE was performed every 4-6 wk when the patient had good hepatic function (Child-Pugh class A or B) until disease progression occurred.

According to Common Terminology Criteria for Adverse Events (CTCAE), v5.0, adverse events of patients during treatment were evaluated and graded. For grade 1 to grade 2 adverse events, there was no need to stop medication and symptomatic treatment was performed. If grade 3 or higher adverse reactions occur, medication should be suspended until the adverse reactions improve to grade 0-1 or baseline. For grade 4 or more adverse reactions, medication should be stopped and symptomatic treatment should be carried out actively.

Outcomes and assessments

The primary outcomes were OS and PFS. The definition of OS was the time from the first TACE therapy to death or the last follow-up.Tumor response was evaluated according to contrast-enhanced computed

tomography or magnetic resonance imaging. The definition of PFS was the time between the first TACE therapy and disease recurrence or the last follow-up. The secondary outcome was the frequency of main adverse events (AEs), which were evaluated by the CTCAE, v 5.0. The key AEs after the initiation of combination therapy were observed. We conducted follow-up every 3 wk to assess outcome variables until tumor progression, intolerable AEs, or death.

Statistical analysis

We used *T* tests to compare continuous data conforming to the normal distribution. χ^2 tests were performed to compare categorical variables. We used Mann-Whitney *U* tests to compare the continuous variables that did not conform to the normal distribution. Fisher's exact test was used when the sample size is less than 40 or the theoretical frequency was less than 1. The survival rates were evaluated by the Kaplan-Meier curve. We identified independent prognostic factors related to OS by univariate and multivariate analyses based on the Cox regression model. All statistical analyses were performed by SPSS, v 25.0.

RESULTS

Patient characteristics

We finally enrolled 65 patients with uHCC according to the criteria; 45 received the PD-1 inhibitors, lenvatinib, TACE therapy (PD-1-Lenv-T), and 20 received the lenvatinib, TACE (Lenv-T therapy) (Figure 1). The clinical information and data at baseline of all patients are shown in Table 1. The median age in the PD-1-Lenv-T group was 54 years old and in the Lenv-T group was 62 years old. At baseline, tumors were considered BCLC stage A in 6.67%, stage B in 17.78% and stage C in 75.56% of patients receiving PD-1-Lenv-T and stage A in 10%, stage B in 15% and stage C in 75% of patients receiving Lenv-T (P = 0.88). The ECOG-PSs were 0 in 57.78% and 1 in 42.22% of patients treated with PD-1-Lenv-T and 0 in 35.0% and 1 in 65.0% of patients treated with Lenv-T (P = 0.154). The numbers of TACE treatments were < 3 in 62.22% and ≥ 3 in 37.78% of patients received PD-1-Lenv-T and < 3 in 80.0% and ≥ 3 in 20.0% of patients received Lenv-T therapy (P = 0.26). The tumor numbers were 1 in 37.78% and more than 1 in 62.22% of patients who were treated with PD-1-Lenv-T and 1 in 20.00% and more than 1 in 80.00% of patients who were treated with Lenv-T (P = 0.26). Baseline characteristics of tumor burden score (TBS) group, extrahepatic metastasis rate and portal vein tumor thrombus rate were similar between the two groups. Median administration time of lenvatinib was 5.9 mo (range: 1.0-15.9 mo) and 7.8 mo (range: 1.1-28.2 mo) in the Lenv-T group and the PD-1-Lenv-T group (P = 0.07), respectively. The median injection timesof PD-1 inhibitors in the group that received PD-1 inhibitor therapy was 6 (range: 1-29).

Efficacy

The primary clinical outcomes of this research were OS and PFS. The median follow-up time for all enrolled patients was 25.2 mo [95% confidence interval (CI): 18.8-31.7]. The duration of OS and PFS for all included patients were 17.9 mo (95%CI: 11.8-24.1) and 11.0 mo (95%CI: 7.6-14.4), respectively (Figure 2). The median OS time in the PD-1-Lenv-T group was 26.8 mo (95%CI: 14.4-39.1), while that in the Lenv-T group was 14.0 mo [(95%CI: 10.3-17.8), P = 0.027] (Figures 2A and C). A remarkable median OS improvement of 12.8 mo was observed, suggesting that the PD-1-Lenv-T regimen may have advantage over the Lenv-T regimen. In addition, the median PFS time was different between the two treatment groups [11.7 mo (95%CI: 7.7-15.7) in the PD-1-Lenv-T group *vs* 8.5 mo (95%CI: 3.0-13.9) in the Lenv-T group (P = 0.028)] (Figures 2B and D).

The best tumor responses of all patients with uHCC are shown in Table 2. The ORR in the PD-1-Lenv-T group was 44.4%, which was obviously higher than the ORR of 20% in the Lenv-T group (P = 0.059) according to the mRECIST criteria (Table 2). The disease control rates (DCRs) were 93.3% in the PD-1-Lenv-T group and 64.0% in the Lenv-T group (P = 0.003). When stratified by BCLC stage, the DCR differed between the two groups (patients with BCLC stage A or B *vs* patients with BCLC stage C) (Table 3). A total of nine patients changed medications after disease progression, with two patients switching from lenvatinib to apatinib, two receiving donafenib, one receiving regofenib, and three receiving bevacizumab. Another patient stopped TACE and switched to HAIC (hepatic arterial infusion chemotherapy).

Prognostic factors for OS and PFS in the subgroups

We identified independent prognostic factors related to OS by univariate and multivariate analyses based on the Cox regression model (Figure 3). Basic clinical characteristics (gender, age, *etc.*), tumor characteristics (tumor size, tumor number, *etc.*) and treatment status (number of TACE treatments, *etc.*) were included in the analyses. Univariate analysis suggested that OS was related to the treatment option [P = 0.032, hazard ratio (HR) = 0.44, 95%CI: 0.21-0.93), ECOG-PS score (P = 0.003, HR = 0.34, 95%CI: 0.16-0.69), BCLC stage (P = 0.059, HR = 2.75, 95%CI: 0.96-7.86) and TBS group (P = 0.065, HR = 0.47,

Table 1 The baseline characteristics of included patients (*n*, %)

	Overall	Lenvatinib + TACE + PD-1	Lenvatinib + TACE	*P* value
Patient characteristics				
Number (*n*)	65	45	20	
Age, median (range), yr	57 (18-79)	54 (18-79)	62 (26-75)	0.066[1]
< 65	50 (76.92)	38 (84.44)	12 (60.00)	
≥ 65	15 (23.08)	7 (15.56)	8 (40.00)	
Gender				0.095[1]
Female	8 (12.31)	3 (6.67)	5 (25.00)	
Male	57 (87.69)	42 (93.33)	15 (75.00)	
ECOG-PS				0.154[1]
0	33 (50.77)	26 (57.78)	7 (35.00)	
1	32 (49.23)	19 (42.22)	13 (65.00)	
Etiology				0.002[1]
HBV	54 (83.08)	42 (93.33)	12 (60.00)	
HCV	4 (6.15)	2 (4.44)	2 (10.00)	
Non-HBV, non-HCV	7 (10.77)	1 (2.22)	6 (30.00)	
Hepatic cirrhosis				0.879[1]
Yes	43 (66.15)	29 (64.44)	14 (70.00)	
No	22 (33.85)	16 (35.56)	6 (30.00)	
AFP				0.551[1]
< 400	21 (32.31)	13 (28.89)	8 (40.00)	
≥ 400	44 (67.69)	32 (71.11)	12 (60.00)	
Child-Pugh score				0.095[1]
A5	48 (73.85)	30 (66.67)	18 (90.00)	
A6 or B7	17 (26.15)	15 (33.33)	2 (10.00)	
BCLC stage				1.000[1]
A or B	16 (24.62)	11 (24.44)	5 (25.00)	
C	49 (75.38)	34 (75.56)	15 (75.00)	
Tumor characteristics				
Tumor size				0.035[1]
< 7 cm	25 (38.46)	13 (28.89)	12 (60.00)	
≥ 7 cm	40 (61.54)	32 (71.11)	8 (40.00)	
Tumor number				0.260[1]
Single	21 (32.31)	17 (37.78)	4 (20.00)	
Multiple	44 (67.69)	28 (62.22)	16 (80.00)	
TBS group				0.173[1]
L	23 (35.38)	13 (28.89)	10 (50.00)	
H	42 (64.62)	32 (71.11)	10 (50.00)	
Portal vein tumor thrombus				0.108[1]
Presence	24 (36.92)	20 (44.44)	4 (20.00)	
Absence	41 (63.08)	25 (55.56)	16 (80.00)	
Extrahepatic metastasis				0.376[1]

Yes	23 (35.38)	18 (40.00)	5 (25.00)	
No	42 (64.62)	27 (60.00)	15 (75.00)	
TACE times				0.260[1]
< 3	44 (67.69)	28 (62.22)	16 (80.00)	
≥ 3	21 (32.31)	17 (37.78)	4 (20.00)	

[1]χ^2 test.
AFP: Alpha-fetoprotein; BCLC: Barcelona Clinic Liver Cancer; ECOG-PS: Eastern Cooperative Oncology Group performance status; HBV: Hepatitis B virus; HCV: Hepatitis C virus; PD-1: Programmed death receptor-1; TACE: Transarterial chemoembolization; TBS: Tumor burden score.

Table 2 Therapeutic efficacy in all patients, *n* (%)

	Lenvatinib + TACE + PD-1 (*n* = 45)	Lenvatinib + TACE (*n* = 20)	*P* value
CR	0 (0)	0 (0)	
PR	20 (44.4)	4 (20)	
SD	22 (48.9)	9 (45)	
PD	3 (6.7)	7 (35)	
ORR	20 (44.4)	4 (20)	0.059[1]
DCR	42 (93.3)	13 (64)	0.003[1]

[1]χ^2 test.
[2]Fisher's exact test.
BCLC: Barcelona Clinic Liver Cancer; DCR: Disease control rate; ORR: Objective response rate; PD: Progressive disease; PR: Partial response; SD: Stable disease; PD-1: Programmed death receptor-1; TACE: Transarterial chemoembolization.

Table 3 Therapeutic efficacy in patients with stage A or B and stage C, *n* (%)

	BCLC A or B			BCLC C		
	Lenvatinib + TACE + PD-1 (*n* = 11)	Lenvatinib + TACE (*n* = 5)	*P* value	Lenvatinib + TACE + PD-1 (*n* = 34)	Lenvatinib + TACE (*n* = 15)	*P* value
CR	0 (0)	0 (0)		0 (0)	0 (0)	
PR	8 (72.7)	1 (20)		12 (35.3)	3 (20.0)	
SD	3 (27.3)	2 (40)		19 (55.9)	7 (46.7)	
PD	0 (0)	2 (40)		3 (8.8)	5 (33.3)	
ORR	8 (72.7)	1 (20)	0.077[2]	12 (35.3)	3 (20)	0.235[2]
DCR	11(100)	3 (60)	0.083[2]	31 (91.2)	10 (66.7)	0.047[2]

[1]χ^2 test.
[2]Fisher's exact test.
BCLC: Barcelona Clinic Liver Cancer; DCR: Disease control rate; ORR: Objective response rate; PD: Progressive disease; PR: Partial response; SD: Stable disease; PD-1: Programmed death receptor-1; TACE: Transarterial chemoembolization.

95%CI: 0.21-1.05). We subsequently included factors with a *P* value < 0.1 in the multivariate analysis and found that only the treatment option was an independent prognostic factor for OS (*P* = 0.031, HR = 0.43, 95%CI: 0.2-0.93). Similarly, univariate analysis revealed that PFS was related to the treatment option (*P* = 0.031, HR = 0.47, 95%CI: 0.23-0.93), ECOG-PS score (*P* = 0.006, HR = 2.42, 95%CI: 1.29-4.55), BCLC stage (*P* = 0.09, HR = 2.02, 95%CI: 0.9-4.56), Child-Pugh score (*P* = 0.071, HR = 1.86, 95%CI: 0.95-3.65) and number of TACE treatments (*P* = 0.017, HR = 0.44, 95%CI: 0.22-0.86). We subsequently included factors with a *P* value < 0.1 in the multivariate analysis and found that only the number of TACE treatments was an independent prognostic factor for PFS (*P* = 0.049, HR = 0.46, 95%CI: 0.21-1).

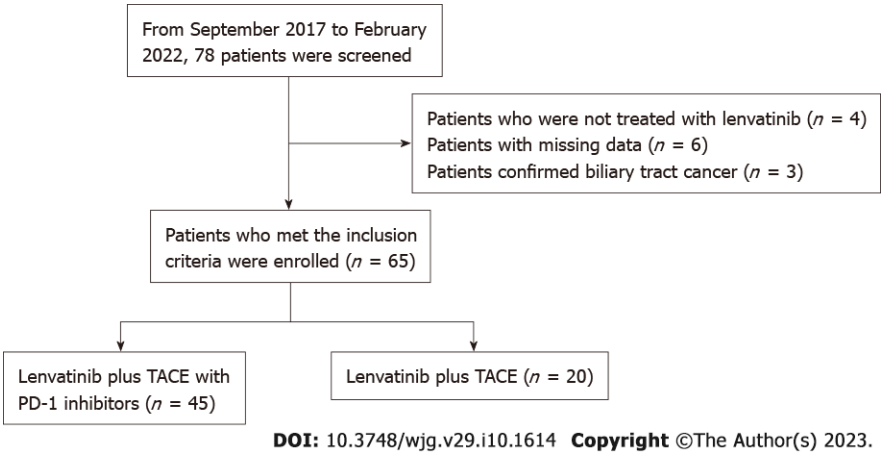

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Figure 1 Workflow of this study. TACE: Transarterial chemoembolization; PD-1: Programmed death receptor-1.

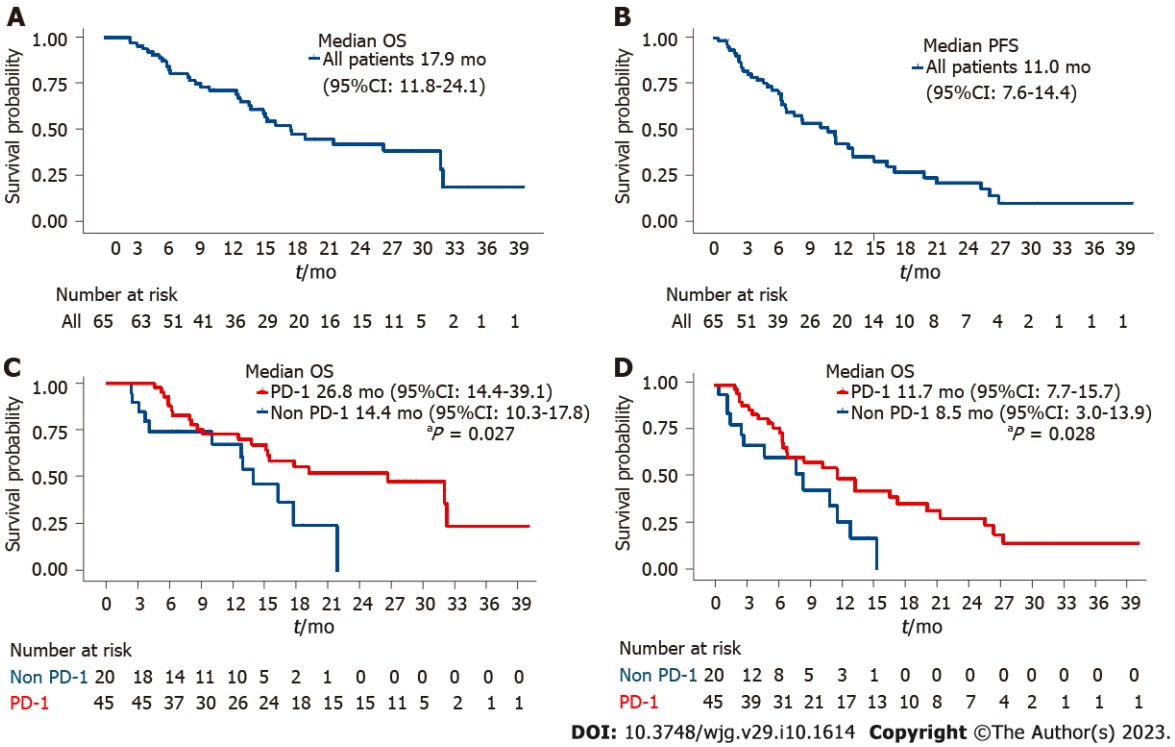

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Figure 2 The overall survival and progression-free survival times of all included patients and programmed death receptor-1-lenvatinib-transarterial chemoembolization group and lenvatinib-transarterial chemoembolization group. A: The overall survival (OS) times of all included patients; B: The progression-free survival (PFS) times of all included patients; C: The OS times of programmed death receptor-1 (PD-1)-lenvatinib-transarterial chemoembolization (TACE) group and lenvatinib-TACE group; D: The PFS times of PD-1-lenvatinib-TACE group and lenvatinib-TACE group. OS: Overall survival; PFS: Progression-free survival; CI: Confidence interval; PD-1: Programmed death receptor-1.

Safety

In total, 61 patients (93.8%) experienced AEs of any grade (Table 4). The top five most frequent treatment-related AEs in the PD-1-Lenv-T group were decreased appetite (42.2%), elevated aspartate aminotransferase (AST) (40.0%), decreased albumin (40.0%), hypertension (28.9%) and diarrhea (28.9%). In the Lenv-T group, fatigue (40.0%), decreased appetite (35.0%), and decreased albumin (30.0%) were the most frequent treatment-related AEs. Diarrhea (11.1%), decreased appetite (6.7%), elevated AST (6.7%), fatigue (6.7%), and hypertension (6.7%) were the most frequent grade 3/4 AEs in the PD-1-Lenv-T group. Decreased appetite (10.0%), decreased albumin (5.0%), diarrhea (5.0%), fatigue (5.0%), decreased platelet count (5.0%) and abdominal pain (5.0%) were the most frequent grade 3/4 AEs in the Lenv-T group. A total of three patients, two in the PD-1-Lenv-T group and one in the Lenv-T group, stopped treatment or changed treatment regimens because of intolerable AEs. Overall, the type and frequency of AEs was relatively similar between the two groups.

Table 4 Adverse events, *n* (%)

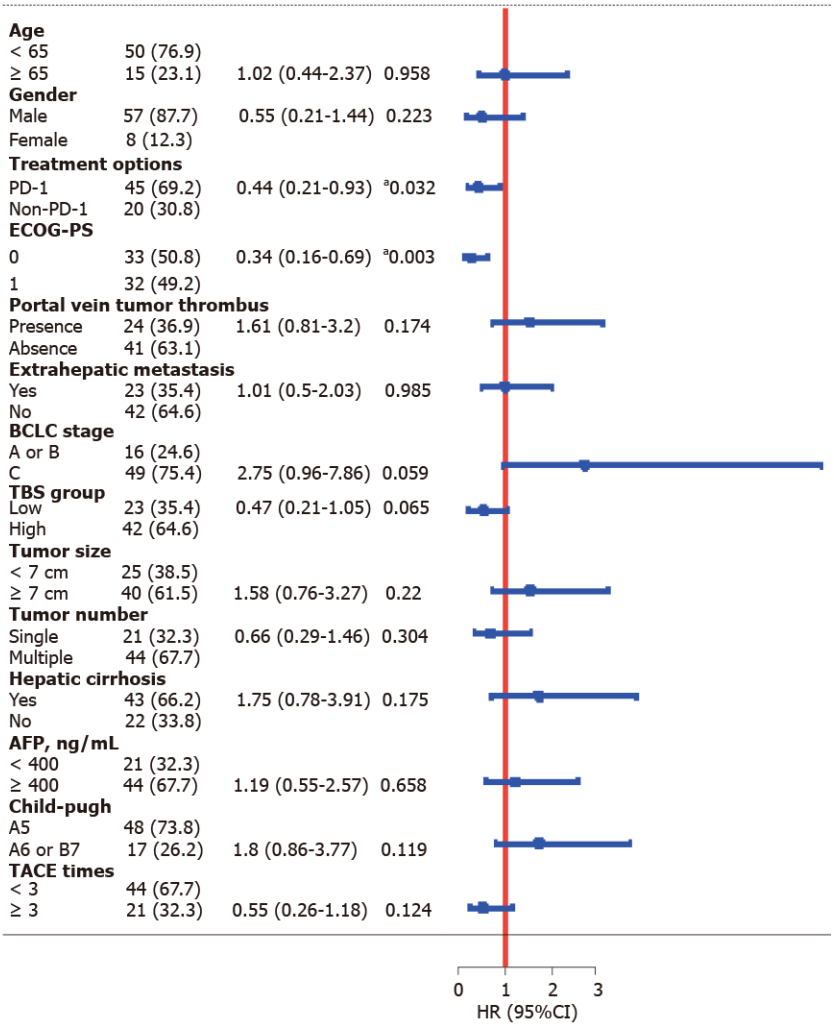
AEs	Lenvatinib + TACE + PD-1		Lenvatinib + TACE	
	All grades	Grade 3/4	All grades	Grade 3/4
Decreased appetite	19 (42.2)	3 (6.7)	7 (35.0)	2 (10.0)
Elevated AST	18 (40)	3 (6.7)	5 (25.0)	0 (0)
Decreased albumin	18 (40)	2 (4.4)	6 (30.0)	1 (5.0)
Fatigue	17 (37.8)	3 (6.7)	8 (40.0)	1 (5.0)
Diarrhoea	13 (28.9)	5 (11.1)	4 (20.0)	1 (5.0)
Hypertension	13 (28.9)	3 (6.7)	3 (15.0)	0 (0)
Elevated blood bilirubin	13 (28.9)	2 (4.4)	5 (25.0)	0 (0)
Decreased platelet count	13 (28.9)	2 (4.4)	5 (25.0)	1 (5.0)
Hypothyroidism	12 (26.7)	2 (4.4)	3 (15.0)	0 (0)
Abdominal pain	11 (24.4)	2 (4.4)	4 (20.0)	1 (5.0)
Elevated ALT	11 (24.4)	2 (4.4)	4 (20.0)	0 (0)
Rash	8 (17.8)	2 (4.4)	2 (10.0)	0 (0)
Decreased WBC	7 (15.6)	0 (0)	4 (20.0)	0 (0)
Vomiting	7 (15.6)	0 (0)	3 (15.0)	0 (0)
Hypocalcemia	6 (13.3)	0 (0)	5 (25.0)	0 (0)
Palmar-plantar erythrodysaesthesia	5 (11.1)	0 (0)	1 (5.0)	0 (0)
Nausea	4 (8.9)	0 (0)	4 (20.0)	0 (0)
Elevated WBC	4 (8.9)	0 (0)	3 (15.0)	0 (0)
Gingival bleeding	4 (8.9)	0 (0)	1 (5.0)	0 (0)
Gastrointestinal hemorrhage	4 (8.9)	1 (2.2)	0 (0)	0 (0)
Decreased weight	3 (6.7)	0 (0)	2 (10.0)	0 (0)
Dysphonia	3 (6.7)	0 (0)	2 (10.0)	0 (0)
Proteinuria	1 (2.2)	1 (2.2)	1 (5.0)	0 (0)

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; WBC: White blood cell; AEs: Adverse events; PD-1: Programmed death receptor-1; TACE: Transarterial chemoembolization.

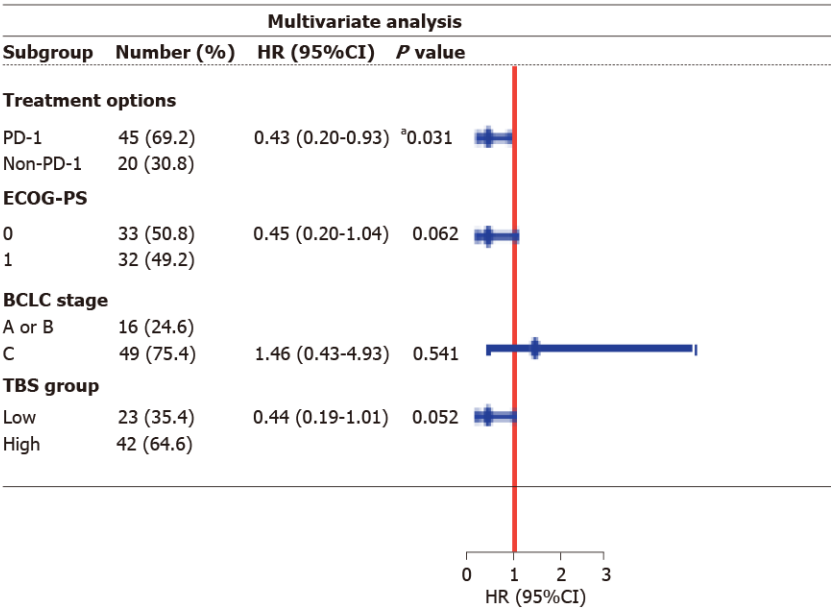
DISCUSSION

With the progress of new tyrosine kinase inhibitors and immunotherapy, individualized strategies for uHCC have improved. A recent randomized phase III trial, LAUNCH, comparing local therapy plus lenvatinib with lenvatinib monotherapy, demonstrated that TACE plus lenvatinib showed better overall survival in patients with uHCC (median OS: 17.8 *vs* 11.5 mo; HR = 0.45, $P < 0.001$)[22]. In our real-world study, patients in the TACE plus lenvatinib group had shorter OS and PFS than those in the LAUNCH trial (median OS: 17.8 *vs* 14.0 mo; median PFS: 10.6 *vs* 8.5 mo). A preclinical study showed that lenvatinib can blocke FGFR4 to reduce tumor PD-L1 expression and Treg differentiation, thus improving anti-PD-1 efficacy[18]. In addition to local therapies such as TACE, systemic therapy with PD-1 inhibitors is also being explored as a first-line therapy for uHCC. At the ESMO congress 2022, LEAP-002, a randomized phase III trial, enrolled 794 patients with advanced HCC who were not systematically treated and received lenvatinib plus pembrolizumab or lenvatinib alone in a 1:1 ratio [23]. Although the survival curve was initially higher in the Len + pembro group than in the monotherapy group approximately 15 mo after treatment, the prespecified statistical end point was not reached (median OS: 21.2 mo *vs* 19.0 mo, HR = 0.840, 95%CI: 0.708-0.997, $P = 0.0227$). However, in the subgroup analysis, HCC patients with HBV background benefited more in the Len + pembro group (HR = 0.75, 95%CI: 0.58-0.97). In our study, HBV-related HCC patients accounted for 93.33% in the PD-1-lenvatinib-TACE group, indicating that in the real world, Chinese HBV-related HCC patients are more likely to benefit from PD-1 inhibitors. This retrospective study estimated the clinical outcomes and safety of TACE in combination with lenvatinib and PD-1 inhibitors *vs* lenvatinib plus TACE in the

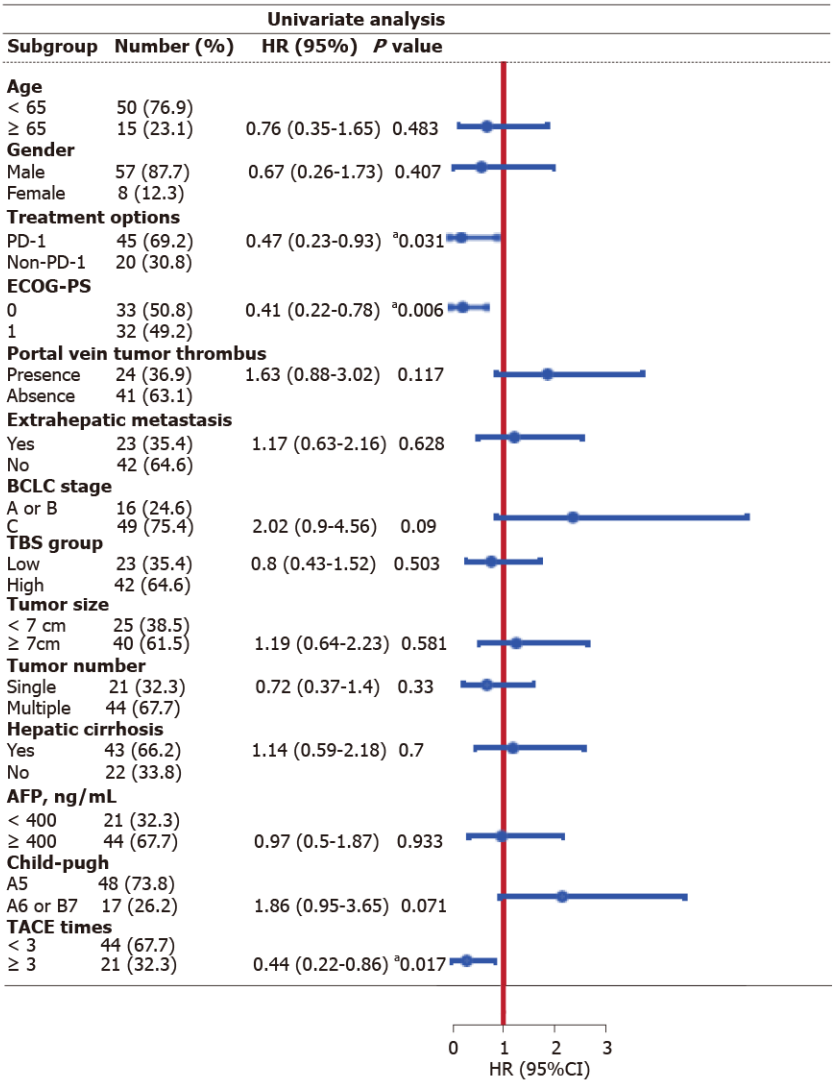
A


Univariate analysis
Subgroup Number (%) HR (95%) P value
Age
< 65 50 (76.9)
≥ 65 15 (23.1) 1.02 (0.44-2.37) 0.958
Gender
Male 57 (87.7) 0.55 (0.21-1.44) 0.223
Female 8 (12.3)
Treatment options
PD-1 45 (69.2) 0.44 (0.21-0.93) a0.032
Non-PD-1 20 (30.8)
ECOG-PS
0 33 (50.8) 0.34 (0.16-0.69) a0.003
1 32 (49.2)
Portal vein tumor thrombus
Presence 24 (36.9) 1.61 (0.81-3.2) 0.174
Absence 41 (63.1)
Extrahepatic metastasis
Yes 23 (35.4) 1.01 (0.5-2.03) 0.985
No 42 (64.6)
BCLC stage
A or B 16 (24.6)
C 49 (75.4) 2.75 (0.96-7.86) 0.059
TBS group
Low 23 (35.4) 0.47 (0.21-1.05) 0.065
High 42 (64.6)
Tumor size
< 7 cm 25 (38.5)
≥ 7 cm 40 (61.5) 1.58 (0.76-3.27) 0.22
Tumor number
Single 21 (32.3) 0.66 (0.29-1.46) 0.304
Multiple 44 (67.7)
Hepatic cirrhosis
Yes 43 (66.2) 1.75 (0.78-3.91) 0.175
No 22 (33.8)
AFP, ng/mL
< 400 21 (32.3)
≥ 400 44 (67.7) 1.19 (0.55-2.57) 0.658
Child-pugh
A5 48 (73.8)
A6 or B7 17 (26.2) 1.8 (0.86-3.77) 0.119
TACE times
< 3 44 (67.7)
≥ 3 21 (32.3) 0.55 (0.26-1.18) 0.124
0 1 2 3
HR (95%CI)

B


Multivariate analysis
Subgroup Number (%) HR (95%CI) P value
Treatment options
PD-1 45 (69.2) 0.43 (0.20-0.93) a0.031
Non-PD-1 20 (30.8)
ECOG-PS
0 33 (50.8) 0.45 (0.20-1.04) 0.062
1 32 (49.2)
BCLC stage
A or B 16 (24.6)
C 49 (75.4) 1.46 (0.43-4.93) 0.541
TBS group
Low 23 (35.4) 0.44 (0.19-1.01) 0.052
High 42 (64.6)
0 1 2 3
HR (95%CI)

C



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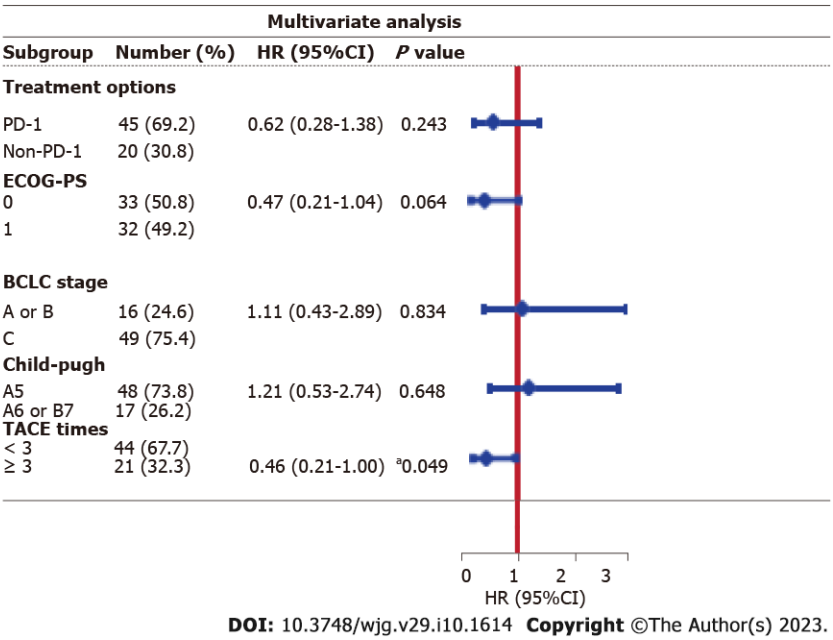

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Figure 3 Univariate and multivariable Cox regression analysis for overall survival and progression-free survival. A: Univariate Cox regression analysis for overall survival (OS); B: Multivariable Cox regression analysis for OS; C: Univariate Cox regression analysis for progression-free survival (PFS); D:

Multivariable Cox regression analysis for PFS. [a]*P* < 0.05. HR: Hazard ratio; CI: Confidence interval; PD-1: Programmed death receptor-1; TACE: Transarterial chemoembolization; ECOG-PS: Eastern Cooperative Oncology Group performance status; BCLC: Barcelona Clinic Liver Cancer; AFP: Alpha-fetoprotein; TBS: Tumor burden score.

remedy of uHCC. The results suggested that the PD-1-Lenv-T regimen significantly prolonged survival time in patients with uHCC, without unexpected safety-related complications.

After stratification according to BCLC stage, the ORR and DCR were 72.7% and 100%, respectively, in the PD-1-Lenv-T group of uHCC patients with BCLC stage A or B, indicating that combination early treatment with PD-1 inhibitors has a good control effect on lesions. In contrast to proportions in previous studies, 75.4% of patients in this study had BCLC stage C tumors, and 67.7% of patients had multiple tumors, which indicates greater clinical significance for the treatment of patients with advanced HCC. When identifying independent factors associated with OS, similar to previous studies, univariate log-rank analysis indicated that the treatment option (P = 0.032, HR = 0.44, 95%CI: 0.21-0.93) and ECOG-PS score (P = 0.003, HR = 0.34, 95%CI: 0.16-0.69) were associated with OS. This might be accounted for the fact that patients with a good ECOG-PS score have enough physical strength to tolerate treatment. The subsequent multivariate analysis showed that the treatment option was an independent prognostic risk factor for OS. When determining independent prognostic factors associated with PFS, the multivariate analysis suggested that only the number of TACE treatments was an independent prognostic factor for PFS (P = 0.049, 95%CI: 0.21-1); specifically, undergoing TACE ≥ 3 times prolonged the PFS of patients with uHCC. It may be that patients who respond to TACE treatment are more likely to undergo repeated TACE treatments.

According to our data, the PD-1-Lenv-T regimen performed well in terms of safety, and grade 3 or 4 AEs were rare. AEs of any grade occurred more frequently in the PD-1-Lenv-T group than in the pembrolizumab monotherapy group in the KEYNOTE-240 clinical trial, but there was no obvious difference in grade 3/4 AEs. The timely monitoring of and intervention for AEs by the treatment team also played a key role in the remedy of all patients. From the information collected, more symptomatic AEs, such as decreased appetite and fatigue, occurred with this treatment regimen. This study also exists several limitations. For example, this research was a retrospective study based on a single medical center with a limited number of patients.

CONCLUSION

In conclusion, the study results suggest that early combination treatment with PD-1 inhibitors has manageable toxicity and promising efficacy in patients with uHCC.

ARTICLE HIGHLIGHTS

Research background

Programmed death receptor-1 (PD-1) inhibitors have been approved as second-line treatment regimen in hepatocellular carcinoma (HCC), and it is still worth studying whether patients can benefit from PD-1 inhibitors as first-line drugs combined with targeted drugs and local therapy.

Research motivation

To provide more options and references for the therapy of patients with unresectable HCC (uHCC).

Research objectives

Aim to evaluate the clinical outcomes and safety of transarterial chemoembolization (TACE) and lenvatinib plus PD-1 inhibitors for patients with uHCC.

Research methods

We carried out a retrospective investigation of 65 patients with uHCC who were treated at Peking Union Medical College Hospital from September 2017 to February 2022.

Research results

Patients with uHCC who received PD-1 inhibitors, lenvatinib, TACE therapy (n = 45) had a clearly longer overall survival than those who underwent lenvatinib, TACE therapy (n = 20, 26.8 *vs* 14.0 mo; P = 0.027). The type and frequency of adverse events showed little difference between the two treatment groups.

Research conclusions

Our results suggest that the early combination of PD-1 inhibitors has manageable toxicity and promising efficacy in patients with uHCC.

Research perspectives

Patients with uHCC may obtain benefit a lot from the early combination of PD-1 inhibitors with TACE and lenvatinib.

FOOTNOTES

Author contributions: Wang YY participated in the study design and drafting of the manuscript; Yang X, Wang YC, Long JY, Sun HS, Li YR, Xun ZY, Zhang N, Xue JN and Ning C participated in data collection and statistical analysis; Zhang JW, Zhu CP, Yang XB, and Zhao HT participated in study supervision; and all authors have read and approved the final manuscript.

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Institutional review board statement: This study was approved by the Ethical Review Board at Peking Union Medical College Hospital (No.SK-2000).

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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ORCID number: Yan-Yu Wang 0000-0003-0599-9629; Xu Yang 0000-0001-5278-7667; Jun-Yu Long 0000-0001-5745-7165; Jun-Wei Zhang 0000-0002-2833-0090; Xiao-Bo Yang 0000-0003-1929-8866; Hai-Tao Zhao 0000-0002-3444-8044.

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ORIGINAL ARTICLE

Retrospective Study

Clinical features, diagnosis, and treatment of Peutz-Jeghers syndrome: Experience with 566 Chinese cases

Zu-Xin Xu, Li-Xin Jiang, Yu-Rui Chen, Yu-Hui Zhang, Zhi Zhang, Peng-Fei Yu, Zhi-Wei Dong, Hai-Rui Yang, Guo-Li Gu

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Zu-Xin Xu, Fifth Clinical College of Anhui Medical University, Air Force Clinical College of Anhui Medical University, Beijing 100142, China

Li-Xin Jiang, Yu-Rui Chen, Air Force Clinical College of China Medical University, Beijing 100142, China

Yu-Hui Zhang, Zhi Zhang, Peng-Fei Yu, Zhi-Wei Dong, Hai-Rui Yang, Guo-Li Gu, Department of General Surgery, Air Force Medical Center, Chinese People's Liberation Army, Beijing 100142, China

Corresponding author: Guo-Li Gu, FRCS (Gen Surg), Chief Doctor, Department of General Surgery, Air Force Medical Center, Chinese People's Liberation Army, No. 30 Fucheng Road, Haidian District, Beijing 100142, China. kzggl@163.com

Abstract

BACKGROUND

Peutz-Jeghers syndrome (PJS) is a clinically rare disease with pigmented spots on the lips and mucous membranes and extremities, scattered gastrointestinal polyps, and susceptibility to tumors as clinical manifestations. Effective preventive and curative methods are still lacking. Here we summarize our experience with 566 Chinese patients with PJS from a Chinese medical center with regard to the clinical features, diagnosis, and treatment.

AIM

To explore the clinical features, diagnosis, and treatment of PJS in a Chinese medical center.

METHODS

The diagnosis and treatment information of 566 cases of PJS admitted to the Air Force Medical Center from January 1994 to October 2022 was summarized. A clinical database was established covering age, gender, ethnicity, family history, age at first treatment, time and sequence of appearance of mucocutaneous pigmentation, polyp distribution, quantity, and diameter, frequency of hospitalization, fre-quency of surgical operations, *etc.* The clinical data was retrospectively analyzed using SPSS 26.0 software, with $P < 0.05$ considered statistically significant.

RESULTS

Of all the patients included, 55.3% were male and 44.7% were female. Median time to the appearance of mucocutaneous pigmentation was 2 years, and median time from the appearance of mucocutaneous pigmentation to the occurrence of abdominal symptoms was 10 years. The vast majority (92.2%) of patients underwent small bowel endoscopy and treatment, with 2.3% having serious complications. There was a statistically significant difference in the number of enteroscopies between patients with and without canceration (P = 0.004, Z = -2.882); 71.2% of patients underwent surgical operation, 75.6% of patients underwent surgical operation before the age of 35 years, and there was a statistically significant difference in the frequency of surgical operations between patients with and without cancer (P = 0.000, Z = -5.127). At 40 years of age, the cumulative risk of intussusception in PJS was approximately 72.0%, and at 50 years, the cumulative risk of intussusception in PJS was approximately 89.6%. At 50 years of age, the cumulative risk of cancer in PJS was approximately 49.3%, and at 60 years of age, the cumulative risk of cancer in PJS was approximately 71.7%.

CONCLUSION

The risk of intussusception and cancer of PJS polyps increases with age. PJS patients ≥ 10 years old should undergo annual enteroscopy. Endoscopic treatment has a good safety profile and can reduce the occurrence of polyps intussusception and cancer. Surgery should be conducted to protect the gastrointestinal system by removing polyps.

Key Words: Peutz-Jeghers syndrome; Management; Intussusception; Canceration; *STK11*

Core Tip: Peutz-Jeghers syndrome (PJS) is a clinically rare autosomal dominant inherited disease with pigmented spots on the lips and mucous membranes and extremities, scattered gastrointestinal polyps, and susceptibility to tumors as clinical manifestations. Effective pre-ventive and curative methods are still lacking. Here we summarize our experience with 566 Chinese patients with PJS from a Chinese medical center with regard to the clinical features, diagnosis, and treatment, in order to improve the understanding of PJS in Chinese patients and improve its clinical diagnosis and treatment.

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INTRODUCTION

Peutz-Jeghers syndrome (PJS) is clinically characterized by labial mucosa and extremity terminal pigmentation and gastrointestinal multiple hamartoma polyposis[1-3]. Mucocutaneous pigmentation generally does not require specific treatment, but PJS polyposis is clinically serious. Gastrointestinal polyps cause secondary rupture, bleeding, intussusception, intestinal obstruction, abdominal pain, abdominal distension, hematochezia, and other symptoms, and their further progression causes enteric necrosis, intestinal perforation, and even cancer[4-7]. Therefore, it is essential to develop safe and effective treatments for PJS polyps. From January 1994 to October 2022, we diagnosed and treated 566 patients with PJS at the Air Force Medical Center, China, thereby accumulating some clinical experience for the management of this disease. This paper report our experience with this disease in order to explore the clinicopathological features, diagnosis, and treatment of PJS in Chinese patients.

MATERIALS AND METHODS

Materials

Patients diagnosed with PJS (ICD-9, ICD-10 disease code Q85.801) from January 1994 to October 2022 in the Air Force Medical Center's electronic medical record system were screened according to the standard reported previously[8,9]. Suspicious diagnoses and misdiagnoses were excluded, leaving a

total of 566 patients. Statistical parameters explored in this study included: (1) General information: Gender, age, ethnicity, family history, *etc.*; (2) Clinical information: Age at onset, location of mucocutaneous pigmentation, time from mucocutaneous pigmentation to the appearance of abdominal symptoms (such as abdominal pain, intestinal obstruction, and gastrointestinal bleeding), polyp distribution, polyp burden, maximum- polyp diameter, polyp pathology and canceration *etc.*; and (3) Diagnosis and treatment information: Age at initial treatment, age at follow-up, frequency of hospitalization, frequency of surgical operations, frequency of endoscopy and comorbidities, *etc.* A database of clinical parameters was created and retrospective statistical analysis was performed.

Statistical analysis

SPSS 26.0 software was used for descriptive statistical analyses. A normal testing method was applied to evaluate the quantitative data, which are expressed as the mean ± standard deviation (SD); *t*-test was used for comparisons between groups. Skewed distribution data are described as medians (interquartile ranges); the Mann-Whitney *U* test was used for comparisons between groups. Qualitative data are expressed as percentages; the comparison of proportion and correlation analysis was evaluated by the χ^2 test. $P < 0.05$ was considered statistically significant.

RESULTS

General data

The general information of 566 patients with PJS is shown in Table 1. As of the final follow-up age, 236 cases were married and 330 were unmarried. There were 183 cases married with children and 106 patients with healthy children after marriage; 77 children born after marriage had PJS; considering that some healthy children born to 106 patients were still too young for disease characteristics to appear, the diagnosis may not be clear, and the actual proportion of children with the disease may be higher.

Clinical data

The clinical data of 566 patients with PJS is shown in Table 2. Age distribution ranged from 0.5 to 60 years old, with a median age of 15 years old.

First treatment age: A total of 407 patients (71.9%) received initial treatment before 20 years old, and 513 (90.6%) received initial treatment before 30 years old. There was a statistically significant difference in the age of initial treatment between patients with and without a family history of PJS ($P = 0.035$, $Z = -2.114$); the age of initial treatment of patients with a family history of PJS was later than that of patients without. There was no significant difference in first treatment age according to gender ($Z = -0.105$, $P = 0.310$). There was no significant difference in first treatment age according to blood group ($H = 1.652$, $P = 0.648$). There was a significant difference in first treatment age between patients with and without malignant tumors ($P = 0.009$, $Z = -2.631$). The median age of patients with malignant tumors was significantly higher than that of patients without, which suggests that the later the age of initial treatment, the more likely the occurrence of malignancy.

Mucocutaneous pigmentation: A total of 563 cases (99.5%) had mucocutaneous pigmentation, which appeared before 7 years old in 507 cases (90.1%). As to the order of the appearance of mucocutaneous pigmentation, 402 cases (71.0%) had mucocutaneous pigmentation on both the lips and limbs, 45 (8.0%) had mucocutaneous pigmentation on the lips and then on the limbs, 4 (0.7%) had mucocutaneous pigmentation on the limbs and then on the lips, and 16 cases (2.8%) developed mucocutaneous pigmentation in an unknown order. There was a statistically significant difference in the age of the appearance of mucocutaneous pigmentation age between patients with and without a family history of PJS ($P = 0.016$, $Z = -2.415$), and the age of the appearance of mucocutaneous pigmentation in cases with a family history of PJS was significantly lower than that of patients without. There was no significant difference in the age of the appearance of mucocutaneous pigmentation and gender ($P = 0.686$, $Z = -0.404$). There was no significant difference in the age of the appearance of mucocutaneous pigmentation according to blood group ($H = 2.3$, $P = 0.512$). The age of the appearance of mucocutaneous pigmentation was positively correlated with the age of initial treatment ($r = 0.197$, $P = 0.000$). The age of initial treatment was positively correlated with small intestinal polyp burden ($r = 0.097$, $P = 0.034$) and colorectal polyp burden ($r = 0.208$, $P = 0.000$), and negatively correlated with the maximum diameter of colorectal polyps ($r = -0.120$, $P = 0.024$).

Interval time between age of mucocutaneous pigmentation and occurrence of gastrointestinal symptoms: A total of 529 cases (93.5%) had clear records; 3 cases (0.5%) had no data on gastrointestinal symptoms and 34 (6.0%) had missing data. The interval time was negatively correlated with the age of pigmentation ($r = -0.175$, $P = 0.000$). There was a statistical difference in the interval time of gastrointestinal symptoms according to the burden of colorectal polyps ($\chi^2 = 70.476$, $P = 0.015$). The greater the burden of colorectal polyps, the shorter the interval until the occurrence of gastrointestinal symptoms.

Table 1 General data of 566 patients with Peutz-Jeghers syndrome

General data	Classification	Cases	Percentage (%)
Gender	Male	313	55.3
	Female	253	44.7
Ethnic distribution	Han nationality	539	95.4
	Manchu	11	1.9
	Hui nationality	7	1.2
	Mongolian	4	0.7
	Tibetan	2	0.4
	Tujia nationality	1	0.2
	Zhuang nationality	1	0.2
	She minority	1	0.2
Family history of PJS	Yes	330	58.3
	No	236	41.7
ABO blood group	A	149	27.1
	B	175	31.8
	O	167	30.4
	AB	59	10.7
	Deletion	16	2.8
Rh blood group	Rh^+	530	97.2
	Rh^-	0	0
	Deletion	16	2.8
Marital history	Unmarried	330	58.3
	Married	236	41.7
Offspring inheritance	Yes	77	42.1
	No	106	57.9

PJS: Peutz-Jeghers syndrome.

Gastrointestinal polyps: PJS polyps were distributed in the stomach in 421 cases (74.4%), in the small intestine in 546 (96.5%), and in the colorectum in 445 (78.6%). There were 26 cases (4.6%) with gallbladder polyps, 4 (0.7%) with nasal polyps, and 2 (0.35%) with uterine polyps. Regarding pathological type, there were 433 cases of hamartoma, 75 cases of adenoma, 32 cases of canceration, and 23 cases of hyperplastic and inflammatory polyps. There was no statistically significant difference between the maximum diameter of gastric polyps and that of small intestinal polyps (χ^2 = 656.319, P = 0.991), but there was a statistically significant difference between the maximum diameter of gastric polyps and that of colorectal polyps (χ^2 = 639.396, P = 0.026). There was a statistically significant difference between the diameters of small intestinal polyps and colorectal polyps (χ^2 = 1443.082, P = 0.000). There was a statistically significant difference between the gastric polyp burden and small intestinal polyp burden (χ^2 = 1000.592, P = 0.000), between the gastric polyp burden and colorectal polyp burden (χ^2 = 468.22, P = 0.000), and between the small intestinal polyp burden and colorectal polyp burden (χ^2 = 1739.598, P = 0.000).

Endoscopic examination and treatment: Among the 566 patients in this study, 522 (92.2%) underwent enteroscopy and treatment, and a total of 1381 small intestinal enteroscopies were completed (841 transoral small intestinal enteroscopies and 533 transanal small intestinal enteroscopies). About 7236 polyps were found through endoscopy (1959 gastric polyps, 3555 small intestinal polyps and 1722 colorectal polyps). 346 polyps < 5 mm were not treated, 1489 polyps between 5-10 mm were electrocoagulated and removed by argon plasma coagulation (APC), 917 polyps between 10-20 mm were removed by endoscopic mucosal resection (EMR) and snare polypectomy (SP), and 4484 polyps > 20 mm were removed by endoscopic mucosal dissection (ESD). After endoscopic treatment, 60 cases

Table 2 Clinical data of 566 patients with Peutz-Jeghers syndrome

Clinical data	Median	(P_{25}, P_{75})	Extreme value
First treatment age	15	(9, 22)	0.5, 60
Follow-up age	26	(18, 34)	4, 65
Age of appearance of mucocutaneous pigmentation	2	(1, 4)	0, 33
Interval time between age of mucocutaneous pigmentation and occurrence of gastrointestinal symptoms	10	(5.5, 18)	0, 58
Gastric polyp burden	2	(1, 5)	1, 100
Maximum diameter of gastric polyps (mm)	8	(5, 15)	2, 80
Small intestinal polyp burden	3	(2, 8)	1, 100
Maximum diameter of small intestinal polyps (mm)	40	(25, 50)	1, 160
Colorectal polyps burden	2	(1, 5)	1, 100
Maximum diameter of colorectal polyps (mm)	30	(15, 45)	1, 120
Cumulative hospitalizations	2	(1, 3)	1, 11
Number of operations	1	(0, 2)	0, 7
Frequency of small intestinal enteroscopic examinations	2	(1, 3)	0, 12
Frequency of gastroenterographic examinations	1	(0, 2)	0, 16
Frequency of colonoscopic examinations	1	(0, 2)	0, 16

developed clinical symptoms (10 cases of abdominal discomfort, 35 cases of incomplete intestinal obstruction and 15 cases of gastrointestinal perforation and bleeding), and 47 cases (91.7%) were cured. However, 13 cases (2.3%) developed gastrointestinal perforation and bleeding, and accepted surgical operations for the perforation repair or partial resection of the small intestine. 329 patients (58.1%) underwent gastroscopy 673 times, 393 patients (69.4%) underwent colonoscopy 868 times, 38 patients (6.7%) underwent capsule endoscopy 41 times and 9 patients (1.6%) underwent gastroenterography examinations. There was a statistically significant difference in the number of small intestinal enteroscopies between patients with and without canceration (P = 0.004, Z = -2.882), which suggests that the greater number of follow-up enteroscopies, the easier the detection of polyp canceration.

Surgical treatment

Laparotomy is the most common type of surgery for the treatment of PJS, in which multiple polyps in the gastrointestinal are treated by intraoperative endoscopic resection or small incision removal, and it is necessary to removal part of the bowel in some severe cases. Due to the progress of endoscopic techniques in the treatment of PJS, we advise removing all the polyps that can be reached at the same time. Current indications for surgery include: (1) The endoscope could not reach the lesion; (2) The occurrence of acute intussusception and intestinal obstruction symptoms prevents endoscopic treatment; (3) Patients with sessile flat polyps, giant polyps, or densely distributed polyps which are difficult to remove by endoscopy; (4) Polyps pathologically confirmed to have become malignant or highly suspected of canceration under endoscopic observation; (5) The occurrence of perforation during endoscopic polyp resection or postoperative perforation which could not be treated conservatively; (6) The occurrence of major bleeding during or after endoscopic treatment which does not respond to conservative medical treatment; and (7) Patients or their family members request the disposable surgical removal of polyps. A total of 405 patients (71.6%) underwent 655 surgical procedures, 305 (75.6%) underwent abdominal surgery before the age of 35, 168 (29.7%) underwent surgical treatment twice or more, and 1 underwent surgical treatment seven times. With regard to the number of times of operation, these patients underwent nasopharyngeal polypectomy (n = 1), subtotal gastrectomy (n = 2), partial small bowel resection (n = 570), pancreaticoduodenectomy (n = 7), partialcolectomy (n = 22), left-semicolon resection (n = 2), right hemicolectomy resection (n = 4), pancolectomy (n = 11), radical resection of small bowel cancer (n = 18), radical operations for colon cancer (n = 6), radical resection of rectal carcinoma (n = 2), radical surgery for ovarian cancer (n = 3), radical hysterectomy (n = 3), radical resection of pulmonary carcinoma (n = 3), and modified radical mastectomy (n = 1). There was a statistically significant difference in the number of operations between patients with and without cancer (P = 0.000, Z = -5.127), and patients with cancer required more surgery.

Drug therapy

Of nine patients who were treated with oral celecoxib capsules 400 mg once daily after small intestinal

enteroscopies, three developed allergic reactions and three developed hematochezia, for which the treatment was ceased. Two patients completed a 6-mo course of treatment and one completed a 9-mo course of treatment. The reexamination of small intestinal enteroscopies showed that the drug therapy was effective in only one case. Traditional Chinese medicine (TCM) was used in 30 cases and was shown to inhibit the growth of some small polyps < 1 cm, but its inhibitory effects on the growth of polyps ≥ 1 cm were unsatisfactory, with an overall effective rate of only 40.0%.

Follow-up

As of the final follow-up date of this study, 361 patients were followed more than twice at our hospital, and no death occurred. A total of 338 cases (189 males and 149 females with a median age of 27.5 years) had previously or concurrently developed intussusception, intestinal obstruction, or intestinal perforation. Kaplan-Meier survival analysis showed that, at the age of 40 years, the cumulative risk of intussusception in PJS patients could reach 72.0%, and at 50 years, the cumulative risk of intussusception could reach 89.6% (Figure 1A). Forty-six patients had malignant tumors (27 males and 19 females with a median age of 36.5 years); there were 18 cases of small bowel cancer, 8 cases of colorectal cancer, 6 cases of duodenal cancer, 3 cases of ovarian cancer, 3 cases of cervical cancer, 3 cases of lung cancer, 2 cases of gastric cancer, and 1 case each of cholangiocarcinoma, breast cancer, and nasopharyngeal cancer. Kaplan-Meier survival analysis showed that, at the age of 50 years, the cumulative risk of cancer in PJS patients could reach 49.3%, and at 60 years, the cumulative risk of cancer could reach 71.7% (Figure 1B).

DISCUSSION

This study found that 90.1% of PJS patients developed mucocutaneous pigmentation before the age of 7 years. The median time between the appearance of mucocutaneous pigmentation and the occurrence of abdominal symptoms was 10 years. Therefore, as PJS patients are often outpatients undergoing dermatological and stomatological treatment for mucocutaneous pigmentation, which is easy for doctors unfamiliar with the disease to misdiagnose, and valuable early treatment opportunities may thus be missed, clinicians should be more vigilant. PJS that does not cause the malignant transformation of mucocutaneous pigmentation on the lips and limbs generally does not need to be treated[10]. Some scholars used a 755 nm picosecond laser to treat mucocutaneous pigmentation on the lips, which showed a reduction in pigmentation of 50%-75% after 3 mo, and good recovery after surgery[11].

At present, there is no effective treatment for PJS polyps. Some scholars used everolimus (mechanistic target of rapamycin inhibitor, a derivative of rapamycin) to treat two PJS patients, and the results showed that pancreatic cancer progressed after 2 mo in one patient, while the other refused to continue treatment due to severe toxicity, so the trial was discontinued[12]. One study[13] found that patients with PJS (2/6) responded well to celecoxib and had fewer gastric polyps, suggesting that COX-2 inhibitors may be beneficial in PJS therapy. However, in our study, celecoxib capsules were used to prevent polyps, and it was effective in only one case. This suggests that COX-2 inhibitors may not be ideal drugs for PJS therapy, as they had a high proportion of side effects (6/9) such as allergic reaction and gastrointestinal bleeding. We also treated 30 PJS patients with TCM, which had an effective rate for small polyps of only 40%, while being basically ineffective for large polyps. This study showed that PJS polyps could be distributed in the stomach (74.4%), small intestine (96.5%), and colorectum (78.6%). The burden and diameter of small intestine polyps were much higher than those of stomach polyps, which was the root cause of intussusception and intestinal obstruction. We found that some PJS patients had concurrent gallbladder polyps, nasal polyps, cervical polyps, *etc*. Whether these are the extra-gastrointestinal manifestations of PJS polyps still needs to be supported by pathological evidence. Endoscopic treatment methods for PJS polyps include EMR and ESD[14,15]. Our study showed that the incidence of gastrointestinal bleeding, perforation, and other serious complications during colonoscopy and endoscopic treatment was 2.3%. Overall, the endoscopic treatment of PJS polyps is safe and feasible.

Due to gastrointestinal polyps occurring in multiple places, patients often undergo multiple laparotomy operations, which can cause severe intraperitoneal adhesion. Laparoscopic surgery is difficult and not recommended as a routine treatment method for PJS. Laparoscopic surgery and small intestinal endoscopic surgery could be used for abdominal exploration or patients with moderate abdominal adhesions. Laparoscopic perforation repair or hemostatic suture can be used as adjuvant treatments for endoscopic complications when removing large localized polyps and dense intestinal segments of polyps with perforation or bleeding during endoscopic treatment.

Open surgery for PJS patients should be explored to determine the distribution of polyps in the whole gastrointestinal tract. Following the principle of organ protection, it is recommended to perform intestinal adhesion release and try to avoid large sections of intestinal resection causing short bowel syndrome. Multiple small incisions can be made in the intestinal wall to remove all palpable polyps, but when encountering necrotic, perforated, or dense bowel polyps, bowel segment resection is recommended. For patients diagnosed with malignant transformation, radical resection and regional lymph node dissection should be performed on cancerous intestinal segments.

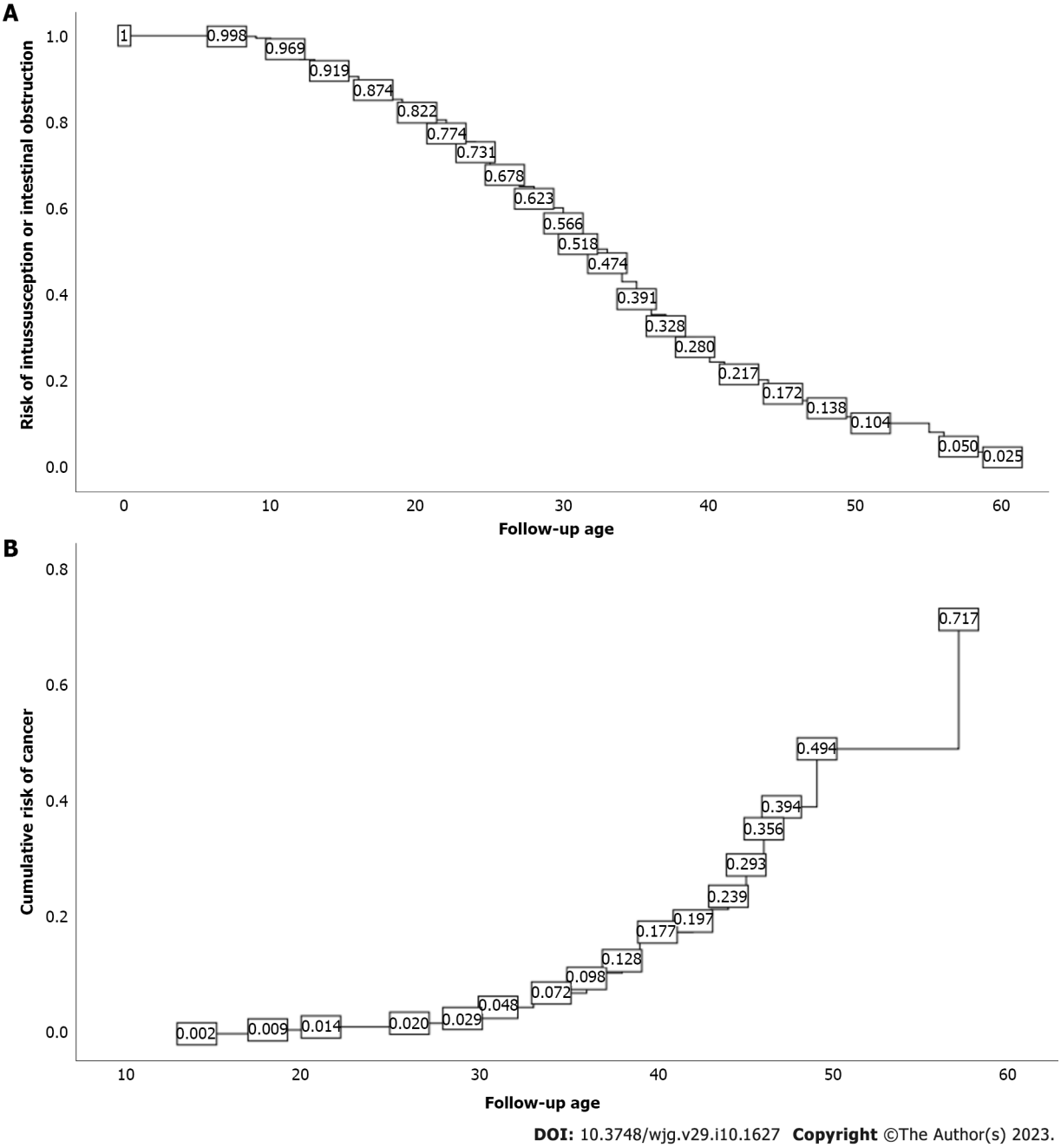

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Figure 1 Cumulative risk. A: Intussusception; B: Cancer.

PJS is a tumor-susceptible syndrome[16-18], and it has been previously reported that PJS polyps have a evolvement sequence of hamartoma-adenoma-cancer[19]. In our study, 46 cases were pathologically confirmed to have a malignant tumor, of which 35 (76.1%) were malignant transformation of PJS polyps and the rest were breast cancer, cervical cancer, lung cancer, ovarian cancer, nasopharyngeal cancer, and cholangiocarcinoma. The cumulative cancer risk of PJS patients reached 49.3% at the age of 50 years and 71.7% at the age of 60 years. Considering the short follow-up time for some cases, the actual cumulative risk of carcinogenesis may be higher. For PJS patients, we should also pay attention to their mental health, especially adolescent PJS patients. With the deepening of their understanding of PJS, *e.g.*, it is a genetic disease which requires long-term hospitalization, they may gradually develop the psychology of hating their parents and retaliating against society. We also found that PJS patients have different degrees of concern about the risk of cancer, which reminds us that we should strengthen psychological counseling and provide professional interpretation for PJS patients to reduce their anxiety and depression, and where necessary, psychologists should be involved in the treatment process of PJS patients.

Our study showed that there was a statistically significant difference in the age of the occurrence of mucocutaneous pigmentation between patients with and without a family history of PJS ($P = 0.016$, $Z = -2.415$). As such, we suggest that suspected or already confirmed PJS patients should undergo next

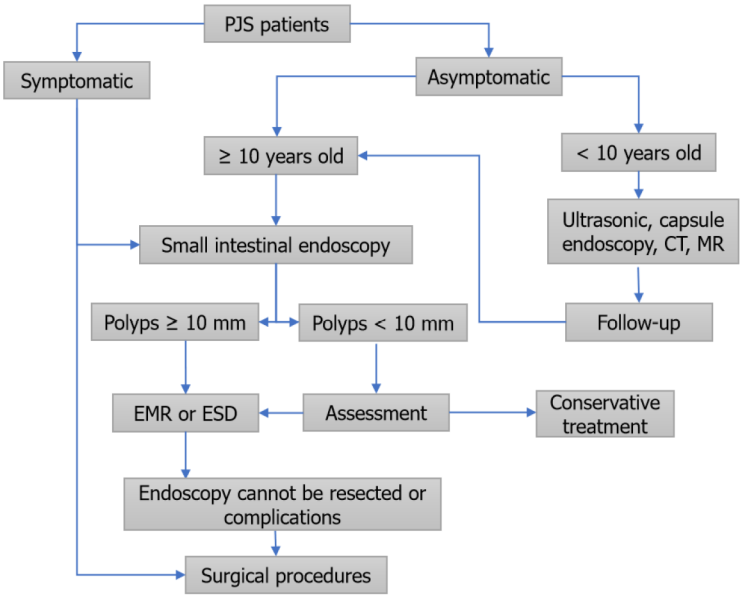

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Figure 2 Diagnosis and treatment process of Peutz-Jeghers syndrome patients. PJS: Peutz-Jeghers syndrome; CT: Computed tomography; MR: Magnetic resonance imaging; EMR: Endoscopic mucosal resection; ESD: Endoscopic mucosal dissection.

generation sequencing, which can reliably quantify the incidence, penetrance, mutation type, and expression of PJS. When adult patients are pregnant, they can receive preimplantation genetic testing or preimplantation genetic screening to predict whether there is a correlation with *STK11* or other gene mutation[20,21]. If screening finds that the fetus is a carrier of the *STK11* gene mutation, the parents can terminate the pregnancy; thus, this approach makes it possible to have healthy children born.

At present, there is no unified protocol for the follow-up of PJS polyps. Our study showed that the occurrence time of mucocutaneous pigmentation in most patients is earlier than the occurrence time of gastrointestinal symptoms, which is a good window period in which to intervene in the development of polyps. We suggest that PJS patients should start endoscopic examinations when they are 10 years old, and the endoscopic treatment of gastrointestinal polyps ≥ 10 mm in diameter should be performed to prevent intussusception and intestinal obstruction. The whole gastrointestinal tract should be explored by oral and transanal small intestinal enteroscopy as far as possible. Cold forceps polypectomy and cold SP or APC can be used on polyps < 10 mm which are sessile or flat; combined with high-frequency electroresection, effective eradication can be achieved without complications such as bleeding and perforation. If small intestinal enteroscopy exploration finds moderate intussusception, incomplete intestinal obstruction, and a perforation area < 1 cm, conservative treatment methods can be used, such as fasting, fluid infusion, gastrointestinal decompression, endoscopic balloon reduction, or perforation repair with titanium clips. If polyps ≥ 10 mm are detected, EMR or ESD will be required to remove them, but the possibility of perforation should be noted. Early surgical treatment should be performed for irretrievable intussusception, complete intestinal obstruction, intestinal perforation area ≥ 1 cm, malignant polyps, and failure of conservative treatment. PJS patients < 10 years old may undergo noninvasive examinations such as abdominal ultrasound, CT, capsule endoscopy, gastrointestinal contrast, or magnetic resonance imaging to assess the burden and diameter of their gastrointestinal polyps. If polyps < 10 mm are found, resection can be attempted for a smaller quantity of polyps, but if they cannot be removed, a biopsy will be required to determine the pathology of the polyps and estimate the next follow-up time, and the growth rate of the polyps needs to be closely monitored. For giant polyps, multiple endoscopic resections should be carried out, but for diffuse distributions of polyps, the complete resection of all polyps in the entire intestinal canal should not be excessively pursued in order to prevent short bowel syndrome, and the patients should be monitored every 1 to 2 years (Figure 2).

CONCLUSION

The development of gastrointestinal symptoms in PJS patients is closely related to the age of the appearance of mucocutaneous pigmentation and polyp burden and diameter. The later the age of mucocutaneous pigmentation, the more severe the gastrointestinal symptoms, and patients receive more frequent operations and hospitalization. As PJS polyps have a high risk of intussusception and carcinogenesis, patients aged ≥ 10 years should undergo a small intestinal enteroscopy every 1–2 years.

Endoscopic treatment has a good safety profile and can have significant beneficial effects for PJS patients, and timely endoscopic treatment can reduce the risk of the intussusception and carcinogenesis of polyps.

ARTICLE HIGHLIGHTS

Research background

Peutz-Jeghers syndrome (PJS) is a clinically rare autosomal dominant inherited disease with pigmented spots on the lips and mucous membranes and extremities, scattered gastrointestinal polyps, and susceptibility to tumors as clinical manifestations. Effective preventive and curative methods are still lacking.

Research motivation

Here we summarize out experience with 566 Chinese patients with PJS from a Chinese medical center our experience with 566 Chinese patients with PJS from a Chinese medical center with regard to the clinical features, diagnosis, and treatment, in order to promote the clinical understanding of PJS and improve its clinical diagnosis and treatment.

Research objectives

To explore the clinical features, diagnosis, and treatment of PJS in Chinese patients.

Research methods

The clinical data of 566 PJS cases admitted to the Air Force Medical Center from January 1994 to October 2022 was retrospectively analyzed, including age, gender, ethnicity, family history, first treatment age, time and sequence of appearance of mucocutaneous pigmentation, polyp distribution, polyp quantity and diameter, frequency of hospitalization, frequency of surgical operations, *etc.*

Research results

Of all the patients included, 55.3% were male and 44.7% were female. Median time to the appearance of mucocutaneous pigmentation was 2 years, and median time from the appearance of mucocutaneous pigmentation to the occurrence of abdominal symptoms was 10 years. The vast majority (92.2%) of patients underwent small bowel endoscopy and treatment, with 2.3% having serious complications. There was a statistically significant difference in the number of enteroscopies between patients with and without canceration (P = 0.004, Z = -2.882); 71.2% of patients underwent surgical operation, 75.6% of patients underwent surgical operation before the age of 35 years, and there was a statistically significant difference in the frequency of surgical operations between patients with and without cancer (P = 0.000, Z = -5.127). At 40 years of age, the cumulative risk of intussusception in PJS was approximately 72.0%, and at 50 years, the cumulative risk of intussusception in PJS was approximately 89.6%. At 50 years of age, the cumulative risk of cancer in PJS was approximately 49.3%, and at 60 years of age, the cumulative risk of cancer in PJS was approximately 71.7%.

Research conclusions

The risk of intussusception and cancer of PJS polyps increases with age. PJS patients ≥ 10 years old should undergo annual enteroscopy. Endoscopic treatment has a good safety profile and can reduce the occurrence of polyp intussusception and cancer. Surgery should be conducted to protect the gastrointestinal system by removing polyps.

Research perspectives

The clinical data of 566 PJS cases diagnosed and treated in a Chinese medical center were retrospectively analyzed, and the clinical characteristics and diagnosis and treatment process of Chinese PJS are summarized.

FOOTNOTES

Author contributions: Xu ZX, Jiang LX, and Chen YR contributed equally to this study; Gu GL designed the research; Xu ZX, Jiang LX, Chen YR, Zhang YH, Zhang Z, Yu PF, Dong ZW, and Yang HR collected and analyzed the clinical data; Xu ZX, Jiang LX, and Chen YR wrote the manuscript; Gu GL and Dong ZW revised the manuscript.

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ORCID number: Yu-Hui Zhang 0000-0002-3224-9017; Zhi Zhang 0000-0001-5870-1940; Peng-Fei Yu 0000-0002-0528-1839; Zhi-Wei Dong 0000-0001-7009-9331; Hai-Rui Yang 0000-0003-2768-8493; Guo-Li Gu 0000-0002-9998-047X.

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ORIGINAL ARTICLE

Observational Study

Intraprocedural gastric juice analysis as compared to rapid urease test for real-time detection of *Helicobacter pylori*

Riccardo Vasapolli, Florent Ailloud, Sebastian Suerbaum, Jens Neumann, Nadine Koch, Lukas Macke, Jörg Schirra, Julia Mayerle, Peter Malfertheiner, Christian Schulz

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Riccardo Vasapolli, Nadine Koch, Lukas Macke, Jörg Schirra, Julia Mayerle, Peter Malfertheiner, Christian Schulz, Medical Department II, University Hospital LMU Munich, Munich 81377, Germany

Riccardo Vasapolli, Sebastian Suerbaum, Lukas Macke, Christian Schulz, Deutsches Zentrum für Infektionsforschung, Partner Site Munich, Munich 81377, Germany

Florent Ailloud, Sebastian Suerbaum, Max von Pettenkofer Institute, Faculty of Medicine, Ludwig-Maximilians University of Munich, Munich 80336, Germany

Florent Ailloud, Sebastian Suerbaum, National Reference Center for Helicobacter pylori, Munich 81377, Germany

Jens Neumann, Institute of Pathology, Faculty of Medicine, Ludwig-Maximilians-University of Munich, Munich 81377, Germany

Corresponding author: Christian Schulz, MD, Deputy Director, Medical Department II, University Hospital LMU Munich, Marchionini Strasse 15, Munich 81377, Germany.
chr.schulz@med.uni-muenchen.de

Abstract

BACKGROUND

Endofaster is an innovative technology that can be combined with upper gastrointestinal endoscopy (UGE) to perform gastric juice analysis and real-time detection of *Helicobacter pylori* (*H. pylori*).

AIM

To assess the diagnostic performance of this technology and its impact on the management of *H. pylori* in the real-life clinical setting.

METHODS

Patients undergoing routine UGE were prospectively recruited. Biopsies were taken to assess gastric histology according to the updated Sydney system and for rapid urease test (RUT). Gastric juice sampling and analysis was performed using the Endofaster, and the diagnosis of *H. pylori* was based on real-time ammonium measurements. Histological detection of *H. pylori* served as the diagnostic gold standard for comparing Endofaster-based *H. pylori* diagnosis with RUT-based *H.*

pylori detection.

RESULTS

A total of 198 patients were prospectively enrolled in an *H. pylori* diagnostic study by Endofaster-based gastric juice analysis (EGJA) during the UGE. Biopsies for RUT and histological assessment were performed on 161 patients (82 men and 79 women, mean age 54.8 ± 19.2 years). *H. pylori* infection was detected by histology in 47 (29.2%) patients. Overall, the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value (NPV) for *H. pylori* diagnosis by EGJA were 91.5%, 93.0%, 92.6%, 84.3%, and 96.4%, respectively. In patients on treatment with proton pump inhibitors, diagnostic sensitivity was reduced by 27.3%, while specificity and NPV were unaffected. EGJA and RUT were comparable in diagnostic performance and highly concordant in *H. pylori* detection (κ-value = 0.85).

CONCLUSION

Endofaster allows for rapid and highly accurate detection of *H. pylori* during gastroscopy. This may guide taking additional biopsies for antibiotic susceptibility testing during the same procedure and then selecting an individually tailored eradication regimen.

Key Words: *Helicobacter pylori* diagnostic; Chronic gastritis; Gastric juice; Endofaster; Rapid urease test; Antimicrobial susceptibility testing

Core Tip: Diagnosis of *Helicobacter pylori* (*H. pylori*) infection can be rapidly achieved within the framework of gastroscopy by rapid urease test (RUT) or by gastric juice analysis with Endofaster. In this prospective observational study, we compared the accuracy of these two methods. Gastric juice analysis with Endofaster could reliably detect *H. pylori* with high accuracy, showing a diagnostic performance comparable to that of RUT and a major advantage of an immediate result. Intraprocedural *H. pylori* detection (or exclusion) is crucial to optimize the diagnostic approach and improve the management of infection. The diagnosis of Endofaster may guide additional sampling for antibiotic susceptibility testing in positive patients or avoid unnecessary biopsies in negative patients.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infects nearly half of the world's population, with variable prevalence rates ranging from 20%-30% in Western countries to > 70% in Africa[1]. *H. pylori* infection causes chronic active gastritis and may lead to severe complications including gastroduodenal ulcers, gastric cancer and mucosa-associated lymphoid tissue lymphoma[2]. The diagnosis of active *H. pylori* infection is achieved by non-invasive tests such as the urea breath test (UBT) and stool antigen tests (SAT), as well as invasive methods based on endoscopy and gastric biopsies for histological assessment, rapid urease test (RUT), culture and molecular tests.

Current guidelines recommend testing for *H. pylori* in all patients undergoing upper gastrointestinal endoscopy (UGE)[3]. The Endofaster has been introduced as new diagnostic device, which consents the detection of *H. pylori* by performing biochemical analysis of gastric juice aspirated during gastroscopy. Previous validation studies have shown that this device has high accuracy for *H. pylori* detection and reported diagnostic values similar to those of UBT and histology[4,5]. The diagnostic performance of the Endofaster has not been compared with that of the RUT, which shares a similar characteristic in terms of providing results in a short-term temporal context through endoscopic examination. This allows for therapeutic management immediately after the diagnostic procedure.

The aim of this prospective study was to validate the diagnostic performance of the Endofaster for *H. pylori* detection in patients who underwent UGE compared to conventional RUT.

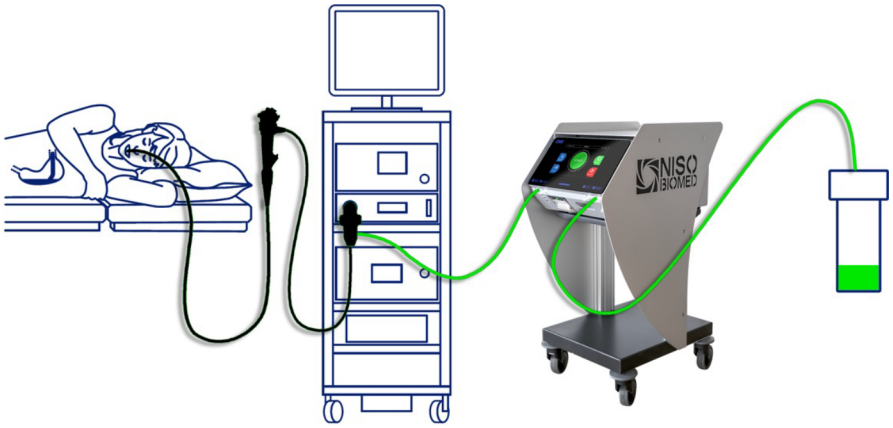

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Figure 1 Schematic diagram of Endofaster device's montage.

MATERIALS AND METHODS

Study population

Consecutive patients undergoing routine UGE to investigate dyspepsia or other alarming symptoms (weight loss, anemia, vomiting, abdominal pain, or dysphagia) were prospectively recruited at the Ludwig Maximilians University Hospital in Munich from January to June 2022.

Subjects were recruited within the ERANET Bavaria and Helicopredict projects (German clinical trials register, DRKS-ID: DRKS00028629), large-scale prospective studies focused on studying different aspects of *H. pylori* infection, including improving the diagnosis and management of *H. pylori*, determining local antibiotic resistance spectrum, with the aim of developing a genotypic resistance testing database for predicting antibiotic susceptibility and evaluating the impact of the microbiome of the upper gastrointestinal tract on gastric carcinogenesis.

The study was approved by the local ethics committee and government authorities and was conducted in accordance with current Good Clinical Practice guidelines and the Declaration of Helsinki [6]. All recruited subjects provided written informed consent for participation. Previous gastric surgery and intake of anticoagulants or any antibiotic therapy within 4 wk prior to endoscopy were exclusion criteria. Regular use of proton pump inhibitors (PPI) or previous *H. pylori* eradication therapy did not represent exclusion criteria, but were recorded in detail. Only patients not taking a PPI or *H. pylori* treatment-naïve patients were considered to meet the desired minimum sample size.

Endoscopic procedure and histological assessment

Enrolled patients underwent a diagnostic UGE using standard video gastroscopes (GIF-HQ190, Olympus, Tokyo, Japan). All examinations were performed with sedation using Propofol and/or Midazolam. An analysis of gastric juice was performed at the beginning of the UGE by Endofaster. Special attention was paid during intubation: The stomach was handled first and no fluid was allowed to be sucked during passage through the oral cavity or esophagus. In order to avoid possible dilution of gastric juice prior to collection the administration of endoscopic premedications (*i.e.* dimethicone, N-acetylcysteine, pronase *etc.*) before endoscopy were not allowed. Furthermore, washing with water and cleaning the endoscopic lens were avoided until sampling was completed. After endoscopic assessment of the mucosa, gastric biopsies were obtained. Two biopsies - one from antrum and one from corpus (both from the greater curvature) - were taken for the RUT (Pronto Dry® New, Medical Instruments Corporation, Herford, Germany). RUT was performed according to the manufacturer's instructions and assessed for positive response during gastroscopy and 1 h after biopsy sampling. The inspection time taken to perform the diagnostic UGE (excluding the time spent on gastric juice aspiration and on biopsy sampling) and the time it took until first detection of *H. pylori* positivity by RUT were recorded. Further biopsies (2 from antrum, 1 from angulus and 2 from corpus) were subjected to routine histology according to the updated Sydney system[7] and current guidelines[3]. In each biopsy sampling set the following stainings were performed: Hematoxylin and eosin, periodic acid-Schiff and a *H. pylori* specific staining (modified Giemsa staining).

Endofaster analysis

Real-time gastric juice analysis was performed using an Endofaster 21-42 (NISO Biomed, Turin, Italy), which is interposed between the endoscope and the suction system (Figure 1). This innovative device analyzes the first 3.3 mL of gastric juice aspirated at the beginning of the UGE. The Endofaster provides information regarding gastric pH based on hydrogen ion concentration and *H. pylori* detection based on

the measurement of ammonium derived from bacterial urease activity within 60-90 s[4,5]. Considering that approximately 10-20 s (max 30 s) are needed to aspirate the gastric juice through the scope a final *H. pylori* diagnosis is provided within the first 2 min from the beginning of the endoscopic procedure. Except for the time spent on the initial gastric juice collection no additional time is required for Endofaster use during the endoscopic procedure. In line with previous studies, we used a cut-off value of > 62 ppm/mL to indicate the presence of *H. pylori*[8].

Statistical analyses

Descriptive statistical analysis was performed using IBM SPSS Statistics 23.0.0 (IBM Corporation, New York, NY, United States). Numerical variables were expressed as mean ± SD. Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were calculated for both Endofaster and RUT using histology as the gold standard. The concordance between Endofaster and RUT results was assessed by using Cohen's κ-value. The McNemar test was used to compare sensitivities and specificities between the two tests.

Sample size estimation was based on a 95%CI and the calculation methods of Buderer *et al*[9] were applied using following formula:

$$n_{Se} = \frac{Z^2_{\frac{\alpha}{2}} \hat{Se}\,(1 - \hat{Se})}{d^2 \times Prev}$$

$$n_{Sp} = \frac{Z^2_{\frac{\alpha}{2}} \hat{Sp}\,(1 - \hat{Sp})}{d^2 \times (1 - Prev)}$$

Where Z is the normal distribution value set to 1.96, corresponding to the 95%CI, and d is the maximum acceptable width of the 95%CI, set at 10%. Based on a previous study, Endofaster had a sensitivity (*Se*) of 97.1% and a specificity (*Sp*) of 89.7% for *H. pylori* detection[5]. Recently, the prevalence of *H. pylori* infection (*Prev*) in Germany was estimated to be 35.3% (95%CI: 31.2-39.4)[1]. As a result, using the criteria listed above, this study required a minimum of 31 *H. pylori*-positive patients (n_{Se}) and 55 *H. pylori*-negative patients (n_{Sp}), resulting in a minimum total sample size of 86 subjects. Patients with PPI use or prior *H. pylori* eradication therapy were not considered to achieve the minimum sample size required. All authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

Characteristics of the study cohort

A total of 198 patients undergoing elective UGE were enrolled. Of these, 182 underwent gastric juice analysis with the Endofaster. After excluding patients who reported antibiotic intake within the last 4 wk (n = 10, 5.2%), patients who could not undergo biopsy due to anticoagulation therapy (n = 8, 4.1%) and patients with insufficient volume of aspirated gastric juice for Endofaster analysis (n = 13, 6.7%) a total of 161 patients (male: 82, female: 79, mean age 54.8 ± 19.2 years) were included in the analysis. 67 (41.6%) patients were on ongoing PPI therapy and 94 patients (58.4%) did not report any PPI therapy. The demographic, endoscopic, and histopathological characteristics of the study cohort are shown in Table 1. A flow chart of the study's recruitment is shown in Figure 2.

Diagnostic performance of Endofaster and RUT for H. pylori detection

The average duration of the diagnostic UGE was 8.5 min. *H. pylori* infection was diagnosed in 47 (29.2%) patients on histopathology. Endofaster results were positive in 51 patients (31.6%), while RUT was positive in 45 (28.0%) cases. A positive RUT reaction was detected during endoscopy in 37 subjects (78.7%), with a mean positive reaction time of 16.4 min. The overall diagnostic performances of Endofaster and RUT for *H. pylori* detection as compared to histology (gold standard) are shown in Table 2. Sensitivity, specificity, accuracy, PPV and NPV were 91.5%, 93.0%, 92.6%, 84.3% and 96.4% for Endofaster, and 93.6%, 99.1%, 97.5%, 97.8% and 97.4% for RUT, respectively. No significant differences were observed in the diagnostic performances of the Endofaster and the RUT (P > 0.05). This was confirmed by an almost perfect agreement of *H. pylori* detection between the two tests (κ-value = 0.85).

Both Endofaster and RUT showed excellent diagnostic performances when considering only patients without ongoing PPI therapy (n = 94). In this subgroup, 37 (39.4%) subjects were histopathologically diagnosed as positive for *H. pylori*.

Among patients treated with PPI (n = 67), the presence of *H. pylori* was detected by histology in 10 subjects (14.9%). In this subgroup, a reduction in sensitivity, PPV and accuracy was observed for both Endofaster and RUT, whereas specificity and NPV remained almost unchanged (Table 2). Again, in the subgroup analysis, there were no significant differences in diagnostic performances between Endofaster and RUT (P > 0.05).

Table 1 Demographic, endoscopic and histopathological characteristics of the patients included in the study, *n* (%)

Characteristics	Value
Overall	161
Male	82 (50.9)
Female	79 (49.1)
Age, mean ± SD (range) yr	54.8 ± 19.2 (19-90)
H. pylori positive	47 (29.2)
H. pylori negative	114 (70.8)
Patients without PPI therapy	94 (58.4)
Male	46 (48.9)
Female	48 (51.1)
Age, mean ± SD (range) yr	50.3 ± 19.2 (19-86)
H. pylori positive	37 (39.4)
H. pylori negative	57 (60.6)
Patients with PPI therapy	67 (41.6)
Male	36 (53.7)
Female	31 (46.3)
Age, mean ± SD (range) yr	58.9 ± 19.2 (23-90)
H. pylori positive	10 (14.9)
H. pylori negative	57 (85.1)
Endoscopic and histopathological findings[1]	
Normal	13 (8.1)
Gastroesophageal reflux disease	26 (16.1)
Chronic gastritis	84 (52.2)
Erosive gastritis	32 (19.9)
Gastric ulcer	5 (3.1)
Duodenal ulcer	3 (1.9)
Gastritis with low-grade PL	36 (22.4)
Gastritis with high-grade PL	4 (2.5)
Others[2]	6 (3.7)

[1]Different conditions may coexist. Precancerous lesions include gastric atrophy and/or intestinal metaplasia, assessed according to the Operative Link on Gastritis Assessment (OLGA) and Operative Link on Gastric Intestinal Metaplasia systems (low grade if OLGA/IM < 3, high grade if OLGA/IM ≥ 3)[23, 24].

[2]"Others" include patients with gastric cancer (*n* = 1), gastric lymphoma (*n* = 1), Barrett's esophagus (*n* = 2), gastric hyperplastic polyps (*n* = 1) and gastric neuroendocrine tumors (*n* = 1).

H. pylori: *Helicobacter pylori*; PPI: Proton pump inhibitors.

DISCUSSION

Several diagnostic methods are performed on biopsies obtained during the UGE to detect *H. pylori* with high accuracy. They are highly accurate, but have the limitation to delay even a few days in providing diagnostic results, thus not allowing an immediate therapeutic decision. RUT is the only exception in clinical practice that allows relatively rapid detection of *H. pylori*, usually within 1 h after UGE[10-12].

Here, we report on the diagnostic performance of Endofaster-based gastric juice analysis (EGJA), an innovative technology that allows intraprocedural *H. pylori* detection compared to RUT. We found that the high accuracy (> 90%) of EGJA was comparable to that of RUT for *H. pylori* detection, confirming previous reports of the high accuracy of EGJA compared to histology[4,5,8,13]. A previous prospective study of EGJA in 182 patients determined the sensitivity, specificity and accuracy of *H. pylori* to be 97.1%, 89.7% and 92.6%, respectively, compared to histology being used as the gold standard as well as

Table 2 Diagnostic performance of the Endofaster® and rapid urease test (ProntoDry®) for the diagnosis of *Helicobacter pylori* infection in the study cohort, % (95%CI)

	Endofaster			Rapid urease test		
	Overall	No PPI	Ongoing PPI therapy	Overall	No PPI	Ongoing PPI therapy
Sensitivity	91.5 (79.6-97.6)	97.3 (85.8-99.9)	70.0 (34.8-93.3)	93.6 (82.5-98.7)	97.3 (85.8-99.9)	80.0 (44.4-97.5)
Specificity	93.0 (84.6-96.9)	96.5 (87.9-99.6)	89.5 (78.5-96.0)	99.1 (95.2-100)	100 (93.7-100)	98.3 (90.6-100)
PPV	84.3 (73.3-91.3)	94.7 (82.2-98.6)	53.9 (33.1-73.4)	97.8 (86.2-99.7)	100 (-)	88.9 (52.8-98.3)
NPV	96.4 (91.2-98.6)	98.2 (88.8-99.7)	94.4 (86.8-97.8)	97.4 (92.7-99.1)	98.3 (89.2-99.8)	96.6 (89.0-99.0)
Accuracy	92.6 (87.3-96.1)	96.8 (91.0-99.3)	86.6 (76.0-93.7)	97.5 (93.8-99.3)	98.9 (94.2-100)	95.5 (87.5-99.1)

Overall (n = 161), in patients with ongoing proton pump inhibitors (PPI) therapy (n = 67) and in patients without PPI therapy (n = 94). Values are shown as percentages with 95%CI. PPI: Proton pump inhibitor; PPV: Positive predictive value; NPV: Negative predictive value.

UBT, which was used for reclassification of *H. pylori* status in case of discordance between EGJA and histology results[5]. A multicenter study of 525 consecutive patients reported an overall sensitivity, specificity and accuracy of 87%, 84% and 85%, respectively, when compared to histology[8].

We have observed impaired diagnostic sensitivity in patients with PPI in EGJA and RUT, which is a common phenomenon for all tests, including non-invasive tests[3,14]. In the context of PPI intake, only histology remains highly sensitive when gastric biopsies are taken from the proximal stomach[15,16]. This is related to the PPI-induced shift from antrum-predominant to corpus-predominant gastritis. We found that 3 out of 4 false negatives and 6 out of 8 false positives (75%) in EGJA were registered in patients on PPI therapy. Two of the three false negatives (66%) diagnosed by the RUT were PPI users. EGJA and RUT rely on the same principles related to ammonium concentration and *H. pylori* urease activity. Therefore, both tests are influenced by the reduction of bacterial load by PPI, which may lead to false negative results. Furthermore, an elevated pH in the stomach environment may lead to an overgrowth of other non-*H. pylori* bacteria with urease activity[17]. Several different urease-positive bacterial strains, such as *Staphylococcus capitis subsp. urealyticus* and *Streptococcus salivarium*, have been isolated in gastric juice and mucosal samples from patients with gastric hypochloridria[18]. The higher abundance of these strains may interfere with urea metabolism and explain the increased number of false-positive cases among patients on PPI therapy. It is necessary to analyze the gastric microbiota and functionality profiles of PPI patients in order to further address this interesting topic. In our study, the low prevalence of *H. pylori*-infected subjects (only 14.9%) within the group of patients on PPI therapy is a limitation because of an underpowered statistical analysis. Using histology as the gold standard for *H. pylori*-diagnosis in a cohort with relatively low-prevalence of *H. pylori* may represent a further limitation of this study. Histopathological diagnosis of *H. pylori* may suffer from potential sampling error due to the patchy distribution of the bacterium[19]. However, by using the updated Sydney system based on biopsies from 5 different sites and applying different staining methods for *H. pylori* detection the accuracy of *H. pylori*-diagnosis by histology is not inferior to any non-invasive test (13C-UBT/SAT). In support for the validity of histology as gold standard for *H. pylori* detection, we found also no indirect signs of *H. pylori*-gastritis (*i.e.* neutrophils infiltration in the gastric mucosa) in the absence of *H. pylori*.

EGJA has the advantage of obtaining more rapid diagnostic results when performing endoscopy compared to RUT. During endoscopy (within a time period of approximately 10 min), a positive signal in the RUT for the presence of *H. pylori* was recorded in 78.7% of those producing *H. pylori* positivity at the end of the reading time in our study, consistent with the time interval of response reported in previous validation studies[12,20], whereas EGJA resulted in the diagnosis of *H. pylori* within 2 min after starting with UGE. The intraprocedural detection of *H. pylori* infection combined with measurement of gastric pH can guide the endoscopist on the most appropriate approach to complete the diagnostic assessment, *i.e.*, whether or not to carry out additional biopsies for gastritis severity staging and antibiotic susceptibility testing (AST). This has become an absolute requirement for the selection of the eradication regimen due to the high antibiotic resistance rates of clarithromycin, metronidazole and fluoroquinolones[21]. Real-time detection of *H. pylori* suggests carrying out additional biopsies for AST during UGE and selecting an *H. pylori* eradication regimen accordingly.

Such a strategy would have a substantial impact on cost-effectiveness by reducing the duration of the procedure and lowering costs due to histological or microbiological analysis of negative gastric biopsies, an aspect that has been previously addressed by others[8].

Future studies will explore the possibility of combining EGJA with *in situ* molecular genetic antibiotic resistance testing. Promising data in this field were revealed by a recent meta-analysis of four studies that evaluated gastric juice-based genotypic detection of *H. pylori* antibiotic resistance to clarithromycin compared to standard culture-based methods[22].

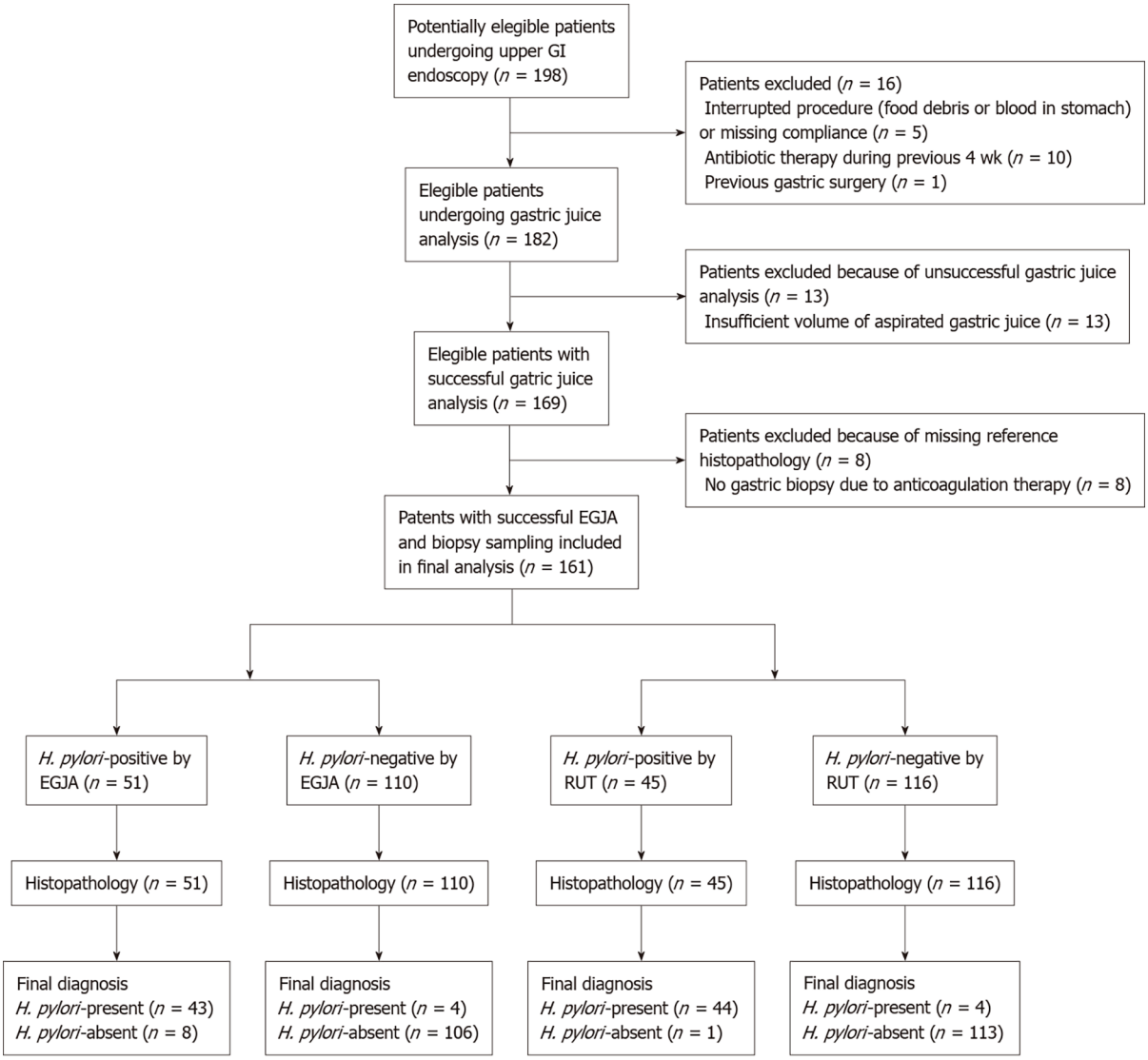

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Figure 2 Flowchart according to Standards for Reporting Diagnostic Accuracy Studies guidelines of patient inclusion in the study and analysis. EGJA: Endofaster-based gastric juice analysis; *H. pylori*: *Helicobacter pylori*; RUT: Rapid urease test.

CONCLUSION

In conclusion, Endofaster's gastric juice analysis is a highly accurate method for the diagnosis of *H. pylori* infection, comparable to RUT. EGJA-based *H. pylori* diagnosis has an advantage in terms of on-site immediacy of diagnosis. In patients on PPI therapy, sensitivity is reduced, but NPV and specificity are not affected. Real-time detection of *H. pylori* along with the determination of gastric pH during endoscopy adds important information on the need for additional biopsies for more detailed histological assessment and antibiotic susceptibility testing.

ARTICLE HIGHLIGHTS

Research background

Diagnosis of *Helicobacter pylori* (*H. pylori*) infection can be rapidly achieved within the framework of gastroscopy by rapid urease test (RUT) or by gastric juice analysis with Endofaster.

Research motivation

The diagnostic performance of the Endofaster has not been compared with that of the RUT, which shares a similar characteristic in terms of providing results in a short-term temporal context through endoscopic examination.

Research objectives

The objective of this prospective study was to validate the diagnostic performance of the Endofaster for *H. pylori* detection in patients who underwent gastroscopy compared to the diagnostic accuracy of a standard RUT.

Research methods

Patients undergoing routine upper gastrointestinal endoscopy were prospectively recruited. Biopsies were taken to assess gastric histology according to the updated Sydney system and for RUT. Gastric juice sampling and analysis was performed using the Endofaster, and the diagnosis of *H. pylori* was based on real-time ammonium measurements. Histological detection of *H. pylori* served as the diagnostic gold standard for comparing Endofaster-based *H. pylori* diagnosis with RUT-based *H. pylori* detection.

Research results

Gastric juice analysis with Endofaster could reliably detect *H. pylori* with an overall sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of 91.5%, 93.0%, 92.6%, 84.3%, and 96.4%, respectively. Gastric juice analysis with Endofaster and RUT were comparable in diagnostic performance and highly concordant in *H. pylori* detection (κ-value = 0.85).

Research conclusions

Endofaster's gastric juice analysis is a highly accurate method for the diagnosis of *H. pylori* infection, comparable to RUT. EGJA-based *H. pylori* diagnosis has an advantage in terms of on-site immediacy of diagnosis.

Research perspectives

Intraprocedural diagnosis of *H. pylori*-infection by Endofaster may guide additional sampling for antibiotic susceptibility testing in positive patients or avoid unnecessary biopsies in negative patients.

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FOOTNOTES

Author contributions: Vasapolli R, Schulz C, and Malfertheiner P designed the study, interpreted the data and drafted the manuscript; Vasapolli R, Suerbaum S, Schirra J, Mayerle J, Schulz C, and Malfertheiner P obtained funding and supervised the study procedures; Vasapolli R recruited suitable subjects, performed endoscopy, and collected samples; Vasapolli R, Macke L, Ailloud F, and Koch N registered data and performed statistical analyses; Neumann J performed the histological analysis; All authors read and approved the final version of the manuscript.

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Institutional review board statement: This study was approved by the local ethics committee and government authorities and was conducted in accordance with current Good Clinical Practice guidelines and the Declaration of Helsinki. All recruited subjects provided written informed consent for participation.

Informed consent statement: Written informed consent was obtained from the participants.

Conflict-of-interest statement: The authors have nothing to disclose.

Data sharing statement: Data and analytic methods can be made available to other researchers upon request.

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Country/Territory of origin: Germany

ORCID number: Riccardo Vasapolli 0000-0001-7150-5864; Florent Ailloud 0000-0001-9063-0839; Sebastian Suerbaum 0000-0001-9583-7505; Jens Neumann 0000-0001-9200-441X; Nadine Koch 0000-0002-3654-0733; Lukas Macke 0000-0002-7620-1744; Jörg Schirra 0000-0002-6887-2791; Julia Mayerle 0000-0002-3666-6459; Peter Malfertheiner 0000-0001-8439-9036; Christian Schulz 0000-0003-1841-1337.

Corresponding Author's Membership in Professional Societies: Deutsche Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten; European Helicobacter and Microbiota Study Group; United European Gastroenterology.

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LETTER TO THE EDITOR

Reporting the cases of alcohol-associated hepatitis using the National Inpatient Sample data

Natalie Marlowe, Wei-Qi Lin, Suthat Liangpunsakul

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

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Natalie Marlowe, Wei-Qi Lin, Durect Corporation, Cupertino, CA 95014, United States

Suthat Liangpunsakul, Division of Gastroenterology and Hepatology, Department of Medicine, Indiana University, Indianapolis, IN 46202, United States

Corresponding author: Suthat Liangpunsakul, MD, Full Professor, Division of Gastroenterology and Hepatology, Department of Medicine, Indiana University, 550 University Blvd, Indianapolis, IN 46202, United States. sliangpu@iu.edu

Abstract

The letter is to respond to the recent publication "Trends in hospitalization for alcoholic hepatitis from 2011 to 2017: A USA nationwide study" (*World J Gastroenterol* 2022; 28: 5036-5046). We noticed a significant difference in the total numbers of reported hospitalized alcohol-associated hepatitis (AH) patients between this publication and our publication on *Alcohol Clin Exp Res* (2022; 46: 1472-1481). We believe the number of "AH-related hospitalizations" inflated by the inclusion of patients with non-AH forms of alcohol-associated liver disease.

Key Words: Hospitalization; Alcoholic hepatitis; Alcohol-associated liver disease

Core Tip: We analyzed the most recent National Inpatient Sample data from 2015-2019 using International Classification of Diseases-10 codes and found an increase in alcohol-associated hepatitis (AH) cases from 110135 to 136620 in 2015 and 2019, respectively. The total numbers of reported AH patients in the retrospective study entitled "Trends in hospitalization for alcoholic hepatitis from 2011 to 2017: A USA nationwide study", we believe, included patients with non-AH forms of alcohol-associated liver disease.

Citation: Marlowe N, Lin WQ, Liangpunsakul S. Reporting the cases of alcohol-associated hepatitis using the National Inpatient Sample data. *World J Gastroenterol* 2023; 29(10): 1648-1650

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TO THE EDITOR

We read with great interest the retrospective study entitled "Trends in hospitalization for alcoholic hepatitis from 2011 to 2017: A USA nationwide study" by Wakil *et al*[1]. In this study the authors examined inpatient admission trends for alcohol-associated hepatitis (AH), using the National Inpatient Sample (NIS) database data from 2011 to 2017. The study population were those with aged ≥ 21 years who were hospitalized with either a primary or secondary diagnosis of AH identified by the International Classification of Diseases (ICD)-9 and its corresponding ICD-10 codes.

The authors reported that AH-related hospitalization demonstrated a significant increase from 281506 in 2011 to 324050 hospitalizations in 2017 with an overall increase in the financial burden and cost. We agree with the authors' opinion that AH-related hospitalizations are on the rise, and that they are associated with escalating healthcare costs and utilization. In fact, our recent paper published in *Alcohol Clin Exp Res*, 2022, came to the same conclusions when we analyzed the most recent NIS data for AH hospital discharges from 2015-2019 using a similar methodology[2]. However, we noticed a significant difference in the total numbers of reported AH patients between these studies. In our study, we reported an increase in total hospitalized AH cases from 110135 to 136620 in 2015 and 2019, respectively [2], which was consistent with another recent paper by Ali *et al*[3] in *Annals of Gastroenterology*, 2022. The numbers from both Ali *et al*[3] and our study were much smaller than the numbers quoted by Wakil *et al* [1]. The difference is explained by the ICD codes used in the studies. We included only patients hospitalized with or without cirrhosis under ICD-9 571.1 (AH) and ICD-10 K70.1 (AH with/without ascites). In contrast, Wakil *et al*[1] included patients admitted with AH, alcoholic fatty liver disease, and alcohol-associated cirrhosis [an advanced chronic form of associated liver disease (ALD)]. In our opinion, the number of "AH-related hospitalizations" reported by this study is inflated by the inclusion of patients with non-AH forms of ALD.

Alcohol use and ALD are global public health issues associated with high morbidity and mortality [4]. Retrospective studies investigating AH hospitalization trends and associated healthcare costs should be interpreted with caution because the results may be influenced significantly by the ICD codes used to identify AH patients. Reporting using the most appropriate codes is the best use of these large national databases.

FOOTNOTES

Author contributions: Marlowe N and Lin WQ drafted the manuscript; Liangpunsakul S criticized and edited the manuscript.

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Country/Territory of origin: United States

ORCID number: Suthat Liangpunsakul 0000-0002-6504-8123.

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