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SYSTEMATIC REVIEWS

Environmental tobacco smoke exposure and heart disease: A systematic review

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Author contributions: Lee PN and Forey BA planned the study; Thornton AJ and Hamling JS carried out the literature searches, assisted by Lee PN and Forey BA; Forey BA, Hamling JS and Thornton AJ carried out the data entry which was independently checked by one of these or Lee PN; Lee PN and Forey BA discussed any difficulties in interpreting published data or in the appropriate methods for derivation of RRs; Forey BA and Hamling JS conducted the main statistical analyses along lines discussed and agreed with Lee PN; Lee PN drafted the paper which was critically reviewed by the other authors.

Conflict-of-interest statement: Lee PN, Director of P.N. Lee Statistics and Computing Ltd., is an independent consultant in statistics and an advisor in the fields of epidemiology and toxicology to a number of tobacco, pharmaceutical and chemical companies including the sponsors of this study; Forey BA and Hamling JS are employees of, and Thornton AJ, a consultant to, P.N. Lee Statistics and Computing Ltd.

Data sharing statement: Supplementary File 1 provides a description of the reasons for rejection of some papers. Supplementary File 2 gives full details of the meta-analyses conducted. Supplementary File 3 gives full details of the stepwise multiple regression analysis. Supplementary File 4 gives some results for less commonly used indices of ETS exposure. Copies of the database files are available on request from the corresponding author at peterLee@pnlee.co.uk.

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Abstract

AIM

To review evidence relating passive smoking to heart disease risk in never smokers.

METHODS

Epidemiological studies were identified providing estimates of relative risk (RR) of ischaemic heart disease and 95%CI for never smokers for various indices of exposure to environmental tobacco smoke (ETS). "Never smokers" could include those with a minimal smoking experience. The database set up included the RRs and other study details. Unadjusted and confounder-adjusted RRs were entered, derived where necessary using standard methods. The fixed-effect and random-effects meta-analyses conducted for each exposure index included tests for heterogeneity and publication bias. For the main index (ever smoking by the spouse or nearest equivalent, and preferring adjusted to unadjusted data), analyses investigated variation in the RR by sex, continent, period of publication, number of cases, study design, extent of confounder adjustment, availability of dose-response results and biomarker

data, use of proxy respondents, definitions of exposure and of never smoker, and aspects of disease definition. Sensitivity analyses were also run, preferring current to ever smoking, or unadjusted to adjusted estimates, or excluding certain studies.

RESULTS

Fifty-eight studies were identified, 20 in North America, 19 in Europe, 11 in Asia, seven in other countries, and one in 52 countries. Twenty-six were prospective, 22 case-control and 10 cross-sectional. Thirteen included 100 cases or fewer, and 11 more than 1000. For the main index, 75 heterogeneous ($P < 0.001$) RR estimates gave a combined random-effects RR of 1.18 (95%CI: 1.12-1.24), which was little affected by preferring unadjusted to adjusted RRs, or RRs for current ETS exposure to those for ever exposure. Estimates for each level of each factor considered consistently exceeded 1.00. However, univariate analyses revealed significant ($P < 0.001$) variation for some factors. Thus RRs were lower for males, and in North American, larger and prospective studies, and also where the RR was for spousal smoking, fatal cases, or specifically for IHD. For case-control studies RRs were lower if hospital/diseased controls were used. RRs were higher when diagnosis was based on medical data rather than death certificates or self-report, and where the never smoker definition allowed subjects to smoke products other than cigarettes or have a limited smoking history. The association with spousal smoking specifically (1.06, 1.01-1.12, $n = 34$) was less clear in analyses restricted to married subjects (1.03, 0.99-1.07, $n = 23$). In stepwise regression analyses only those associations with source of diagnosis, study size, and whether the spouse was the index, were independently predictive (at $P < 0.05$) of heart disease risk. A significant association was also evident with household exposure (1.19, 1.13-1.25, $n = 37$). For those 23 studies providing dose-response results for spouse or household exposure, 11 showed a significant ($P < 0.05$) positive trend including the unexposed group, and two excluding it. Based on fewer studies, a positive, but non-significant ($P > 0.05$) association was found for workplace exposure (RR = 1.08, 95%CI: 0.99-1.19), childhood exposure (1.12, 0.95-1.31), and biomarker based exposure indices (1.15, 0.94-1.40). However, there was a significant association with total exposure (1.23, 1.12-1.35). Some significant positive dose-response trends were also seen for these exposure indices, particularly total exposure, with no significant negative trends seen. The evidence suffers from various weaknesses and biases. Publication bias may explain the large RR (1.66, 1.30-2.11) for the main exposure index for smaller studies (1-99 cases), while recall bias may explain the higher RRs seen in case-control and cross-sectional than in prospective studies. Some bias may also derive from including occasional smokers among the "never smokers", and from misreporting smoking status. Errors in determining ETS exposure, and failing to update exposure data in long term prospective studies, also contribute to the uncertainty. The tendency for RRs to increase as more factors are adjusted for, argues against the association being due to uncontrolled confounding.

CONCLUSION

The increased risk and dose-response for various exposure indices suggests ETS slightly increases heart disease risk. However heterogeneity, study limitations and possible biases preclude definitive conclusions.

Key words: Passive smoking; Heart disease; Dose-response; Meta-Analysis; Review

Core tip: We present an up-to-date meta-analysis of the evidence relating environmental tobacco smoke (ETS) exposure to heart disease risk in never smokers. An association is evident for smoking by the spouse (or nearest equivalent) with the relative risk estimated as 1.18 (95%CI: 1.12-1.24), and also with some other indices of ETS exposure. Though the findings suggest a causal relationship, data limitations and bias limit interpretation.

Lee PN, Forey BA, Hamling JS, Thornton AJ. Environmental tobacco smoke exposure and heart disease: A systematic review. *World J Meta-Anal* 2017; 5(2): 14-40 Available from: URL: http://www.wjgnet.com/2308-3840/full/v5/i2/14.htm DOI: http://dx.doi.org/10.13105/wjma.v5.i2.14

INTRODUCTION

This review concerns studies of environmental tobacco smoke (ETS) and heart disease in lifelong non-smokers ("never smokers"). In the 1990s some reviewers[1-4] concluded that exposure of non-smokers to ETS increases risk of heart disease, based partly on meta-analyses of epidemiological data from between 12 and 19 studies which reported statistically significant overall increases of about 25%, and partly on evidence from experimental and clinical studies. Their conclusions were accepted by some major bodies[5-8], and supported by some other reviewers[9-13]. However, other reviewers[14-18] disagreed, pointing to omission of relevant studies, inclusion of inappropriate estimates, heterogeneity of findings, study weaknesses and various sources of bias, as well as limitations in the experimental and clinical evidence.

Since then, the number of relevant epidemiological studies has increased, with over 50 now published. However, no recent comprehensive meta-analysis has been conducted, one published in 2015[13] including fewer studies than in some earlier reviews.

Our main objective is to present an updated meta-analysis of the epidemiological data, although we also briefly discuss the experimental evidence, and studies

of smoking bans.

MATERIALS AND METHODS

Study inclusion and exclusion criteria

Attention is restricted to epidemiological prospective, case-control or cross-sectional studies providing relative risk (RR) estimates for never smokers for one or more of these ETS exposure indices: Spouse (including cohabiting partner), other at home exposure, at work, in adulthood, in childhood, in total, and biomarker based. We use the term "relative risk" to include estimates of it, such as the odds ratio or hazard ratio. Results must be available for a disease definition sufficiently close to ischaemic heart disease (IHD) as currently defined. Studies using a near equivalent definition of "never smokers" are accepted when results for stricter definitions are unavailable. Thus, never smokers may include occasional smokers, those with a minimal lifetime duration of smoking or number smoked, or those who quit at least 5 years ago.

Literature searches

At intervals until July 2016 potentially relevant papers were regularly sought from Medline searches, from extensive in-house files accumulated over many years and from references cited in papers obtained. At the end of the process no paper examined cited a possibly relevant paper not previously examined. The latest search used the terms ["tobacco smoke pollution" (MeSH terms)] AND {["heart diseases"(MeSH Terms)] OR ["cardiovascular diseases" (MeSH Terms)] OR ["myocardial infarction" (MeSH Terms)]} AND ("2012/0101"[Date-MeSH]:"3000" [Date-MeSH]), restricted to humans, and published in the last 5 years.

Study identification

Relevant publications were separated into studies, noting multiple papers per study or multiple studies per paper, and any study overlaps.

Data recorded

Details were extracted on study author, publication year, study location and design, sexes included, number of cases, potential confounding variables considered, and definitions of disease and of never smoker. RR estimates, together with associated 95%CIs were obtained, where available, for ETS exposure at home, at work, in childhood, and in total, and using biomarker based estimates (cotinine or COHb). Separate estimates were extracted or calculated for fatal, non-fatal and overall outcomes and for both unadjusted (or for prospective studies, age-adjusted) and covariate-adjusted RRs. If a study provided more than one adjusted estimate, we used that adjusted for most covariates.

RR derivation

Where studies report RRs/CIs only by level of exposure, those for the overall unexposed/exposed comparisons were estimated[19,20]. These methods were also used to estimate significance of dose-related trends, if not given in the source. Similar methods were used to estimate RRs and CIs excluding stroke from a broader circulatory disease definition.

Meta-analyses

Pre-planned fixed-effect and random-effects meta-analyses were conducted using standard methods[21]. Heterogeneity between RR estimates was assessed by the heterogeneity χ^2, the ratio of which to its degrees of freedom, H, relates to the I^2 statistic[22] by I^2 = 100 (H-1)/H. Publication bias tests were also carried out[23].

For our main analyses, we aimed to produce an exposure index most closely equivalent to "spouse ever smoked", since spousal smoking is the traditional index for studying ETS effects, women married to a smoker having a markedly higher ETS exposure, as measured by cotinine, than women married to a non-smoker[24]. Thus, results (sex-specific if available, otherwise combined sex) were selected in the following order of preference for: Exposure (spouse, household, total), time of exposure (ever, during marriage, current, in the past, in the last 10 years, in adulthood), disease type (fatal or non-fatal, fatal only, non-fatal only), disease definition (circulatory disease minus stroke, overall circulatory disease), and definition of no ETS exposure (unexposed to the specific ETS exposure, unexposed to any ETS, low exposure to the specific ETS exposure, never exposed to the specific ETS exposure, unexposed to ETS at home and at work). In addition, results selected were those adjusted for the most confounders for which results were given. This approach of selecting the most relevant result allowed the meta-analyses to include results from each study. Apart from conducting meta-analyses based on all selected estimates, additional meta-analyses using the same set of estimates, investigated variation in RR by the factors sex, continent, publication period, number of cases, study type, number of confounders considered in the study, availability of dose-response results, whether the spouse was the index, and whether (where the spouse was the index), analyses excluded unmarried subjects. Variation was also studied by fatality of cases, definition of disease, whether biomarker data was used to exclude smokers, use of proxy respondents, type of control used, source of diagnosis, and never smoker definition.

Sensitivity analyses repeated the complete set of meta-analyses described above for the main index of exposure with the order of preference for time of exposure revised to favour current rather than ever exposure (current, during marriage, ever, in the past, in the last 10 years, in adulthood), and also preferring unadjusted (or least adjusted) estimates. Further sensitivity analyses were carried out omitting results from: (1) studies by Layard[25] and LeVois *et al*[26]; (2) a study by Enstrom *et al*[27]; or (3) all three studies. These studies have been criticised (see discussion).

For the main exposure index stepwise regression analysis using forward selection[28] was also used to

determine factors independently predicting risk of heart disease.

Similar meta-analyses were also conducted for other indices with sufficient data (household, workplace, childhood, total, biomarker based), though the meta-analyses by subset were more limited.

Results of meta-analyses are displayed in forest plots. Within each plot, study estimates are listed in increasing order of RR. For the main index, the estimates are grouped by location. The estimates are shown both as numbers and in graphical form logarithmically. In the latter representation an RR is shown as a square with area proportional to its inverse-variance weight. Arrows warn if a CI extends outside the range of the plot. Random-effects estimates are also presented, overall and by location, shown by a diamond whose width indicates the 95%CI.

RESULTS

Studies identified

Fifty-eight studies met the inclusion criteria. These come from 57 publications[25-27,29-82], one publication[66] describing results from two studies. Table 1 gives study details including author, reference(s), publication year, location, design, sexes included, disease definition and fatality, and numbers of cases in never smokers. The studies are listed in chronological order of publication and given consecutive study numbers. Minor overlap between cases in studies 16 and 30, was ignored. Table 2 gives variables adjusted for and never smoker definitions. Supplementary File 1 describes why other publications which might be thought possibly relevant are not included.

Of the 58 studies, 10 were published in the 1980s, 15 in the 1990s, 21 between 2000 and 2009 and 12 more recently. Twenty studies were in North America (19 United States, one Canada), 19 in Europe (10 United Kingdom, two Sweden, two Greece, one each in Albania, Germany, Italy and Norway and one in multiple countries), 11 in Asia (two Hong Kong, five in the rest of China, and one each in Iran, Japan, Pakistan and Singapore) and eight in other countries (three in each of Australia and New Zealand, one in Argentina, and one in 52 countries worldwide).

Twenty six studies were prospective, with lengths of follow-up from three to 39 years, while 22 were case-control, and 10 cross-sectional. Thirteen studies were of females, and four of males. The rest included both sexes, though some did not report sex-specific results. Twenty studies considered only fatal cases and 26 only non-fatal cases, the other 12 including both. As shown in Table 1, although IHD specifically was the disease definition used in almost half the studies, various other definitions were used. The studies varied considerably in size, with 13 of < 100 cases and 11 of > 1000 cases, the largest being of 14891, 6280 and 5932 cases.

As Table 2 shows, two studies only provided un-adjusted results. While in a number of the mainly earlier studies there was quite limited adjustment, many studies adjusted for numerous variables. Apart from sex and age, variables adjusted for in > 10 studies included marital status, blood pressure (or hypertension), cholesterol, social class (or similar variables based on education or income), obesity (or weight), alcohol consumption, diabetes, family history of heart disease (or hypertension), race and exercise.

Thirty-five studies were of never smokers, though only nine of these clarified that subjects never smoked cigarettes, pipes or cigars. Nine studies were of never cigarette smokers, 11 allowed a minimal smoking history, such as smoking less than one cigarette a day or fewer than 100 cigarettes in life, while three studies allowed those who quit smoking some time ago. Four studies excluded subjects with cotinine levels indicative of current smoking.

Main exposure index

Our main analyses use an index as close as possible to ever smoking by the spouse. Four studies were not included in the main index analyses, one (study 40) only reporting risk per 10 years living or working with a smoker, and three (studies 33, 36 and 48) providing results only for a biochemical index. Table 3, supported by Figure 1, presents RRs for the main index, and also gives details of ETS exposure, the definitions of the unexposed group being given in Supplementary File 2. RRs for the sensitivity analysis preferring current exposure are also in Table 3, nine studies providing RRs and 95%CIs for both ever and current exposure. RRs for the sensitivity analysis preferring unadjusted to adjusted results are given in Supplementary file 2. Studies 7, 17 and 25 only provided incomplete estimates that could not be included in meta-analyses. Similarly, the result for current exposure from study 4 could not be included in the sensitivity analysis. Otherwise, for each study/sex combination, the RR estimate listed first in Table 3 is that used in the main analysis. Exposure was based on spousal smoking for 24 studies, on at home exposure for 17, and on exposure from multiple sources, including outside the home, for 10. Table 4 presents results of meta-analyses, fuller details being given in Supplementary File 2. Table 5 presents dose-response data, separately for spousal and household exposure.

Table 3 demonstrates clear evidence of a positive association, about three-quarters of the main analysis RR estimates exceeding 1. Seventeen are significantly ($P < 0.05$) increased, and none significantly decreased. Study 16 contributed 31% of the total weight, with studies 20, 27, 30 and 38 each contributing about 10%.

The main meta-analysis (Table 4) shows a clear positive association, with the random-effects RR estimate 1.18 (95%CI: 1.12-1.24) based on 75 individual estimates. The RR is little changed in sensitivity analyses preferring unadjusted to adjusted estimates (1.16, 1.09-1.24), or preferring current to ever exposure estimates (1.19, 1.13-1.26). It is somewhat increased if studies 15, 16 and 30 are excluded (1.23, 1.17-1.29).

Table 1 Studies providing evidence on heart disease and environmental tobacco smoke exposure in never smokers

Study No.	Ref.[1]	Year[2]	Location	Type[3]	Sexes included[4]	Disease fatality[5]	Disease definition[6]	No. of cases[7]
1	Hirayama[29]	1984	Japan	P16	F	F	IHD	494
2	Garland *et al*[30]	1985	United States/California	P10	F	F	IHD	19
3	Lee *et al*[31]	1986	England	CC	M, F	NF	IHD	118
4	Martin *et al*[32]	1986	United States/Utah	CS	F	NF	PHA	23
5	Svendsen *et al*[33]	1987	United States	P9	M	F + NF	IHD	69
6	Butler[34]	1988	United States/California	P6	F	F	IHD	80[8]
7	Palmer *et al*[35]	1988	United States/Not known	CC	F	NF	MI	336
8	Hole *et al*[36]	1989	Scotland	P12	M, F	F, NF	IHD, A/E	120
9	Jackson[37]	1989	New Zealand	CC	M, F	F + NF	IHD + MI	303
10	Sandler *et al*[38]	1989	United States/Maryland	P12	M, F	F	AHD	1358
11	Humble *et al*[39]	1990	United States/Georgia	P20	F	F	CVD	76
12	Dobson *et al*[40]	1991	Australia	CC	M, F	F + NF	IHD + MI	343
13	Gardiner *et al*[41]	1992	Scotland	CC	M+F	F + NF	IHD	12
14	La Vecchia *et al*[42]	1993	Italy	CC	M, F	NF	FMI	113
15	Layard[25]	1995	United States	CC	M, F	F	IHD	1389
16[9]	Le Vois *et al*[26] (CPS I)	1995	United States	P13	M, F	F	AHD	14891
17	Mannino *et al*[43]	1995	United States	CS	M + F	NF	CVD	?
18	Muscat *et al*[44]	1995	United States/4 cities	CC	M, F	NF	NMI	114
19	Tunstall-Pedoe *et al*[45]	1995	Scotland	CS	M + F	NF	IHD	428
20	Steenland *et al*[46]	1996	United States	P7	M, F	F	IHD	3819
21	Janghorbani *et al*[47]	1997	Iran	CC	F	NF	IHD	200
22	Kawachi *et al*[48]	1997	United States	P10	F	F + NF	IHD + MI	152
23	Ciruzzi *et al*[49]	1998	Argentina	CC	M, F	NF	FMI	336
24	McElduff *et al*[50]	1998	Australia	CC	M, F	F + NF	MI	283
25	Spencer *et al*[51]	1999	Australia	CC	M	NF	FMIS	91
26	He *et al*[52]	2000	China/Xi'an	CC	F	NF	MI/CS	115
27	Iribarren *et al*[53]	2001	United States	CS	M, F	NF	HD	4801
28	Rosenlund *et al*[54]	2001	Sweden	CC	M, F	NF	FMI	334
29	Pitsavos *et al*[55]	2002	Greece	CC	M + F	NF	FMI/UA	279
30[9]	Enstrom *et al*[27]	2003	United States/California	P39	M, F	F	IHD	5932
31	Chen *et al*[56]	2004	Scotland	CS	M + F	NF	IHD	385
32	Nishtar *et al*[57][10]	2004	Pakistan	CC	M + F	NF	CAD	?
33[11]	Whincup *et al*[58]	2004	Great Britain	P21	M	F + NF	IHD	111
34	McGhee *et al*[59]	2005	Hong Kong	CC	M, F	F	IHD	584
35	Qureshi *et al*[60]	2005	United States	P11	F	F + NF	CVD	328
							CVD-Stroke	219
36	Hedblad *et al*[61]	2006	Sweden	P19	M	F + NF	IHD + MI, FMI	91
37	Stranges *et al*[62]	2006	United States	CC	M, F	NF	FMI	284
38	Teo *et al*[63]	2006	52 countries	CC	M + F	NF	FMI	6280
39	Wen *et al*[64]	2006	China/Not known	P6	F	F	CVD	272
							CVD-Stroke	115
40	Eisner *et al*[65]	2007	United States	P8	M, F	F	CVD	1057
41	Hill *et al*[66]	2007	New Zealand	P3	M, F	F	IHD	2571
42	Hill *et al*[66]	2007	New Zealand	P3	M, F	F	IHD	1680
43	He *et al*[67]	2008	China/Beijing	CS	F	NF	IHD	431
44	Sulo *et al*[68]	2008	Albania	CC	M + F	NF	ACS	169
45	Vozoris *et al*[69]	2008	Canada	CS	M + F	NF	HD	1773
46	Ding *et al*[70]	2009	Hong Kong	CC	F	NF	IHD	314
47	Gallo *et al*[71]	2010	Europe	P?	M, F	F	CVD[12]	399
					M + F		IHD	81
48	Hamer *et al*[72]	2010	England, Scotland	P7	M + F	F	CVD	96
49[11]	Jefferis *et al*[73]	2010	Great Britain	P11	M + F	F + NF	FMI	74
50	Peineman *et al*[74]	2011	Germany	CS	M + F	NF	IHD	128
51	Chen[75]	2012	China/4 provinces	CS	M + F	NF	IHD	405
							MI	171
52	He *et al*[76]	2012	China/Xi'an	P26	M, F	F	IHD	41
53	Clark *et al*[77]	2013	Singapore	P16	M, F	F	IHD	311
54	Iversen *et al*[78]	2013	Norway	P11	M, F	F + NF	FMI	326
55	Kastorini *et al*[79]	2013	Greece	CC	M + F	NF	ACS	52
56	Rostron[80]	2013	United States	P11	M + F	F	IHD	?
57	Batty *et al*[81][13]	2014	United Kingdom	P17	M, F	F	CVD	98

58	Shiue[82]	2014	Scotland	CS	M + F	NF	MI	255

[1]First author of paper, followed by a number to distinguish multiple studies with the same author; [2]Year of publication; [3]Study types are CC: Case-control, CS: Cross-sectional, P: Prospective. Number after P is estimated mean years of follow-up; ?: Indicates length of follow-up not stated; [4]M + F indicates only results for combined sexes available; M, F indicates separate sex results available; [5]F: Fatal; NF: Non-fatal; F + NF indicates only combined results available; F, NF indicates separate results available; [6]A/E: Angina or ECG abnormality; ACS: Acute coronary syndrome; AHD: Arteriosclerotic heart disease; CAD: Coronary artery disease; CVD: Cardiovascular disease; FMI: First myocardial infarction; FMI/UA: First myocardial infarction or unstable angina; FMIS: First myocardial infarction surviving 28 d, HD: Heart disease; IHD: Ischaemic (coronary) heart disease; MI: Myocardial infarction; MI/CS: Myocardial infarction or coronary stenosis; NMI: Newly diagnosed myocardial infarction; PHA: Previous heart attack. "+" indicates inclusion of cases with either disease, indicates different outcome definitions for fatal and non-fatal analyses respectively; [7]Number of heart disease cases in never smokers are totals in the study. For analyses relating to some exposure indices, numbers may be lower than this. ? indicates numbers not available; [8]For study 6 numbers relate only to the spouse-pairs cohort, the AHSMOG cohort including ex-smokers; [9]Studies 16 and 30 were both part of CPS I. Study 30 covered a smaller geographic area but a longer follow-up period; [10]For study 32, although the source paper does not state that the analyses were restricted to never smokers, this has been confirmed to us by the authors; [11]Study 49 included the same male participants as study 33, but started at the end of the follow-up period of that study, so there was no overlap of cases between the two studies; [12]For study 47, CVD was defined as any circulatory disease excluding cerebrovascular causes; [13]For study 57, results in never smokers were taken from Supplementary tables supplied by the authors.

There is clear (P < 0.001) heterogeneity between estimates for all these analyses. Analyses by subset (based on the main analysis) show highly significant (P < 0.001) variation by various factors:

Sex: Estimates are lower for males than for females or sexes combined.

Continent: Estimates are lower for North America than for Europe, Asia or elsewhere.

Publication period: Estimates are higher for the oldest (1984-1991) and newest (2010-2016) studies than for studies in intermediate periods.

Number of cases: Studies with fewer cases give higher estimates, consistent with the significant (P < 0.001) publication bias for the overall analysis.

Study type: Estimates are lower for prospective than for case-control or cross-sectional studies.

Spouse the index: Estimates are lower where the spouse is the index, and where the analysis is limited to married subjects.

Fatality: Estimates are lower when based on fatal cases.

Heart disease definition: Estimates are lower for IHD specifically than for other definitions.

Type of control: In case-control studies, estimates are lower where hospital/diseased controls rather than healthy controls, are used.

Source of diagnosis: Estimates are lower when diagnosis derives from death certificates or self-report than from medical data.

Definition of never smoker: Estimates are higher where the definition allowed "never smoking" subjects to smoke products other than cigarettes, or to have a limited smoking history.

Despite the heterogeneity, each RR estimate in Table 4 for each data subset exceeds 1.00, generally significantly so. Our analyses demonstrated 11 factors with highly significant (P < 0.001) heterogeneity by level, when considered one at a time. However, many were inter-correlated. To isolate the important factors, stepwise regression analysis was conducted (see Supplementary File 3). Only three of the 11 factors independently predicted heart disease risk at P < 0.05, with source of diagnosis introduced first into the model, then spouse the index, and then number of cases. While, for the factors remaining in the model, the direction of effect remained, the magnitude of variation between levels was slightly reduced from that shown in Table 4.

Further results for exposure at home

Table 3 also shows RRs for household exposure for five studies where separate results are available for both spousal and household exposure. Overall, there are 37 household exposure estimates from 22 studies, 10 showing a significant increase in risk, and none a significant decrease. The combined random-effects estimate is 1.19 (95%CI: 1.13-1.25). There is no marked heterogeneity between the estimates overall, and little indication of variation between males and females, continents, periods of publication or numbers of cases. Estimates do vary by study design (P < 0.01), being higher for case-control studies than other designs.

As shown in Table 5, 13 studies reported dose-response results for smoking by the spouse, 11 for smoking by household members, and one (study 47) for both. While only two studies providing dose-response data for spousal smoking reported a significant (P < 0.05) positive trend, nine did so for exposure to household members. These trend tests included the unexposed group. Had they excluded the unexposed group, they would have been significant for only one (study 26). There were no significant negative trends.

Other exposure indices

Table 6 presents results for ETS exposure at work, in childhood, a combined index of total exposure, and a biochemical index of exposure. For these four indices,

Table 2 Potential confounding variables adjusted for and definition of never smoker

Study No.	Ref.[1]	Variables adjusted for[2]	Definition of never smokers[3]
1	Hirayama[29]	Sex, age, marital status	Never cigarettes
2	Garland *et al*[30]	Sex, age, marital status, blood pressure, cholesterol, obesity	Never cigarettes
3	Lee *et al*[31]	Sex, age, marital status	Never NOS
4	Martin *et al*[32]	Sex, marital status, blood pressure, obesity, alcohol, diabetes, family history of heart disease, exercise	Never NOS
5	Svendsen *et al*[33]	Sex, age, marital status, blood pressure, cholesterol, social class, obesity, alcohol	Never any product
6	Butler[34]	Sex, age, marital status	Never cigarettes
7	Palmer *et al*[35]	Sex, marital status	Never NOS
8	Hole *et al*[36]	Sex, age, blood pressure, cholesterol, social class, obesity	Never NOS
9	Jackson[37]	Sex, age, social class, obesity, family history of heart disease	Never NOS
10	Sandler *et al*[38]	Sex, age, social class, personal history of heart disease	Never any product
11	Humble *et al*[39]	Sex, age, marital status, blood pressure, cholesterol, obesity	Never NOS
12	Dobson *et al*[40]	Sex, age, social class, obesity, personal history of heart disease	Never cigarettes
13	Gardiner *et al*[41]	Sex, age, hospital admission date	Never any product
14	La Vecchia *et al*[42]	Sex, age, marital status, blood pressure, cholesterol, social class, obesity, diabetes, family history of heart disease, coffee	Never NOS
15	Layard[25]	Sex, age, marital status, race	Never 100 cigarettes in lifetime
16	Le Vois *et al*[26] (CPS I)	Sex, age, marital status, race	Never NOS
17	Mannino *et al*[43]	Sex, age, social class, race, housing	Never NOS
18	Muscat *et al*[44]	Sex, age, blood pressure, social class, race	Never one cigarette, pipe or cigar per day for more than a year
19	Tunstall-Pedoe *et al*[45]	Age, blood pressure, cholesterol, housing	Never any product and cotinine < 17.5 mg/mL
20	Steenland *et al*[46]	Sex, age, marital status, blood pressure, social class, obesity, alcohol, diabetes, exercise, personal history of heart disease, occupation, oestrogen use, aspirin use, diuretic use and personal history of arthritis	Never any product daily for as long as a year (men), never cigarettes (women)
21	Janghorbani *et al*[47]	Sex, age, marital status	Never any product
22	Kawachi *et al*[48]	Sex, age, blood pressure, cholesterol, obesity, alcohol, diabetes, family history of heart disease, exercise, occupation, oestrogen use, oral contraceptive use, saturated fat intake, vitamin E intake, menopausal status and use of postmenopausal hormones	Never NOS
23	Ciruzzi *et al*[49]	Sex, age, blood pressure, cholesterol, social class, obesity, diabetes, family history of heart disease, exercise	Never NOS
24	McElduff *et al*[50]	Sex, age, social class, obesity, family history of heart disease	Never cigarettes or quit at least 10 yr ago, and not current other products
25	Spencer *et al*[51]	Sex, age	Never NOS
26	He *et al*[52]	Sex, age, blood pressure, cholesterol, family history of heart disease, personality type	Never NOS
27	Iribarren *et al*[53]	Sex, age, marital status, cholesterol, social class, obesity, alcohol, diabetes, race, exercise, personality type	Never any product
28	Rosenlund *et al*[54]	Sex, age, blood pressure, cholesterol, social class, obesity, diabetes, occupation	Never any product regularly for at least a year
29	Pitsavos *et al*[55]	Sex, age, blood pressure, cholesterol, obesity, alcohol, diabetes, exercise and family history of heart disease	Never cigarettes
30	Enstrom *et al*[27]	Sex, age, marital status, social class, obesity, race, exercise, housing, fruit or fruit juice intake and health status	Never any product[4]
31	Chen *et al*[56]	Sex, age, blood pressure, cholesterol, social class, obesity, alcohol, family history of heart disease, employment status, dietary vitamin C and fibre	Never NOS and cotinine < 17.5 mg/mL
32	Nishtar *et al*[57]	Sex, age, matched pair (conditional logistic regression was used)	Never NOS
33	Whincup *et al*[58]	Sex, age, blood pressure, cholesterol, social class, obesity, alcohol, diabetes, exercise, personal history of heart disease, town of residence, FEV_1, height, triglycerides and white cell count	Never any product and cotinine < 14.1 mg/mL
34	McGhee *et al*[59]	Sex, age, marital status, social class	Never NOS
35	Qureshi *et al*[60]	Sex, age, marital status, blood pressure, cholesterol, obesity, alcohol, diabetes, race	Never NOS
36	Hedblad *et al*[61]	Sex, blood pressure, cholesterol, obesity, alcohol, diabetes, exercise, personal history of heart disease, triglycerides and FEV_1	Never one cigarette per day
37	Stranges *et al*[62]	Sex, blood pressure, cholesterol, social class, obesity, alcohol, diabetes, race, exercise	Never 100 cigarettes in lifetime
38	Teo *et al*[63]	Sex, age, alcohol, exercise, region, consumption of fruits and vegetables	Never any product regularly
39	Wen *et al*[64]	Sex, age, social class, obesity, exercise, occupation, intake of meats, vegetables and fruit	Never NOS
40	Eisner *et al*[65]	Sex, age, marital status, social class	Never cigarettes or quit at least 20 yr ago, and < 10 pack-years
41, 42	Hill *et al*[66]	Sex, age, marital status, social class, race, occupation	Never NOS

43	He *et al*[67]	Sex, age, marital status, blood pressure, cholesterol, social class, obesity, alcohol, diabetes, family history of heart disease, exercise, triglycerides, family history of stroke	Never 100 cigarettes in lifetime
44	Sulo *et al*[68]	Sex, age, blood pressure, social class, obesity, diabetes, family history of heart disease, race, exercise, occupation, financial loss in pyramid schemes, emigration of spouse and/or offspring, religious observance	Never cigarettes
45	Vozoris *et al*[69]	Sex, age, social class, province, immigration status, presence of children younger than 12 yr in household	Never cigarettes
46	Ding *et al*[70]	Sex, age, blood pressure, cholesterol, social class, alcohol, diabetes, family history of heart disease, exercise, oestrogen use, history of stroke, history of gout	Never NOS
47	Gallo *et al*[71]	Sex, age, social class, obesity, exercise, study centre	Never NOS
48	Hamer *et al*[72]	Sex, age, blood pressure, cholesterol, social class, exercise, personality type, survey location, log C-reactive protein, fibrinogen	Never NOS
49	Jefferis *et al*[73]	Sex, age, blood pressure, cholesterol, social class, obesity, alcohol, diabetes, exercise, region, triglycerides, FEV_1, C-reactive protein, interleukin 6, white cell count	Never any product or quit at least 5 yr ago, and cotinine < 15 mg/mL
50	Peinemann *et al*[74]	None	Never NOS
51	Chen[75]	None	Never cigarettes
52	He *et al*[76]	Sex, age, marital status, blood pressure, cholesterol, social class, obesity, alcohol, occupation, triglycerides	Never 100 cigarettes in lifetime
53	Clark *et al*[77]	Sex, age, social class, obesity, dialect, dietary fibre intake	Never NOS
54	Iversen *et al*[78]	Sex, age, blood pressure, cholesterol, obesity, exercise, living with a smoker (for analysis of hours spent in smoke-filled rooms), hours spent in smoke-filled rooms (for analysis of living with a smoker)	Never cigarettes
55	Kastorini *et al*[79]	Sex, age, blood pressure, cholesterol, obesity, diabetes, family history of heart disease, exercise, personality type, Mediterranean Diet Score	Never one cigarette a day
56	Rostron[80]	Sex, age, race, social class, alcohol, blood pressure, obesity, personal history of heart disease	Never 100 cigarettes in lifetime
57	Batty *et al*[81]	Sex, age, social class, alcohol, diabetes, exercise, personal history of heart disease, personal history of cancer	Never NOS
58	Shiue[82]	Sex, age, race, social class, alcohol, survey weighting, exercise, blood pressure, obesity	Never any product

[1]First author of paper; [2]In some cases similar adjustment variables have been considered under one name. Thus blood pressure includes hypertension; social class includes education and income; obesity includes weight; family history of heart disease includes family history of hypertension; and housing includes urban-rural; [3]Never any product: Never smoked cigarettes, pipes or cigars; Never NOS: Never smoked, product unspecified; [4]Questions on pipe and cigar smoking were asked at baseline, but not at the follow-up interviews.

results are available from, respectively, 14, 4, 24 and 8 studies. For some studies the estimates for total exposure are the same as those for the main exposure index. The RRs are supported by Figures 2-5, while Table 7 presents results of meta-analyses, and Table 8 the dose-response data. Again, fuller details of meta-analyses are given in Supplementary File 2. Supplementary File 2 also includes results for spousal smoking specifically.

For workplace exposure, there were 22 estimates, with only one showing a significant increase, the combined estimate of 1.08 (95%CI: 0.99-1.19) being almost significantly raised. There was no evidence of heterogeneity, and little evidence of variation by any factor considered.

For childhood exposure, one of the seven estimates showed a significant increase in risk. However, the combined estimate of 1.12 (95%CI: 0.95-1.31) was not significant.

For total exposure, the 33 estimates showed clear heterogeneity ($P < 0.001$), 11 estimates showing a significant ($P < 0.05$) positive association, and one a significant negative association. However, there was a clear preponderance of positive associations, with the random-effects estimate 1.23 (95%CI: 1.12-1.35). Subgroup analyses showed higher estimates for Asia; for case-control studies, and for females and sexes-combined.

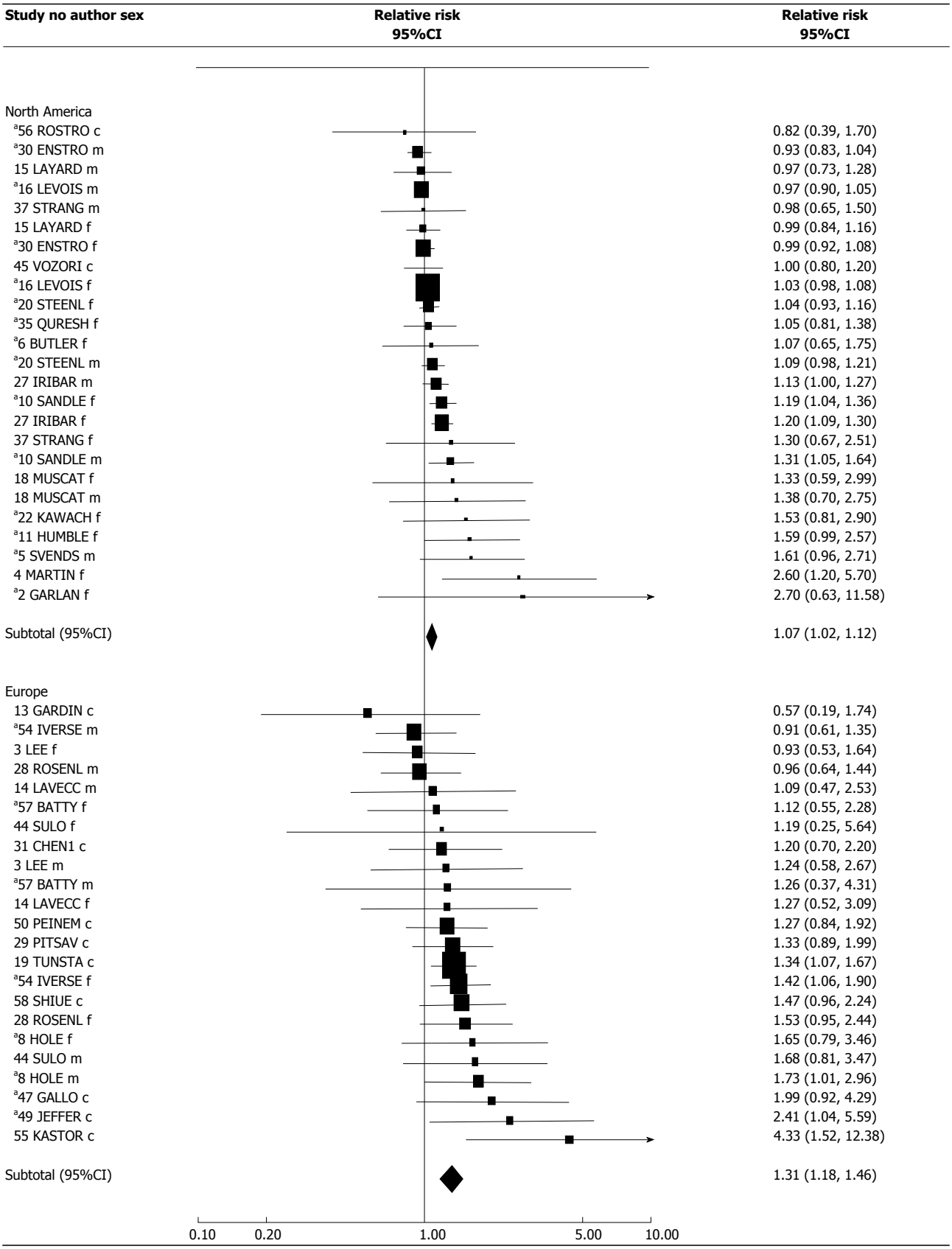
Of nine estimates for biomarker based exposure indices, all were cotinine-based apart from one based on COHb. There was some indication of heterogeneity ($P < 0.1$), the random-effects estimate of 1.15 (95%CI: 0.94-1.40) showing no clear association.

Table 8 presents dose-response data for these exposure indices. For studies reporting dose-response results, significant positive trends were seen (for at least one index) in 12 of 17 studies for total exposure, 3 of 8 studies for biomarker-based exposure, 1 of 5 studies for workplace exposure, and 1 of 2 studies for childhood exposure. No significant negative trends were seen.

Twelve studies presented RR estimates and/or dose-response results for one or more other exposure indices (Supplementary File 4). These results relate to many different indices, and are somewhat variable, with clear evidence of an increase being seen for studies 29 and 32, but a number of other studies showing no relationship with the indices studied.

DISCUSSION

Based on 58 studies, we present meta-analyses relating


Study no author sex
Relative risk 95%CI
Relative risk 95%CI
North America
[a]56 ROSTRO c 0.82 (0.39, 1.70)
[a]30 ENSTRO m 0.93 (0.83, 1.04)
15 LAYARD m 0.97 (0.73, 1.28)
[a]16 LEVOIS m 0.97 (0.90, 1.05)
37 STRANG m 0.98 (0.65, 1.50)
15 LAYARD f 0.99 (0.84, 1.16)
[a]30 ENSTRO f 0.99 (0.92, 1.08)
45 VOZORI c 1.00 (0.80, 1.20)
[a]16 LEVOIS f 1.03 (0.98, 1.08)
[a]20 STEENL f 1.04 (0.93, 1.16)
[a]35 QURESH f 1.05 (0.81, 1.38)
[a]6 BUTLER f 1.07 (0.65, 1.75)
[a]20 STEENL m 1.09 (0.98, 1.21)
27 IRIBAR m 1.13 (1.00, 1.27)
[a]10 SANDLE f 1.19 (1.04, 1.36)
27 IRIBAR f 1.20 (1.09, 1.30)
37 STRANG f 1.30 (0.67, 2.51)
[a]10 SANDLE m 1.31 (1.05, 1.64)
18 MUSCAT f 1.33 (0.59, 2.99)
18 MUSCAT m 1.38 (0.70, 2.75)
[a]22 KAWACH f 1.53 (0.81, 2.90)
[a]11 HUMBLE f 1.59 (0.99, 2.57)
[a]5 SVENDS m 1.61 (0.96, 2.71)
4 MARTIN f 2.60 (1.20, 5.70)
[a]2 GARLAN f 2.70 (0.63, 11.58)
Subtotal (95%CI) 1.07 (1.02, 1.12)
Europe
13 GARDIN c 0.57 (0.19, 1.74)
[a]54 IVERSE m 0.91 (0.61, 1.35)
3 LEE f 0.93 (0.53, 1.64)
28 ROSENL m 0.96 (0.64, 1.44)
14 LAVECC m 1.09 (0.47, 2.53)
[a]57 BATTY f 1.12 (0.55, 2.28)
44 SULO f 1.19 (0.25, 5.64)
31 CHEN1 c 1.20 (0.70, 2.20)
3 LEE m 1.24 (0.58, 2.67)
[a]57 BATTY m 1.26 (0.37, 4.31)
14 LAVECC f 1.27 (0.52, 3.09)
50 PEINEM c 1.27 (0.84, 1.92)
29 PITSAV c 1.33 (0.89, 1.99)
19 TUNSTA c 1.34 (1.07, 1.67)
[a]54 IVERSE f 1.42 (1.06, 1.90)
58 SHIUE c 1.47 (0.96, 2.24)
28 ROSENL f 1.53 (0.95, 2.44)
[a]8 HOLE f 1.65 (0.79, 3.46)
44 SULO m 1.68 (0.81, 3.47)
[a]8 HOLE m 1.73 (1.01, 2.96)
[a]47 GALLO c 1.99 (0.92, 4.29)
[a]49 JEFFER c 2.41 (1.04, 5.59)
55 KASTOR c 4.33 (1.52, 12.38)
Subtotal (95%CI) 1.31 (1.18, 1.46)
0.10
0.20
1.00
5.00
10.00

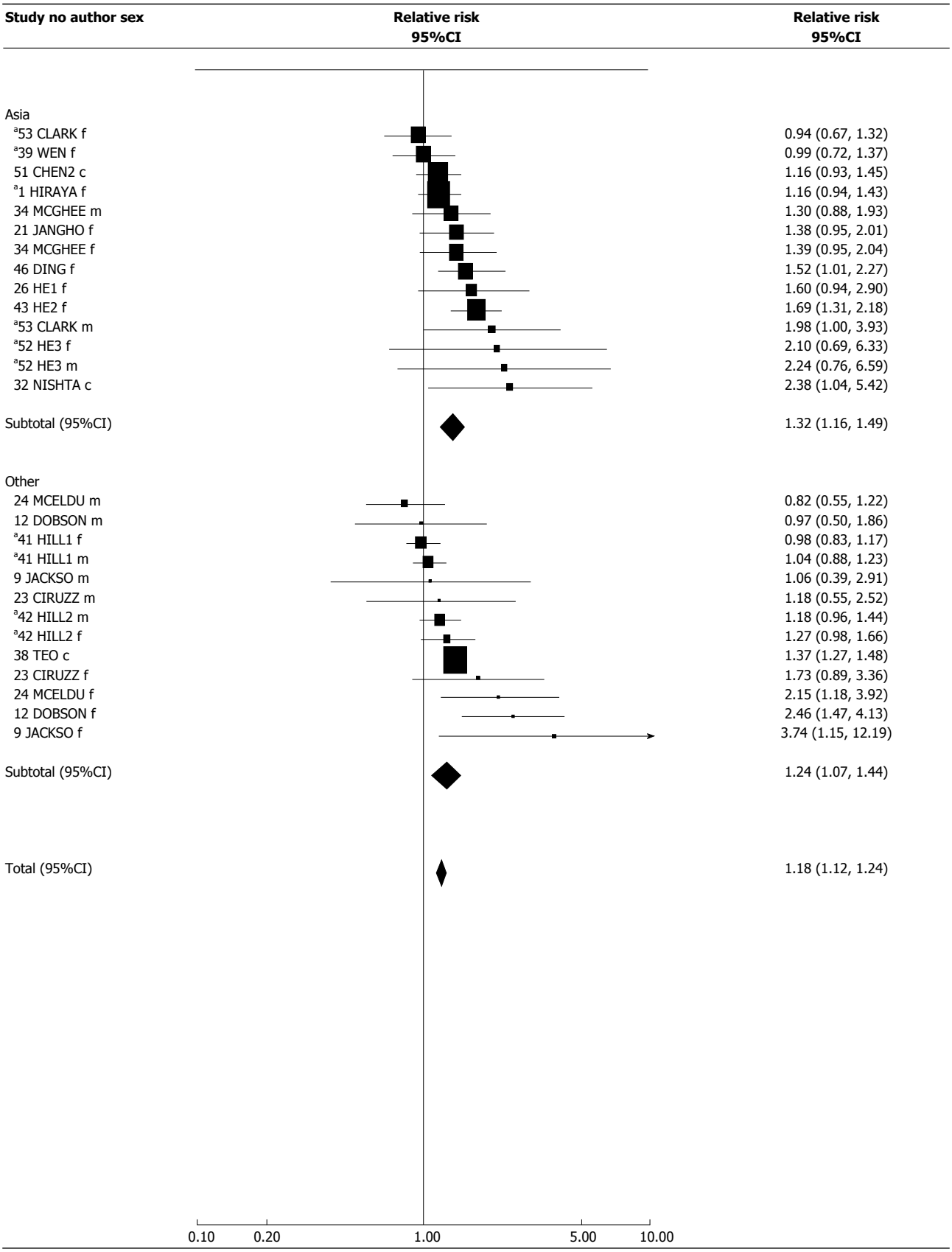


Figure 1 Forest plot for the main index, by continent. Estimates of the RR and its 95%CI are shown separately by continent, sorted in increasing order of RR. These are shown numerically, and also graphically on a logarithmic scale. Estimates are identified by the study number shown in Table 1, an abbreviation of the author name and the sex to which the estimate relates (m = male, f = female, c = combined sex estimate). In the graphical representation, individual RRs are indicated by a solid square, with the area of the square proportional to the weight (the inverse of the variance of log RR). Arrows warn if the CI goes outside the range of the plot. Random-effects estimates (RRs and their 95%CIs) are shown for each continent and overall, represented graphically by a diamond whose width indicates the confidence interval. [a]Prospective study.

Table 3 Heart disease relative risk estimates used in the main analysis for spouse ever smoked (or nearest equivalent) and in sensitivity analyses for spouse a current smoker, as well as additional results for household exposure

Study No.[1]	Author[2]	Sex	Exposure index		Fatality[5]	Relative risk (95%CI)[6]
			Source[3]	Timing[4]		
Results used in the main analysis[7]						
1	Hirayama[29]	F	S	E	F	1.16 (0.94-1.43)[8]
2	Garland *et al*[30]	F	S	E	F	2.70 (0.63-11.58)
3	Lee *et al*[31]	M	S	M	NF	1.24 (0.58-2.67)
		F	S	M	NF	0.93 (0.53-1.64)
4	Martin *et al*[32]	F	S	E	NF	2.60 (1.20-5.70)[9]
5	Svendsen *et al*[33]	M	S	C	F + NF	1.61 (0.96-2.71)
6	Butler[34]	F	S	E	F	1.07 (0.65-1.75)
7	Palmer *et al*[35]	F	S	E	NF	1.20
8	Hole *et al*[36]	M	H[10]	E	F	1.73 (1.01-2.96)[11]
		F	H[10]	E	F	1.65 (0.79-3.46)[11]
9	Jackson[37]	M	H	C	F + NF	1.06 (0.39-2.91)
		F	H	C	F + NF	3.74 (1.15-12.19)
10	Sandler *et al*[38]	M	H	C	F	1.31 (1.05-1.64)
		F	H	C	F	1.19 (1.04-1.36)
11	Humble *et al*[39]	F	S	C(N)	F	1.59 (0.99-2.57)
12	Dobson *et al*[40]	M	H	C	F + NF	0.97 (0.50-1.86)
		F	H	C	F + NF	2.46 (1.47-4.13)
13	Gardiner *et al*[41]	M + F	S	M	F + NF	0.57 (0.19-1.74)
14	La Vecchia *et al*[42]	M	S	E	NF	1.09 (0.47-2.53)
		F	S	E	NF	1.27 (0.52-3.09)
15	Layard[25]	M	S	E	F	0.97 (0.73-1.28)
		F	S	E	F	0.99 (0.84-1.16)
16	Le Vois *et al*[26] (CPS I)	M	S	E	F	0.97 (0.90-1.05)
		F	S	E	F	1.03 (0.98-1.08)
17	Mannino *et al*[43]	M + F	H	C	NF	1.12
18	Muscat *et al*[44]	M	S	E	NF	1.38 (0.70-2.75)
		F	S	E	NF	1.33 (0.59-2.99)
19	Tunstall-Pedoe *et al*[45]	M + F	T	C	NF	1.34 (1.07-1.67)
20	Steenland *et al*[46]	M	S	E	F	1.09 (0.98-1.21)
		F	S	E	F	1.04 (0.93-1.16)
21	Janghorbani *et al*[47]	F	S	E	NF	1.38 (0.95-2.01)
22	Kawachi *et al*[48]	F	H	C	F + NF	1.53 (0.81-2.90)[9]
23	Ciruzzi *et al*[49]	M	S	C	NF	1.18 (0.55-2.52)
		F	S	C	NF	1.73 (0.89-3.36)
24	McElduff *et al*[50]	M	T	C	F + NF	0.82 (0.55-1.22)
		F	T	C	F + NF	2.15 (1.18-3.92)
25	Spencer *et al*[51]	M	H	E	NF	No significant association
26	He *et al*[52]	F	S	E	NF	1.60 (0.94-2.90)
27	Iribarren *et al*[53]	M	H	C	NF	1.13 (1.00-1.27)
		F	H	C	NF	1.20 (1.09-1.30)
28	Rosenlund *et al*[54]	M	S	E	NF	0.96 (0.64-1.44)
		F	S	E	NF	1.53 (0.95-2.44)
29	Pitsavos *et al*[55]	M + F	H	C	NF	1.33 (0.89-1.99)
30	Enstrom *et al*[27]	M	S	E	F	0.93 (0.83-1.04)
		F	S	E	F	0.99 (0.92-1.08)
31	Chen *et al*[56]	M + F	H	C	NF	1.20 (0.70-2.20)
32	Nishtar *et al*[57]	M + F	S	E	NF	2.38 (1.04-5.42)
34	McGhee *et al*[59]	M	H	P	F	1.30 (0.88-1.93)
		F	H	P	F	1.39 (0.95-2.04)
35	Qureshi *et al*[60]	F	S	E	F + NF	1.05 (0.81-1.38)[12]
37	Stranges *et al*[62]	M	H	E	NF	0.98 (0.65-1.50)
		F	H	E	NF	1.30 (0.67-2.51)
38	Teo *et al*[63]	M + F	T	C	NF	1.37 (1.27-1.48)
39	Wen *et al*[64]	F	S	M	F	0.99 (0.72-1.37)[13]
41	Hill *et al*[66]	M	H	C	F	1.04 (0.88-1.23)
		F	H	C	F	0.98 (0.83-1.17)
42	Hill *et al*[66]	M	H	C	F	1.18 (0.96-1.44)
		F	H	C	F	1.27 (0.98-1.66)
43	He *et al*[67]	F	T	T	NF	1.69 (1.31-2.18)
44	Sulo *et al*[68]	M	S	C	NF	1.68 (0.81-3.47)
		F	S	C	NF	1.19 (0.25-5.64)
45	Vozoris *et al*[69]	M + F	T	C	NF	1.00 (0.80-1.20)
46	Ding *et al*[70]	F	H	E	NF	1.52 (1.01-2.27)
47	Gallo *et al*[71]	M + F	S	C	F	1.99 (0.92-4.29)[14]
49	Jefferis *et al*[73]	M + F	S	C	F + NF	2.41 (1.04-5.59)

50	Peinemann *et al*[74]	M + F	T	C	NF	1.27 (0.84-1.92)
51	Chen[75]	M + F	T	E	NF	1.16 (0.93-1.45)[15]
52	He *et al*[76]	M	T	E	F	2.24 (0.76-6.59)
		F	T	E	F	2.10 (0.69-6.33)
53	Clark *et al*[77]	M	H	C	F	1.98 (1.00-3.93)
		F	H	C	F	0.94 (0.67-1.32)
54	Iversen *et al*[78]	M	H	A	F + NF	0.91 (0.61-1.35)
		F	H	A	F + NF	1.42 (1.06-1.90)
55	Kastorini *et al*[79]	M + F	T	E	NF	4.33 (1.52-12.38)
56	Rostron[80]	M + F	H	C	F	0.82 (0.39-1.70)
57	Batty *et al*[81]	M	H	C	F	1.26 (0.37-4.31)
		F	H	C	F	1.12 (0.55-2.28)
58	Shiue[82]	M + F	T	C	NF	1.47 (0.96-2.24)
Alternative result used in the analysis of spouse a current smoker						
2	Garland *et al*[30]	F	S	C(N)	F	2.25 (0.32-15.74)
4	Martin *et al*[32]	F	S	C	NF	3.40
6	Butler[34]	F	S	C(N)	F	1.40 (0.51-3.84)
14	La Vecchia *et al*[42]	M	S	C(N)	NF	1.09 (0.39-3.01)
		F	S	C(N)	NF	1.36 (0.46-4.05)
16	Le Vois *et al*[26] (CPS I)	M	S	C(N)	F	0.98 (0.91-1.06)
		F	S	C(N)	F	1.04 (0.99-1.09)
20	Steenland *et al*[46]	M	S	C(N)	F	1.22 (1.07-1.40)
		F	S	C(N)	F	1.10 (0.96-1.27)
28	Rosenlund *et al*[54]	M	S	C(N)	NF	0.98 (0.57-1.69)
		F	S	C(N)	NF	2.59 (1.27-5.29)
30	Enstrom *et al*[27]	M	S	C(N)	F	0.92 (0.80-1.05)
		F	S	C(N)	F	0.97 (0.89-1.06)
37	Stranges *et al*[62]	M	H	C	NF	0.71 (0.40-1.23)
		F	H	C	NF	0.94 (0.48-1.82)
39	Wen *et al*[64]	F	S	C	F	1.19 (0.84-1.67)[16]
Additional household exposure results						
18	Muscat *et al*[44]	M	H	E	NF	1.40 (0.70-2.81)
		F	H	E	NF	1.55 (0.55-4.37)
20	Steenland *et al*[46]	M	H	C(N)	F	1.15 (1.01-1.32)
		F	H	C(N)	F	1.07 (0.98-1.17)
21	Janghorbani *et al*[47]	F	H	E	NF	1.34 (0.94-1.91)
23	Ciruzzi *et al*[49]	M	H[17]	C	NF	1.89 (1.13-3.18)
		F	H[17]	C	NF	1.54 (0.95-2.51)
47	Gallo *et al*[71]	M + F	H	C	F	1.31 (0.83-2.08)[18]

[1]Study 40 omitted as results only available per 10 years of living with a smoker. Studies 33, 36 and 48 omitted as they only provide results for a biochemical index of ETS exposure; [2]First author of paper; [3]S: Spouse (or partner), H: Household member (or exposure at home), T: Total; [4]E: Ever exposed (compared to never exposed) or unspecified; M: During marriage; C(N): Current exposure (compared to never exposed); C: Current exposure (compared to non-current exposure), P: In the past, T: In the last 10 years, A: In adulthood; [5]F: Fatal; NF: Non-fatal; F + NF indicates combined results were analysed; [6]Relative risks are adjusted for covariates if adjusted data are available. Those without 95%CI are not used in the meta-analyses; [7]Except where lacking a 95%CI, as in studies 7, 17 and 25; [8]Adjusted for the age of the husband. Alternative estimates[115] were very similar; [9]Estimates given by Wells[1]; [10]Cohabitant(s) age 45-64 also attending screening; [11]Estimates given by Wells[116]; [12]Result for CVD - Stroke. Result also available for CVD: 1.00 (0.81-1.24); [13]Result for CVD - Stroke. Result also available for CVD: 1.18 (0.92-1.51); [14]Result for CVD. Result for IHD shown in the "household" section of this table; [15]Result for IHD. Result also available for myocardial infarction: 0.93 (0.66-1.31); [16]Result for CVD - Stroke. Results also available for CVD: 1.37 (1.06-1.78); [17]Smoking by close relatives (although not necessarily living in same home); [18]Result for IHD. Result also available for CVD: 1.82 (1.06-3.12). ETS: Environmental tobacco smoke; CVD: Cardiovascular disease; IHD: Ischaemic heart disease; CPS: Cancer Prevention Studies.

ETS exposure to heart disease risk in never smokers. Using an exposure index as equivalent as possible to having a spouse who ever smoked, a random-effects meta-analysis gave a significantly increased RR of 1.18 (95%CI: 1.12-1.24) based on 75 RR estimates. Positive associations, not all significant at $P < 0.05$, were also noted with spousal exposure specifically (1.10, 1.04-1.17, $n = 34$), household exposure (1.19, 1.13-1.25, $n = 37$), workplace exposure (1.08, 0.99-1.19, $n = 22$), childhood exposure (1.12, 0.95-1.31, $n = 22$), and total exposure (1.23, 1.12-1.35, $n = 33$). The overall estimate was also elevated for a biomarker-based index (1.15, 0.94-1.40, $n = 9$). There was also evidence of dose-response.

While the relationship of smoking with heart disease[83] suggests some effect may be evident for ETS, exposure to smoke constituents from ETS is much less than from active smoking. For example, studies of cotinine indicate relative exposure of ETS compared to smoking of 0.6% to 0.4%[84-86], while studies of particulate matter suggest a lower factor, < 0.02%[87-95]. In interpreting our meta-analyses, one must note the clear heterogeneity between the RR estimates. Thus, for the main exposure index, estimates were higher for females, United States studies, and small studies, and smaller for prospective studies and for fatal cases, and varied by definition of exposure and source of diagnosis. Although these factors are not independent, and the variations may reflect characteristics of studies

Table 4 Meta-analyses of heart disease[1] risk among never smokers in relation to ever smoking by the spouse (or nearest equivalent)

Subgroup	*n*[3]	Fixed-effect Relative risk (95%CI)	Random-effects Relative risk (95%CI)	Publication bias *P*[4] value	Heterogeneity[2] χ^2	DF[5]	*P*[6] value
Main analyses[7]							
All	75	1.10 (1.08-1.13)	1.18 (1.12-1.24)	< 0.001	176.45	74	< 0.001
By sex							
Combined	14	1.32 (1.24-1.40)	1.30 (1.14-1.47)	NS	23.54	13	< 0.05
Males	25	1.04 (1.00-1.09)	1.07 (1.01-1.15)	< 0.05	32.90	24	NS
Females	36	1.09 (1.06-1.12)	1.20 (1.12-1.29)	< 0.001	81.04	35	< 0.001
			Between sexes		38.98	2	< 0.001
By continent							
North America	25	1.05 (1.02-1.08)	1.07 (1.02-1.12)	< 0.05	45.67	24	< 0.01
Europe	23	1.31 (1.18-1.46)	1.31 (1.18-1.46)	NS	20.63	22	NS
Asia	14	1.29 (1.17-1.42)	1.32 (1.16-1.49)	< 0.05	18.94	13	NS
Other	13	1.26 (1.19-1.33)	1.24 (1.07-1.44)	NS	37.12	12	< 0.001
			Between continents		54.09	3	< 0.001
By publication period							
1984-1991	16	1.28 (1.17-1.39)	1.35 (1.18-1.54)	< 0.05	21.29	15	NS
1992-1998	18	1.04 (1.00-1.07)	1.06 (1.00-1.12)	< 0.1	24.86	17	< 0.1
1999-2005	13	1.08 (1.03-1.13)	1.13 (1.02-1.24)	< 0.1	28.86	12	< 0.01
2006-2009	13	1.24 (1.17-1.31)	1.19 (1.06-1.34)	NS	32.96	12	< 0.001
2010-2016	15	1.26 (1.11-1.41)	1.31 (1.11-1.55)	< 0.05	21.07	14	< 0.1
			Between periods		47.42	4	< 0.001
By number of heart disease cases[8]							
1-99	13	1.62 (1.32-1.99)	1.66 (1.30-2.11)	NS	14.83	12	NS
100-199	14	1.33 (1.11-1.58)	1.33 (1.11-1.58)	NS	5.78	13	NS
200-999	30	1.26 (1.17-1.35)	1.27 (1.16-1.39)	NS	44.09	29	< 0.05
1000+	18	1.08 (1.05-1.10)	1.08 (1.02-1.15)	NS	76.70	17	< 0.001
			Between numbers		35.06	3	< 0.001
By study design							
Case-control	32	1.29 (1.21-1.36)	1.28 (1.15-1.42)	NS	52.18	31	< 0.05
Prospective	33	1.04 (1.01-1.07)	1.09 (1.03-1.14)	< 0.001	55.43	32	< 0.01
Cross-sectional	10	1.20 (1.14-1.28)	1.24 (1.12-1.37)	NS	16.78	9	< 0.1
			Between types		52.06	2	< 0.001
By number of confounders considered in the study							
0-2	15	1.03 (0.99-1.07)	1.05 (0.92-1.12)	< 0.1	17.51	14	NS
3-4	10	1.27 (1.16-1.39)	1.32 (1.13-1.55)	NS	16.65	9	< 0.1
5-9	38	1.13 (1.09-1.18)	1.19 (1.09-1.30)	< 0.05	94.55	37	< 0.001
10+	12	1.16 (1.10-1.22)	1.21 (1.10-1.32)	< 0.05	21.01	11	< 0.05
			Between groups		26.72	3	< 0.01
By results available in the study on dose-response							
No	24	1.15 (1.08-1.22)	1.19 (1.08-1.32)	< 0.05	44.81	23	< 0.01
Yes	51	1.10 (1.07-1.12)	1.18 (1.11-1.25)	< 0.01	129.74	50	< 0.001
			Between groups		1.90	1	NS
By spouse the index							
Yes	34	1.03 (1.00-1.06)	1.06 (1.01-1.12)	< 0.001	47.62	33	< 0.05
No	41	1.23 (1.19-1.28)	1.24 (1.16-1.32)	NS	72.59	40	< 0.01
			Between groups		56.24	1	< 0.001
Spouse the index, by whether unmarried subjects were excluded							
Yes	23	1.02 (0.99-1.05)	1.03 (0.99-1.07)	< 0.05	27.88	22	NS
No	11	1.30 (1.10-1.54)	1.35 (1.11-1.63)	< 0.01	12.00	10	NS
			Between groups		7.74	1	< 0.01
By heart disease fatality considered							
Fatal	31	1.04 (1.01-1.07)	1.07 (1.02-1.12)	< 0.001	46.74	30	< 0.05
Non-fatal	31	1.27 (1.22-1.33)	1.27 (1.19-1.36)	NS	39.58	30	NS
Both	13	1.25 (1.10-1.43)	1.34 (1.06-1.68)	NS	28.43	12	< 0.01
			Between groups		61.70	2	< 0.001
By heart disease definition							
IHD	32	1.06 (1.03-1.11)	1.12 (1.05-1.19)	< 0.001	56.92	31	< 0.01
MI	18	1.34 (1.25-1.43)	1.29 (1.14-1.46)	NS	23.10	17	NS
Other/Mixed	25	1.08 (1.05-1.12)	1.20 (1.10-1.30)	< 0.001	58.29	24	< 0.001
			Between definitions		38.14	2	< 0.001
By use of biomarker data to exclude smokers							
Yes	6	1.30 (1.08-1.57)	1.30 (1.08-1.57)	NS	3.89	5	NS
No	69	1.10 (1.08-1.13)	1.18 (1.12-1.24)	< 0.001	169.45	68	< 0.001
			Between groups		3.12	1	< 0.1
By any use of proxy respondents							
Yes	11	1.10 (0.99-1.23)	1.23 (0.98-1.53)	NS	26.38	10	< 0.01
No	64	1.10 (1.08-1.13)	1.18 (1.12-1.24)	< 0.001	150.07	63	< 0.001
			Between groups		0.00	1	NS

By type of control							
Healthy	15	1.30 (1.13-1.50)	1.38 (1.12-1.70)	< 0.1	27.67	14	< 0.05
Diseased/hospital	15	1.12 (1.01-1.24)	1.14 (1.01-1.28)	< 0.1	14.72	14	NS
Both	2	1.37 (1.27-1.48)	1.37 (1.27-1.48)	NC	0.29	1	NS
Prospective/cross-sectional	43	1.07 (1.05-1.10)	1.13 (1.08-1.19)	< 0.001	91.01	42	< 0.001
			Between types		42.78	3	< 0.001
			Between types, excluding prospective/cross-sectional		9.51	2	< 0.01
By source of diagnosis							
Death certificate only	27	1.04 (1.01-1.07)	1.06 (1.02-1.11)	< 0.01	41.57	26	< 0.05
Medical data used	41	1.35 (1.28-1.43)	1.34 (1.23-1.46)	NS	51.49	40	NS
Self-report only	7	1.17 (1.10-1.24)	1.17 (1.07-1.27)	NS	8.11	6	NS
			Between sources		75.29	2	< 0.001
By definition of never smoker							
Never any product	11	1.10 (1.05-1.15)	1.15 (1.05-1.27)	NS	32.42	10	< 0.001
Never, product unstated	33	1.05 (1.02-1.09)	1.15 (1.07-1.24)	< 0.001	49.99	32	< 0.05
Never cigarettes	12	1.17 (1.06-1.30)	1.21 (1.05-1.38)	NS	16.54	11	NS
Other	19	1.20 (1.14-1.25)	1.21 (1.07-1.37)	NS	57.89	18	< 0.001
			Between definitions		19.62	3	< 0.001
Sensitivity analyses							
Preferring unadjusted to adjusted estimates	75	1.06 (1.04-1.08)	1.16 (1.09-1.24)	< 0.01	321.31	74	< 0.001
Preferring current to ever exposure	75	1.12 (1.09-1.14)	1.19 (1.13-1.26)	< 0.001	176.96	74	< 0.001
Excluding studies 15 and 16	71	1.16 (1.12-1.19)	1.21 (1.15-1.28)	< 0.01	144.97	70	< 0.001
Excluding study 30	73	1.12 (1.10-1.15)	1.20 (1.14-1.26)	< 0.001	158.21	72	< 0.001
Excluding studies 15, 16 and 30	69	1.20 (1.17-1.24)	1.23 (1.17-1.29)	< 0.05	109.86	68	< 0.001

[1]Nearest equivalent to IHD as shown in Tables 1 and 3; [2]Heterogeneity relates to variation between studies within subgroup, except for results given in italics which relate to heterogeneity between subgroups; [3]N: Number of estimates in meta-analysis; [4]Egger test *P* expressed as < 0.001, < 0.01, < 0.05, < 0.1 or NS ($P \geqslant 0.1$). NC indicates not calculable as too few data points; [5]DF: Degrees of freedom; [6]Expressed as < 0.001, < 0.01, < 0.05, < 0.1 or NS ($P \geqslant 0.1$); [7]Relative risks are adjusted for covariates if adjusted data are available, with estimates for ever exposure preferred to those for current exposure where there is choice; [8]Number of cases was estimated for Nishtar[57] (as category 1-99) and for Rostron[80] (as category 100-199). MI: Myocardial infarction.

with a large weight, they do add to the difficulties in interpreting the overall estimate.

Below, we comment on various aspects of the findings and discuss potential sources of bias.

Study size and publication bias

For the main exposure index, there was clear publication bias ($P < 0.001$), RRs from smaller studies (more likely not to be published if finding no association) being much greater than from larger studies. Thus, for studies of > 1000 cases of heart disease, the RR was 1.08 (95%CI: 1.02-1.15, $n = 18$) while for studies of < 100 cases it was 1.66 (1.30-2.11, $n = 13$). This variation by study size explains why the random-effects estimate (1.18, 1.12-1.24) was higher than the fixed-effect estimate (1.10, 1.08-1.13), as small studies contribute relatively more to random-effects analyses. The random-effects estimate may be an overestimate, due to publication bias.

Definition of never smoker

Some studies clarified that never smoking related to never smoking any product, and others that never smoking related only to cigarettes. However, many studies merely stated the subjects were never smokers. The distinction is more important in countries where smoking of other products is more common. Some studies also made it clear that the definition allowed inclusion of those with a limited history of smoking, and a few rejected individuals with cotinine levels typical of current smokers. However, the estimated RR for the main index varied little depending on the definition.

Misclassification of never smoking status

No study attempted to determine whether self-reported never smokers had in fact smoked previously. However, as noted above and in Table 2, a few studies excluded those with cotinine levels indicative of current smoking In our recent review of ETS and lung cancer[96], we presented analyses demonstrating that correction for misclassification bias substantially reduced the estimated RR for husband's smoking. We did not attempted such correction here, partly because the extent of bias depends on the magnitude of the active smoking RR, which is much lower for heart disease than for lung cancer. However, we are aware of a study[97] which reported particularly high heart disease mortality among smokers who deny smoking, which, if confirmed, suggests misclassification bias might be of some relevance.

Errors in determining ETS exposure

While random errors in determining ETS exposure will tend to underestimate any association with heart disease, errors may not be random. Thus, studies of case-control or cross-sectional design, are subject to recall bias if subjects with heart disease tend to overestimate their exposure relative to those without heart disease. Only two studies[45,56] used biomarker data to try to avoid recall bias. Some support for the existence of recall bias arises from the RRs for the main index being higher for case-control and cross-sectional studies than for prospective studies.

Table 5 Dose-response evidence for heart disease among never smokers in relation to smoking by the spouse or household members in adulthood

Study No.	Ref.[1]	Sex	Exposure grouping	Relative risks by grouping[2]	Significance (trend)[3]
Smoking by the spouse					
1	Hirayama[29]	F	0, 1-19, 20+ (cigs/d)	1.00, 1.10, 1.31[4]	+
5	Svendsen *et al*[33]	M	0, 1-19, 20+ (cigs/d)	1.00, 1.20, 1.75	
14	La Vecchia *et al*[42]	M + F	0, 1-14, 15+ (cigs/d)	1.00, 1.13, 1.30	
15	Layard[25]	M	0, 1-14, 15-34, 35+ (cigs/d)	1.00, 0.76, 1.07, 0.92	
		F	0, 1-14, 15-34, 35+ (cigs/d)	1.00, 0.85, 1.15, 1.06	
16	Le Vois *et al*[26] (CPS I)	M	0, 1-19, 20-39, 40+ (cigs/d)	1.00, 0.99, 0.98, 0.72	
		F	0, 1-19, 20-39, 40+ (cigs/d)	1.00, 1.04, 1.06, 0.95	
20	Steenland *et al*[46]	M	0, 1-19, 20, 21+ (cigs/d)	1.00, 1.33, 1.17, 1.09	
		F	0, 1-19, 20, 21-39, 40+ (cigs/d)	1.00, 1.15, 1.07, 0.99, 1.04	
		M	0, 1-12, 13-21, 22-29, 30+ (year)	1.00, 1.14, 1.13, 1.14, 1.25	
		F	0, 1-14, 15-25, 26-33, 34+ (year)	1.00, 0.84, 0.99, 1.20, 1.20	
		M	0, 1-5, 6-14, 15-27, 28+ (pack year)	1.00, 1.25, 1.33, 1.13, 1.00	
		F	0, 1-12, 13-25, 26-33, 34+ (pack year)	1.00, 0.83, 1.12, 1.09, 1.26	
21	Janghorbani *et al*[47]	F	0, 1-30, 31+ (year)	1.00, 1.74, 0.85	
		F	0, 1-19, 20+ (cigs/d)	1.00, 1.76, 1.11	
		F	0, 1-10, 11+ (pack year)	1.00, 1.95, 1.17	
23	Ciruzzi *et al*[49]	F	0, 1-20, 21+ (cigs/d)	1.00, 0.82, 3.00	
26	He *et al*[52]	F	0, 1-10, 11-20, 21+ (cigs/d)	1.00, 0.93, 1.40, 3.20	+
			0-5, 6-15, 16-30, 31+ (year)	1.00, 0.80, 2.10, 2.30	+
			0, 1-399, 400-799, 800+ (cigs/day × year)	1.00, 1.20, 1.90, 3.60	+
28	Rosenlund *et al*[54]	M + F	0, 1-19, 20+ (cigs/d)	1.00, 1.02, 1.58	
		M + F	0, 1-32, 33+ (year)	1.00, 1.11, 1.25	
		M + F	0, 1-20, 21+ (pack-year)	1.00, 1.09, 1.33	
30	Enstrom *et al*[27]	M	0, 1-9, 10-19, 20, 21-39, 40+ (cigs/d)	1.00, 0.98, 0.82, 0.89, 1.13, 1.24	
		F	0, 1-9, 10-19, 20, 21-39, 40+ (cigs/d)	1.00, 1.03, 0.99, 1.02, 0.88, 0.80	
39	Wen *et al*[64]	F	0, < 8.8, 8.8-17.9, 18.0+ (pack-year)	1.00, 1.10, 1.12, 1.22[5]	
47	Gallo *et al*[71]	M + F	0, 0.5, 1.0, 1.5+ (packs/d)	1.00, 1.87, 1.89, 2.46[6]	
Smoking by household members					
8	Hole *et al*[36]	F	0, 1-14, 15+ (cigs/d)	1.00, 2.09, 4.12	+
9	Jackson[37]	M	None, low, high (exposure)	1.00, 1.30, 0.90	
		F	None, low, high (exposure)	1.00, 2.10, 7.50	+
18	Muscat *et al*[44]	M	None, 1-20, 21-30, 31+ (year)	1.0, 1.7, 1.5, 1.1	
		F	None, 1-20, 21-30, 31+ (year)	1.0, 2.0, 0.9, 1.7	
22	Kawachi *et al*[48]	F	None, occasional, regular	1.00, 1.19, 2.11	+
		F	< 1, 1-9, 10-19, 20-29, 30+ (year)	1.00, 1.19, 1.54, 1.11, 1.50	
27	Iribarren *et al*[53]	M	0, 1-9, 10-39, 40+ (h/wk)	1.00, 1.12, 1.26, 1.20	+
		F	0, 1-9, 10-39, 40+ (h/wk)	1.00, 1.21, 1.31, 1.36	+
29	Pitsavos *et al*[55]	M + F	0, 1-4, 5-9, 10-19, 20-29, 30-39, 40+ (years living with a regular smoker)	1.00, 1.07, 1.16, 1.39, 1.75, 2.20, 3.09	+
34	McGhee *et al*[59]	M + F	0, 1, 2+ (smokers in the home)	1.00, 1.26, 1.68	+
40	Eisner *et al*[65]	M + F	Per 10 years exposure	1.10	
46	Ding *et al*[70]	F	0, < 1, 1+ (packs/d)	1.00, 1.14, 1.69	+
			0, < 5, 5+, (year)	1.00, 1.26, 1.52	+
			0, < 4, 4+, (h/d)	1.00, 1.28, 1.82	+
			0, < 5, 5+, (pack-year)	1.00, 1.44, 1.53	+
			0, < 20, 20+ (h-year)	1.00, 1.22, 1.61	+
47	Gallo *et al*[71]	M + F	0, < 1, 1-2, 3+ (h/d)	1.00, 1.39, 2.08, 1.94[6]	+
54	Iversen *et al*[78]	M	0, < 10, 10-19, 20-29, 30+ (year)	1.00, 0.70, 1.20, 0.70, 1.10	
		F	0, < 10, 10-19, 20-29, 30+ (year)	1.00, 1.00, 1.40, 1.30, 1.60	+

[1]First author of paper; [2]Relative risks are adjusted for covariates if adjusted data are available; [3]Significant ($P < 0.05$) positive (negative) trends are indicated by + (or -). Blank entries indicate non-significance. The trend test includes the unexposed group. Significant trends excluding the unexposed group are only evident for study 26 (all exposed indices); [4]The 1-19 cigs/d group includes ex-smokers. Estimates are adjusted for the age of the husband. Alternative estimates, adjusted for the age of the subject are also given by Hirayama[115]; [5]Results for CVD. Not available for CVD - Stroke; [6]Results for CVD. Not available for IHD. M: Male; F: Female; CVD: Cardiovascular disease; IHD: Ischaemic (coronary) heart disease.

Weaknesses in prospective studies

While prospective studies avoid recall bias, they may underestimate any true association if ETS exposure is determined only at baseline, and not updated. This was the case for the great majority of such studies. Thus, RRs for the index "spouse current smoker" may be underestimated by inclusion of some spouses who give up after baseline. However, the similarity of the RR estimates preferring current to ever spousal exposure and preferring ever to current spousal exposure sug-

Table 6 Relative risk of heart disease among never smokers in relation to four other indices of environmental tobacco smoke exposure

Study No.	Ref.[1]	Sex	Exposure index[2]	Relative risk (95%CI)[3]	Exposure description
3	Lee *et al*[31]	M	Workplace	0.66 (0.26-1.66)	
		F	Workplace	0.69 (0.26-1.87)	
		M	Total	0.39 (0.17-0.90)	Home, work, travel, leisure
		F	Total	0.52 (0.24-1.09)	Home, work, travel, leisure
5	Svendsen *et al*[33]	M	Workplace	1.40 (0.80-2.50)	
		M	Total	1.17 (0.62-1.19)	Spouse, work
9	Jackson *et al*[37]	M	Workplace	1.80 (0.94-3.46)	
		F	Workplace	1.55 (0.48-5.03)	
		M	Total	1.14 (0.76-1.70)	Home, work
		F	Total	1.56 (0.76-3.20)	Home, work
12	Dobson *et al*[40]	M	Workplace	0.95 (0.51-1.78)	
		F	Workplace	0.66 (0.17-2.62)	
		M	Total	1.09 (0.72-1.63)	Home, work
		F	Total	2.24 (1.28-3.91)	Home, work
18	Muscat *et al*[44]	M	Workplace	1.20 (0.60-2.20)	
		F	Workplace	1.00 (0.40-2.50)	
		M	Childhood	0.79 (0.39-1.63)	Mother, father, other relatives
		F	Childhood	0.72 (0.30-1.72)	Mother, father, other relatives
19	Tunstall-Pedoe *et al*[45]	M + F	Total	1.34 (1.07-1.67)	Exposure to tobacco smoke from someone else in the previous three days
		M + F	Biomarker	1.13 (0.93-1.38)	Serum cotinine
20	Steenland *et al*[46]	M	Workplace	1.03 (0.89-1.19)	
		F	Workplace	1.06 (0.84-1.34)	
22	Kawachi *et al*[48]	F	Workplace	1.68 (0.81-3.47)	
		F	Total	1.71 (1.03-2.84)	Home, work
24	McElduff *et al*[50]	M	Total	0.82 (0.55-1.22)	Daily at home, work
		F	Total	2.15 (1.18-3.92)	Daily at home, work
26	He *et al*[52]	F	Workplace	1.85 (0.86-4.00)[4]	
		F	Total	2.87 (1.36-6.05)	Spouse, work
27	Iribarren *et al*[53]	M	Total	1.07 (0.96-1.19)	Home, small spaces, large indoor areas
		F	Total	1.10 (1.01-1.20)	Home, small spaces, large indoor areas
28	Rosenlund *et al*[54]	M	Workplace	1.14 (0.78-1.67)	
		F	Workplace	0.94 (0.59-1.50)	
		M + F	Total	1.18 (0.87-1.60)	Spouse, work
29	Pitsavos *et al*[55]	M + F	Workplace	1.97 (1.16-3.34)	
		M	Total	1.33 (0.94-1.88)	Home, work
		F	Total	1.39 (0.87-2.23)	Home, work
31	Chen *et al*[56]	M + F	Workplace	1.70 (0.90-3.20)	
		M + F	Total	1.50 (1.03-2.20)	Other people's tobacco smoke in the previous three days
		M + F	Biomarker	0.86 (0.64-1.16)	Serum cotinine
32	Nishtar *et al*[57]	M + F	Total	2.87 (1.28-6.42)	Unspecified, but includes spouse and others
33	Whincup *et al*[58]	M	Biomarker	1.67 (1.03-2.72)	Serum cotinine
36	Hedblad *et al*[61]	M	Biomarker	2.22 (1.21-4.09)	Blood carboxyhaemoglobin
37	Stranges *et al*[62]	M	Workplace	0.97 (0.64-1.48)	
		F	Workplace	0.96 (0.60-1.55)	
		M	Childhood	1.04 (0.72-1.52)	Unspecified
		F	Childhood	0.93 (0.57-1.51)	Unspecified
		M	Total	1.11 (0.69-1.77)	Lifetime; home, work, public places; RR is compared to lower tertile of exposure
		F	Total	0.58 (0.33-1.03)	Lifetime; home, work, public places; RR is compared to lower tertile of exposure
38	Teo *et al*[63]	M + F	Total	1.37 (1.27-1.48)	Family, friends, co-workers
39	Wen *et al*[64]	F	Workplace	1.21 (0.74-2.01)[5]	
		F	Childhood	1.49 (1.01-2.22)[5]	In early life from family members
		F	Total	1.25 (0.69-2.25)[5]	Spouse, work, early life
43	He *et al*[67]	F	Total	1.69 (1.31-2.18)	Home, work
45	Vozoris *et al*[69]	M + F	Total	1.00 (0.80-1.20)	Exposed on most days in the previous month
47	Gallo *et al*[71] (EPIC)	M	Workplace	0.93 (0.46-1.90)[6]	
		F	Workplace	0.76 (0.47-1.24)[6]	
		M	Childhood	1.11 (0.72-1.69)[6]	Parents
		F	Childhood	1.18 (0.88-1.57)[6]	Parents
48	Hamer *et al*[72]	M	Biomarker	1.50 (0.85-2.64)	Salivary cotinine
49	Jefferis *et al*[73]	M + F	Biomarker	0.94 (0.59-1.51)	Serum cotinine
50	Peinemann *et al*[74]	M + F	Total	1.27 (0.84-1.92)	Home, work, other

51	Chen[75]	M + F	Total	1.16 (0.93-1.45)[7]	Home, work, other
52	He *et al*[76]	M	Total	2.24 (0.76-6.59)	Lifetime; home, work
		F	Total	2.10 (0.69-6.33)	Lifetime; home, work
54	Iversen *et al*[78]	M	Total	0.97 (0.61-1.55)	Time spent in smoke-filled rooms
		F	Total	0.70 (0.44-1.12)	Time spent in smoke-filled rooms
55	Kastorini *et al*[79]	M + F	Total	4.33 (1.52-12.38)	Partner, parents, children, roommates, colleagues; 30+ min/d
56	Rostron[80]	M + F	Biomarker	1.02 (0.70-1.47)	Serum cotinine
57	Batty *et al*[81]	M	Biomarker	0.49 (0.19-1.25)	Salivary cotinine
		F	Biomarker	1.26 (0.70-2.24)	Salivary cotinine
58	Shiue[82]	M + F	Total	1.47 (0.96-2.24)	Home, work, other

[1]First author of paper; [2]Biomarker RRs are all based on cotinine measurement except for study 36 which is based on COHb; [3]Relative risks are adjusted for covariates if adjusted data are available. Some of the RRs are repeats of those given in Table 3; [4]Estimate given by an earlier report of the same study[117]; [5]Results for CVD-Stroke. Results also available for CVD: workplace 0.92 (0.64-1.32), childhood 1.26 (0.94-1.69), total 1.45 (0.95-2.22); [6]Results for CVD-Stroke. Not available for IHD; [7]Result for IHD. Result for MI also available: 0.93 (0.66-1.31). M: Male; F: Female; CVD: Cardiovascular disease; IHD: Ischaemic (coronary) heart disease; MI: Myocardial infarction.

Table 7 Meta-analyses of heart disease[1] risk among never smokers in relation to four other indices of environmental tobacco smoke exposure

Index of exposure	n[2]	Fixed-effect Relative risk (95%CI)	Random-effects Relative risk (95%CI)	Publication bias P[3]	Heterogeneity χ^2	DF[4]	P[5] value
Workplace	22	1.08 (0.99-1.19)	1.08 (0.99-1.19)	NS	20.12	21	NS
Childhood	7	1.12 (0.95-1.31)	1.12 (0.95-1.31)	< 0.1	4.77	6	NS
Total	33	1.21 (1.16-1.26)	1.23 (1.12-1.35)	NS	90.21	*32*	$P < 0.001$
Biomarker	9	1.11 (0.98-1.26)	1.15 (0.94-1.40)	NS	15.40	8	$P < 0.1$

[1]Nearest equivalent to IHD as shown in Tables 1 and 6; [2]n: Number of estimates in meta-analysis; [3]Egger test P expressed as < 0.001, < 0.01, < 0.05, < 0.1 or NS ($P \geqslant 0.1$); [4]DF: Degrees of freedom; [5]Expressed as < 0.001, < 0.01, < 0.05, < 0.1 or NS ($P \geqslant 0.1$).

gests this is not a major issue.

Inappropriate controls in case-control studies

In some case-control studies using population controls, the control group may not have been fully representative of the population from which the cases derived, while some hospital studies merely ensured that the controls were not suffering from heart disease, and may have included patients with other diseases associated with ETS exposure.

Weaknesses of cross-sectional studies

Ten of the 58 studies considered were of cross-sectional design. Apart from the possibility of recall bias, this design does not exclude the theoretical possibility that disease onset might have occurred before ETS exposure.

Diagnosis and classification of heart disease

A major determinant of heterogeneity for the main index related to source of diagnosis, with RRs substantially lower for estimates based only on death certificates (1.06, 95%CI: 1.02-1.11), than when based on medical data (1.34, 1.23-1.46), the few estimates based on self-report giving intermediate results (1.17, 1.07-1.27). Note, however, that this classification correlates considerably with that for study type. Thus, all the estimates based on self-report are from cross-sectional studies, nearly all those based only on death certificates are from prospective studies, with case-control studies contributing largely to estimates based on medical data.

The actual disease for which results are available varies by study, with some studies presenting results for multiple definitions. Higher RRs were seen for the main index where the definition was based on MI (1.29, 95%CI: 1.14-1.46) rather than on IHD (1.12, 1.05-1.19) or other/mixed definitions (1.20, 1.10-1.30). However, again there is a correlation with study type, there being few prospective studies using a definition of MI.

Confounding by other risk factors

There are manifold risk factors for heart disease, a study published in 1986[98] mentioning over 300. As several studies[53,99-103] showed differences in many lifestyle factors between smoking and non-smoking households, a potential for confounding is certainly present. Though difficult to assess precisely, partly because of the numerous risk factors involved, and partly because studies rarely present results showing the effect of adjustment for individual factors, some insight can be gained by comparing RR estimates across studies according to the number of risk factors adjusted for. Though the number of risk factors may be correlated with other aspects of the study, the results did not suggest the association was due to confounding, RRs being somewhat higher where more confounders were accounted for.

Inclusion of studies rejected in other meta-analyses

Three meta-analyses published in the late 1990s[2-4] deliberately excluded results reported by Layard[25], based on the National Mortality Followback Survey (NMFS), and by LeVois and Layard[26], based on the American Cancer Society (ACS) Cancer Prevention Studies Ⅰ (CPS Ⅰ) and

Table 8 Other indices of environmental tobacco smoke exposure - dose response results among never smokers

Study No.	Author[1]	Sex	Exposure grouping	Relative risk by grouping (95%CI)[2]	Significance[3]
Workplace exposure					
22	Kawachi *et al*[48]	F	No, occasional, regular	1.00, 1.49, 1.92	
26	He *et al*[52]	F	0-5, 6-10, 11-20, 21+ cigs/d	1.00, 0.87, 2.95, 3.56	+
		F	0-5, 6-15, 16+ year	1.00, 3.08, 1.56	
		F	0, 1-2, 3, 4+ smokers	1.00, 1.16, 5.06, 4.11	+
		F	0, 1-2, 3-4, 5+ h/d	1.00, 0.62, 4.03, 21.32	+
		F	0, 1-2000, 2001-4000, 4000+, cigs/d × year × smokers × h	1.00, 1.00, 2.05, 9.23	+
28	Rosenlund *et al*[54]	M + F	0, 1-31, 32+ yr	1.00, 1.04, 1.30	
		M + F	0, 1-68, 69+ h-year (= h/d × year)	1.00, 0.99, 1.48	
39	Wen *et al*[64]	F	0, < 10, 10-24, > 24 yr	1.00, 0.86, 0.96, 0.93[4]	
40	Eisner *et al*[65]	M + F	Per 10 yr exposure	1.04	
Childhood exposure					
18	Muscat *et al*[44]		Exposure to mother, father, other relatives		
		M	None, 1-17, > 17 yr	1.0, 0.9, 0.7	
		F	None, 1-17, > 17 yr	1.0, 0.6, 0.8	
39	Wen *et al*[64]	F	In early life from family members[5]		
			0, < 20, 20+, year	1.00, 1.21, 1.36[4]	+
Total exposure					
3	Lee *et al*[31]		Home, work, travel, leisure combined index		
		M	Score: 0-1, 2-4, 5-12	1.00, 0.43, 0.43	
		F	Score: 0-1, 2-4, 5-12	1.00, 0.59, 0.81	
5	Svendsen *et al*[33]		Spousal and/or workplace exposure		
		M	Neither, spouse, work, both	1.0, 1.2, 1.0, 1.7	
9	Jackson[37]		Exposure at home and/or work[6]		
		M	No, yes	1.00, 1.14 (0.76-1.70)	
		F	No, yes	1.00, 1.56 (0.76-3.20)	
12	Dobson *et al*[40]		Exposure at home and/or work		
		M	No, yes	1.00, 1.09 (0.72-1.63)	
		F	No, yes	1.00, 2.24 (1.28-3.91)	+
19	Tunstall-Pedoe *et al*[45]		Exposure to tobacco smoke from someone else in the previous three days		
		M + F	None, little, some, a lot, (self-classified)	1.00, 1.2, 1.5, 1.6	+
22	Kawachi *et al*[48]		Exposure at home and/or work		
		F	None, occasional, regular	1.00, 1.58, 1.91	+
26	He *et al*[117]		ETS exposure from spouse and/or work		
		F	Neither, spouse, work, both	1.00, 2.07, 2.53, 4.18	+
27	Iribarren *et al*[53]		Exposure at home, in small spaces, in large indoor areas		
		M	0, 1-9, 10-39, 40+ total h/wk	1.00, 0.90, 1.08, 1.13	+
		F	0, 1-9, 10-39, 40+ total h/wk	1.00, 0.86, 1.07, 1.17	+
28	Rosenlund *et al*[54]		Exposure from spouse and/or work		
		M + F	0, > 16, 7-16, 1-6, < 1, year ago	1.00, 0.92, 1.11, 1.30, 1.39	
		M + F	0, 1-12, 13-23, 24-34, 35+, year	1.00, 0.72, 0.97, 1.54, 1.48	+
		M + F	0, 1-17, 18-41, 42-89, 90+, h-year, (= year × h/d)	1.00, 0.70, 1.22, 1.27, 1.55	+
29	Pitsavos *et al*[55]		Exposure at home and/or work		
		M	None, occasional, regular	1.00, 1.25, 1.47	+
		F	None, occasional, regular	1.00, 1.29, 1.56	+
31	Chen *et al*[56]		Exposure to tobacco smoke from someone else in the previous three days		
		M + F	None, a little, some, a lot	1.00, 1.30, 1.50, 1.80	+
			Exposure to other people's tobacco smoke		
		M + F	0, > 0-2, 3-5, ⩾ 6 h/d	1.00, 1.20, 1.60, 1.70	
37	Stranges *et al*[62]		Cumulative lifetime ETS exposure at home, work and in public settings		
		M	Tertile: 1, 2, 3	1.00, 0.93, 1.40	
		F	Tertile: 1, 2, 3	1.00, 0.50, 0.67	
38	Teo *et al*[63]		Exposure from family, friends, co-workers		
		M + F	< 1, 1-7, 8-14, 15-21, 22+ h/wk	1.00, 1.32, 1.52, 1.73, 1.49	+
43	He *et al*[67]		Exposed at home and/or work		
		F	0, 1-9, 10-19, 20+, cigs/d	1.00, 1.41, 1.85, 1.77	+
			0, 1-20, 21-40, 41+, min/d	1.00, 1.46, 1.78, 1.86	+
52	He *et al*[76]		Exposed at home and/or work[7]		
		M + F	None Low Moderate High	1.00, 1.74, 2.25, 3.79	+
54	Iversen *et al*[78]		Time spent in a smoke-filled rooms		
		M	0, 1-6, > 6, h/d	1.00, 1.00, 0.80	
		F	0, 1-6, > 6, h/d	1.00, 0.70, 0.70	
58	Shiue[82]		Exposed at home, work, other people's home		
		M + F	0, 1, 2+ of these places	1.00, 1.37, 2.64	+

Biomarker					
19	Tunstall-Pedoe *et al*[45]		Serum cotinine (ng/mL)		
		M + F	0, > 0-1.05, 1.06-3.97, 3.98-17.49	1.00, 1.00, 1.30, 1.20	
31	Chen *et al*[56]		Serum cotinine (ng/mL)		
		M + F	0, > 0-1.05, 1.06-3.97, 3.98-17.49	1.00, 0.70, 1.00, 1.10	
33	Whincup *et al*[58]		Serum cotinine (ng/mL)		
		M	≤ 0.7, 0.8-1.4, 1.5-2.7, 2.8-14.0	1.00, 1.54, 1.89, 1.67	+
36	Hedblad *et al*[61]		Blood carboxyhaemoglobin (%)		
		M	0.13-0.49, 0.50-0.57, 0.58-0.66, 0.67-5.47 (quartiles)	1.00, 1.26, 1.77, 3.71	+
48	Hamer *et al*[72]		Salivary cotinine (ng/mL)		
		M + F	≤ 0.05, 0.06-0.70, 0.71-14.99	1.00, 1.33, 2.00	+
			Per unit increase in log cotinine	1.60 (1.11-2.31)	
49	Jefferis *et al*[73]		Serum cotinine (ng/mL)		
		M + F	≤ 0.05, 0.06-0.19, 0.20-0.70, 0.71-15	1.00, 0.91, 0.99, 0.94	
			Per doubling of cotinine	1.00 (0.86-1.16)	
56	Rostron[80]		Serum cotinine (ng/mL)		
		M + F	< 0.1, 0.1- < 1, 1- < 15	1.00, 0.97, 1.41	
57	Batty *et al*[81]		Salivary cotinine (ng/mL)		
		M	≤ 0.3, 0.4-1.2, 1.3-15.0	1.00, 0.41, 0.62	
		F	≤ 0.3, 0.4-1.2, 1.3-15.0	1.00, 0.99, 1.70	

[1]First author; [2]Relative risks presented are adjusted for covariates if adjusted data are available. When two groups only are being compared (or results for log cotinine are given), the relative risk and 95%CI limits for the exposed group (per unit increase) are shown; when more than two exposure groups are being compared, only the set of relative risks is shown; [3]Significant ($P < 0.05$) positive (or negative) differences or trends are indicated by + (or -). ? indicates not known whether significant or not. Blank entries indicate non-significance. The trend test includes the unexposed group; [4]Results for CVD. Not available for CVD - Stroke; [5]For study 39 the results for any childhood exposure (yes/no) shown in Table 4 relate to CVD minus stroke but the results by years exposed shown here relate to CVD as a whole; [6]The data shown here for study 9 come from the publication describing study 24; [7]The index of exposure was a combination of exposure at home (four categories of pack-years) and exposure at work (four categories of pack-years × h/d). M: Male; F: Female; CVD: Cardiovascular disease.

Ⅱ (CPS Ⅱ). The results from these studies showed no evidence of a relationship of spousal smoking to heart disease mortality. Though we have not used the cited CPS Ⅱ results, more detailed results being reported later by the ACS[46], we included the results from NMFS[25] and CPS Ⅰ[26]. Apart from wishing to consider all the evidence, and particularly not omit data from the very large CPS Ⅰ, we found the reasons for excluding these studies to be unconvincing.

One reason given[2] was that their results were inconsistent with other data, and reported by tobacco industry consultants. As regards inconsistency, it seems better to include all data, and investigate reasons for inconsistency, than to reject results not fitting in with preconceptions. As regards tobacco industry support, the test is whether the analyses presented were sound. We note no attempt was made by any critic to check the results from the publicly available NMFS, or by the ACS to check results from their CPS Ⅰ. The ACS did conduct their own analyses of CPS Ⅱ[46] using somewhat different methodology, their findings failing to indicate errors in the results of LeVois and Layard[26].

Another reason[4] given was that results were only presented for ever spousal exposure, rather than current spousal exposure. Apart from not noting that results for current spousal exposure were readily available from the CPSI data presented[26], the results being included in our analysis, Thun *et al*[4] also did not mention that their own analyses included results from other studies (studies 1, 2 and 8) based on ever spousal exposure! In fact, as we show, the overall RRs as can be seen in our main analysis, are very similar whether preferring ever to current spousal exposure (1.18, 95%CI: 1.12-1.24), or preferring current to ever spousal exposure (1.19, 1.13-1.26).

We have also included results reported by Enstrom and Kabat[27] in our analysis (Study 30), despite publication of the paper in the BMJ being subject to a large number of critical responses. As the authors noted in a final rapid response in the BMJ, none of the responses identified "any impropriety, bias, or omission in the review process" with "only about 3%" referring to "actual data in the paper". "No one has identified a single error in the paper, not even Thun, who is in a position to check our findings". We agree with Enstrom and Kabat that "the unethical tactics used by the ACS and others, including *ad hominem* attacks and condemnation of legitimate research based solely on the source of funding, have no place in scientific discourse". The authors noted that "Our current research funding comes from Philip Morris USA and three other sources not connected with the tobacco industry". As shown in Table 4, exclusion from our meta-analysis of the three studies in question (studies 15, 16 and 30) slightly increased the RR estimate for our main index, from 1.18 (95%CI: 1.12-1.24) to 1.23 (95%CI: 1.17-1.29), but did not affect the conclusion that there was a clear association of ETS exposure with heart disease risk.

Evidence from studies of smoking bans

Since the first study in 2004[104], which reported a 40% reduction in hospital admissions from AMI following introducing a local law banning smoking in public places and workplaces, numerous further studies have investigated ban effects at national, regional and local level. In a recent review[105], based on 45 studies, we used a

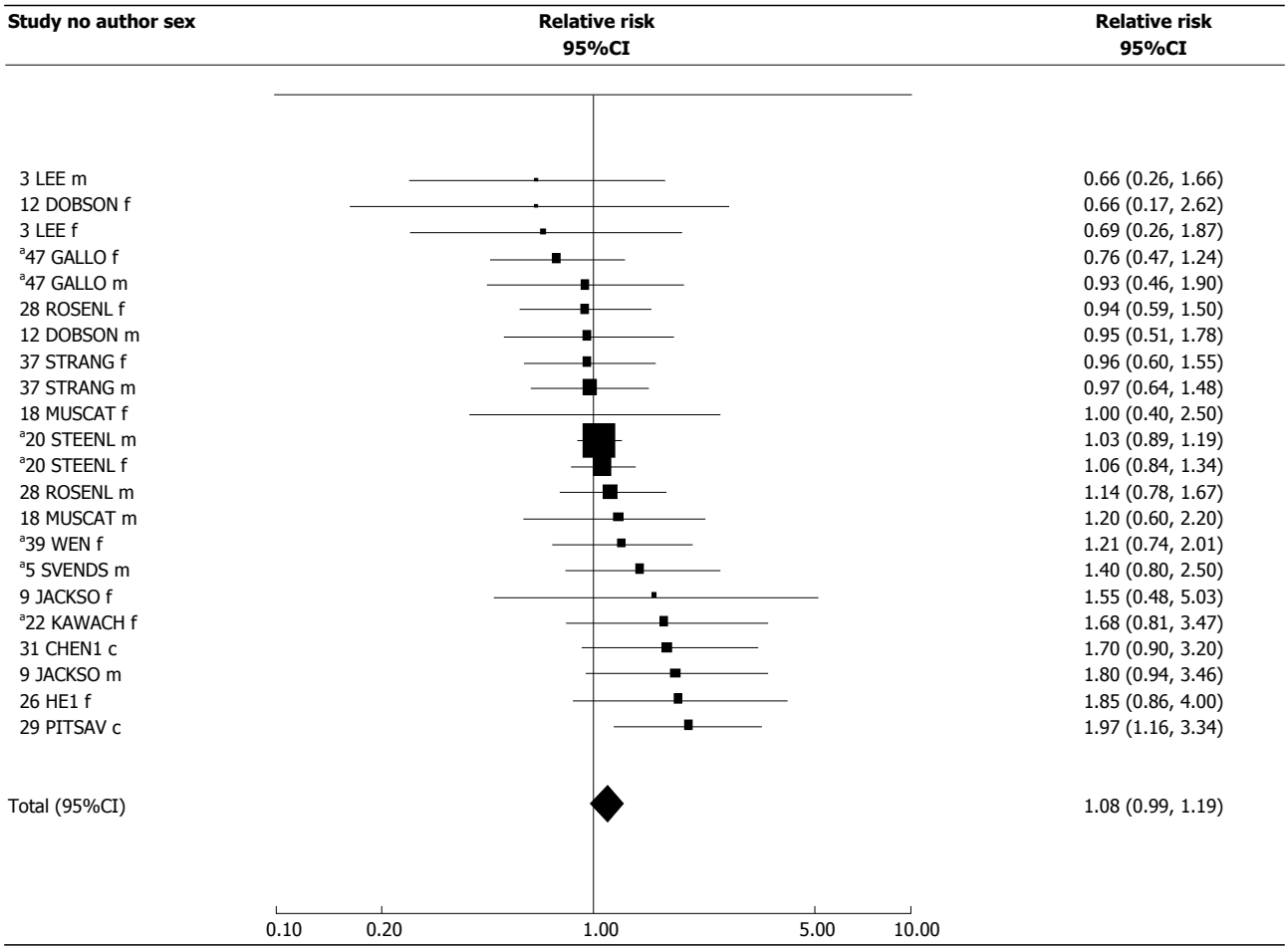


Figure 2 Forest plot for workplace exposure. Estimates of the RR and its 95%CI are shown sorted in increasing order of RR. These are shown numerically, and also graphically on a logarithmic scale. Estimates are identified by the study number shown in Table 1, an abbreviation of the author name and the sex to which the estimate relates (m = male, f = female, c = combined sex estimate). In the graphical representation, individual RRs are indicated by a solid square, with the area of the square proportional to the weight (the inverse of the variance of log RR). The overall random-effects estimate (RRs and 95%CI) is shown, represented graphically by a diamond whose width indicates the confidence interval. [a]Prospective study.

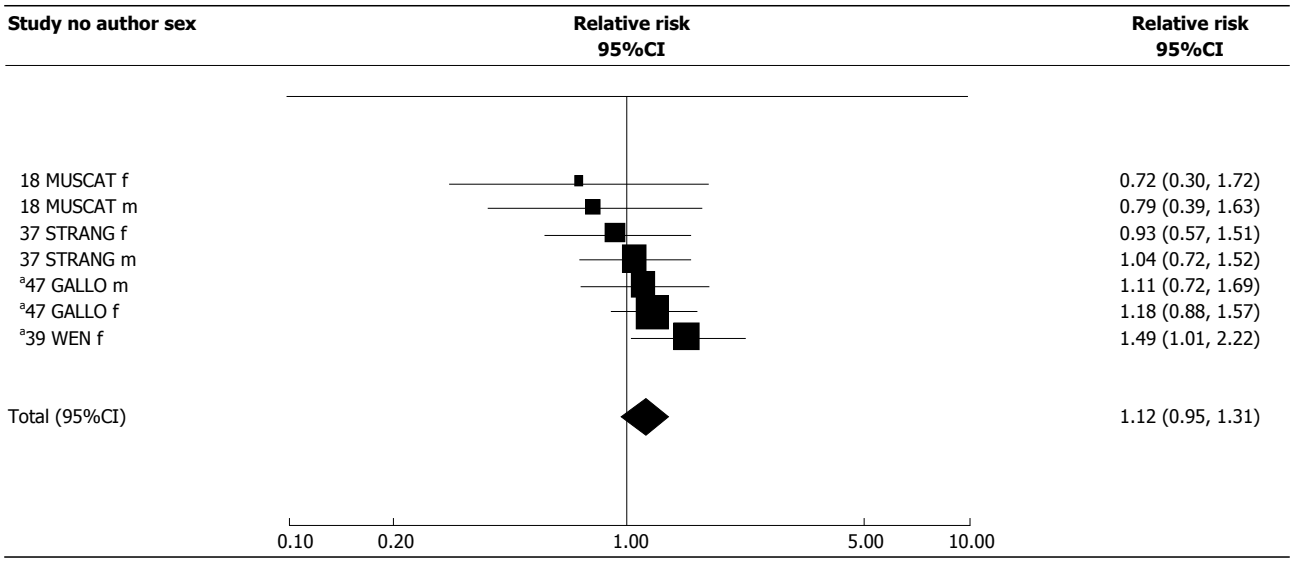


Figure 3 Forest plot for childhood exposure. Estimates of the RR and its 95%CI are shown sorted in increasing order of RR. These are shown numerically, and also graphically on a logarithmic scale. Estimates are identified by the study number shown in Table 1, an abbreviation of the author name and the sex to which the estimate relates (m = male, f = female, c = combined sex estimate). In the graphical representation, individual RRs are indicated by a solid square, with the area of the square proportional to the weight (the inverse of the variance of log RR). The overall random-effects estimate (RRs and 95%CI) is shown, represented graphically by a diamond whose width indicates the confidence interval. [a]Prospective study.

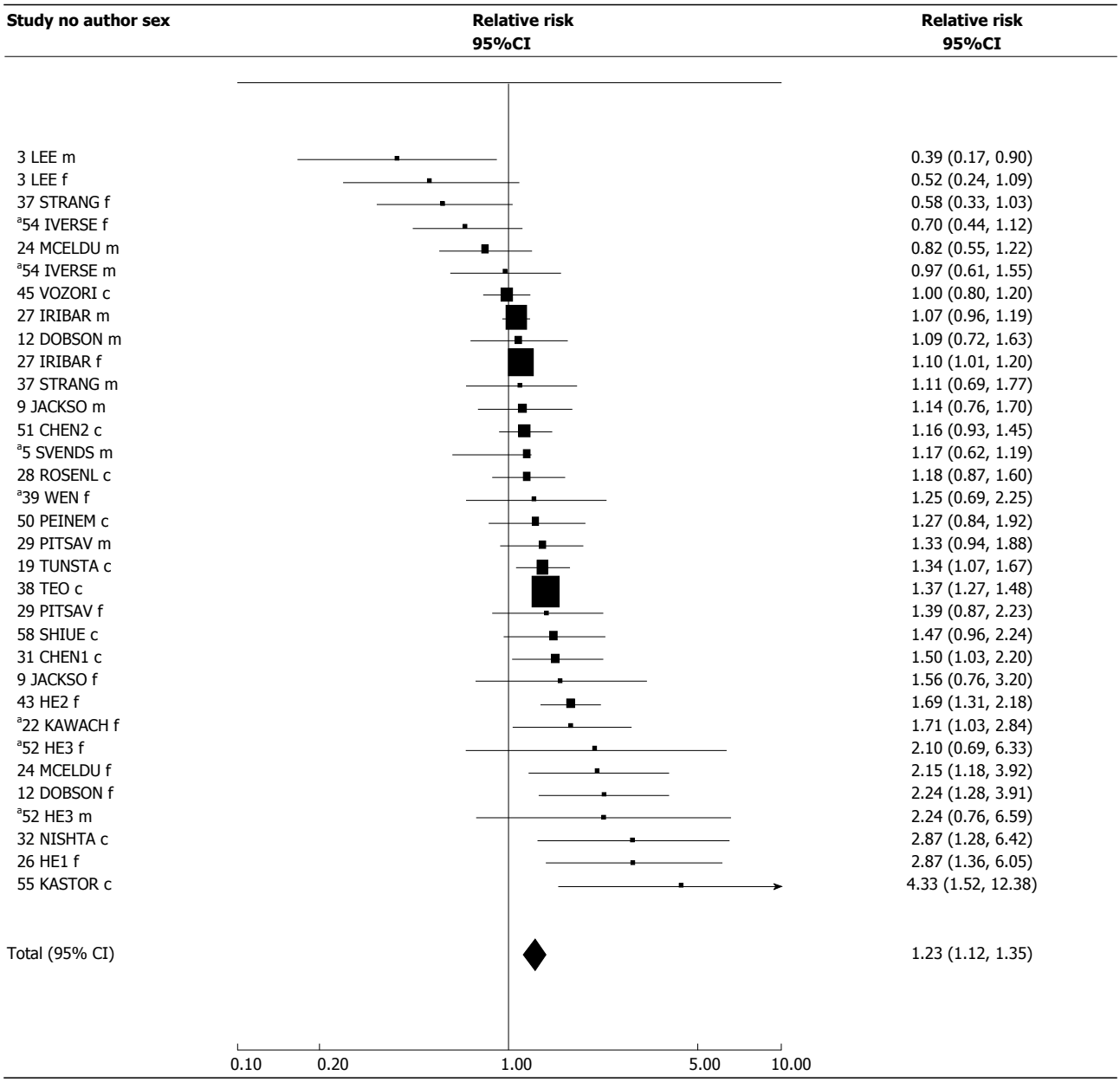


Figure 4 Forest plot for total environmental tobacco smoke exposure. Estimates of the RR and its 95%CI are shown sorted in increasing order of RR. These are shown numerically, and also graphically on a logarithmic scale. Estimates are identified by the study number shown in Table 1, an abbreviation of the author name and the sex to which the estimate relates (m = male, f = female, c = combined sex estimate). In the graphical representation, individual RRs are indicated by a solid square, with the area of the square proportional to the weight (the inverse of the variance of log RR). The overall random-effects estimate (RRs and 95%CI) is shown, represented graphically by a diamond whose width indicates the confidence interval. [a]Prospective study.

consistent approach to adjust for time trends and seasonal effects. We estimated the post-ban risk reduction as 4.2% (95%CI: 1.8%-6.5%) initially, which reduced to 2.6% (1.1%-4.0%) after excluding regional studies where national estimates were available, and also studies where adjustment for the underlying trend in the heart disease rate was not possible. Although these estimates are much less than those from some earlier reviews[106-108] which used less precise techniques, they do suggest a small true ban effect. However, the effect cannot be directly attributed to reductions in risk arising from reduced ETS exposure. Some of the estimated effect might be because smokers reduced their daily cigarette consumption due to the more limited number of places where they are allowed to smoke.

Experimental evidence

The Institute of Medicine (IOM) report[7] discussed "pathophysiologic experiments that have investigated the cardiovascular effects of mainstream and sidestream tobacco smoke in cells, in animals and in humans", noting that cigarette smoke could produce CVD by various "interrelated modes of action, including oxidative stress, hemodynamic and autonomic effects, endothelial dysfunction, thrombosis, inflammation, hyperlipidemia or other effects". While beyond the scope of this paper to

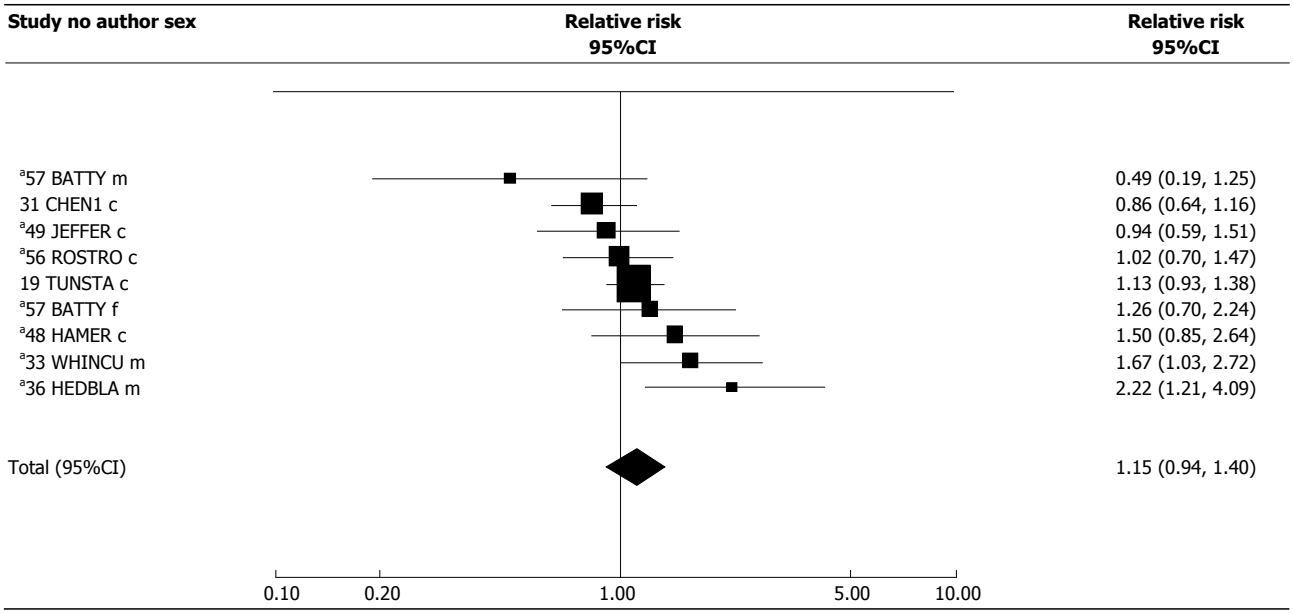


Figure 5 Forest plot for biomarker based indices of environmental tobacco smoke exposure. Estimates of the RR and its 95%CI are shown sorted in increasing order of RR. These are shown numerically and also graphically on a logarithmic scale. Estimates are identified by the study number shown in Table 1, an abbreviation of the author name and the sex to which the estimate relates (m = male, f = female, c = combined sex estimate). In the graphical representation, individual RRs are indicated by a solid square, with the area of the square proportional to the weight (the inverse of the variance of log RR). The overall random-effects estimate (RRs and 95%CI) is shown, represented graphically by a diamond whose width indicates the confidence interval. [a]Prospective study.

consider such evidence, we note that the report states most of the observed changes "have not been formally validated as clinical tests and there is not a consensus within the scientific community that they are predictive of actual clinical disease." While the IOM Committee considered that these effects can "contribute to the biological plausibility that decreasing second-hand smoke could lead to a decrease in acute myocardial infarction", they did not consider that the results, on their own, demonstrated a causal relationship of ETS exposure to heart disease.

Comment on a recent systematic review

In the introduction we referred to various other, conflicting, reviews of ETS and heart disease. Though it is beyond our scope to consider all these in detail, it is worth referring to a recently published systematic review[109] which concluded that ETS exposure "significantly increased the risk for …CVD". This review was limited to prospective and case-control studies, but included studies of stroke, which we have reviewed separately[110]. While the authors' combined RR estimate for cardiovascular disease of 1.23 (95%CI: 1.16-1.31) was similar to our main analysis estimate of 1.18 (1.12-1.24), we note they excluded a number of prospective and case-control studies we included. While some omissions were because they excluded abstracts and theses, and biomarker studies using COHb, we noted eight studies (13, 16, 21, 25, 32, 38, 46 and 55) where there seemed no good reason for the omission. Also, they did not separate results by source of ETS exposure or present any dose-response results.

Association of ETS with other diseases

In recent years, our group has carried out systematic reviews and meta-analyses of the relationship of ETS with various diseases in never smoking adults. These include lung cancer[111], breast cancer[112], other cancers[113], stroke[110] and COPD (submitted for publication). It is of interest to note that spousal smoking is associated with about 20% increased risk in never smokers, not only for heart disease, as we report here, but most studied diseases - stroke, COPD, lung cancer and breast cancer. Estimates are more limited for other cancers, many sites not showing any evidence of an effect, though significant increases were noted for cervix, nasosinus and kidney cancer. Whether evidence of an association for other diseases adds support to the argument that ETS exposure causes heart disease is unclear, as many of the problems of bias noted to affect the association with heart disease may also affect the association with other diseases.

Some, but not all, of the biases may be removed by limiting attention to prospective studies of ETS and total mortality. However, at this point in time, we have not carried out a review of the evidence, though we note that about half the prospective studies cited in Table 1 do give results for total mortality.

Overall assessment

Do the results show that ETS exposure increases risk of heart disease? Here one can usefully cite the classic paper by Hill[114] which specified nine criteria to be considered when attempting to conclude causation. We consider these in turn below.

Strength: The observed association is clearly weak, with our main analyses estimating only an 18% increase in risk associated with ETS exposure.

Consistency: While some studies report no increased risk and a number do not report a statistically significant increased risk, this may reflect the difficulty in demonstrating a weak association, particularly with limited data. Even though there is clear heterogeneity for our main index of exposure, the meta-analysis estimates by level of a range of factors are all increased, and nearly always significantly increased. Thus, for example, significant increases are seen in each sex, in four continents, in prospective, case-control and cross-sectional studies, and in smaller and larger studies. There is certainly an element of consistency.

Specificity: ETS exposure is certainly not a necessary or sufficient cause of heart disease. While it is much easier to demonstrate causation where an agent is such a cause, this criterion is not really relevant here.

Temporality: While theoretically possible in the cross-sectional studies that some cases of heart disease might have preceded exposure to ETS, this could not be so for most cases in the 58 studies we considered.

Biological gradient: Though not all the studies demonstrate a dose-response relationship, many do. However, the significant trends observed are generally calculated including the unexposed group, and evidence of a dose-response within ETS exposed subjects is less clear.

Plausibility: There is clearly plausibility, given smoking causes heart disease and given the experimental evidence referred to above. However, the dose of smoke constituents from ETS is very much less than that from smoking, and it is unclear whether the short-term effects of ETS observed experimentally are actually predictive of heart disease.

Coherence: A cause-and-effect interpretation of the data does not, as far as we are aware, seriously conflict with other generally known facts concerning the history and biology of heart disease.

Experiment: The epidemiological evidence considered lacks any useful material to determine how the risk of heart disease varies following cessation of ETS exposure. However, the evidence from studies of smoking bans suggests that the introduction of smoking bans in public places has caused a modest reduction in risk of heart disease though, as noted, such studies, generally do not separate out effects of reduced ETS exposure in never smokers and of reduced opportunities to smoke in smokers.

Analogy: Whether effects of smoking and of ETS can be regarded as analogous is doubtful, given the substantial differences in extent of exposure and the somewhat different distribution of chemicals for the two types of exposure.

Considering all these points, there seems some inconclusive support for ETS exposure causing heart disease. An important issue not specifically considered in the Bradford Hill criteria, much more relevant for weak than strong associations, is whether the association might be explained by confounding or bias. As regards confounding, the observation that many studies adjusted for numerous risk factors for heart disease, and that RR estimates if anything, increase as more factors are adjusted for, suggests that confounding could not explain the relationship. Nor does it seem likely that the relationship could be fully explained by publication bias or recall bias, though the smaller estimates for large studies and for prospective studies suggest that these biases might have led to some overestimation of the association. Nor is it probable that misclassification of smoking status, or the inclusion of some smokers of products other than cigarettes or occasional or ex-smokers could explain the observed association. While we feel there may well be a true effect of ETS on heart disease risk, it is clear that it is difficult to come to a definitive conclusion, and even more difficult to estimate any true effect precisely.

In conclusion, Taken together with the known relationship of heart disease with smoking, the significantly increased risk for various indices of ETS exposure which can be seen in many study subsets, the evidence of a dose-response relationship, and the lack of any source of bias or confounding that can clearly explain the relationship, the evidence suggests that ETS exposure may cause some increase in the risk of heart disease. That said, the weakness of the overall relationship, the evidence of heterogeneity, the limitations of some of the studies, and the various possibilities of bias, certainly mean that any true effect of ETS exposure is very difficult to quantify precisely.

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COMMENTS

Background

The authors consider evidence that environmental tobacco smoke (ETS)

exposure might cause heart disease by presenting an up-to-date meta-analysis of the available evidence.

Research frontiers

Based on 58 studies providing relevant data, the authors demonstrate an increase in heart disease risk in never smokers associated with ETS exposure by the spouse (or nearest equivalent), with an overall RR estimate of 1.18 (1.12-1.24). While increases were observed in all data subsets considered, there was evidence of heterogeneity, with risk estimates lower for North American studies, larger studies, prospective studies, and when based on fatal cases or death certificate data. Positive associations, not all significant at $P < 0.05$, were also seen with spousal exposure specifically (1.10, 1.04-1.17), workplace exposure (1.08, 0.99-1.19), childhood exposure (1.12, 0.95-1.31), total exposure (1.23, 1.12-1.35) and biomarker-based exposure (1.15, 0.94-1.40) and there was evidence of a dose-response relationship. Although the evidence has various limitations, it is suggestive of a causal relationship. However, the various possibilities of bias mean that any true effect of ETS exposure is very difficult to quantify precisely.

Innovations and breakthroughs

The new feature of the paper is the extent of the evidence considered, and the detail of the analyses conducted.

Applications

The authors analyses emphasise the difficulties in drawing inferences from weak associations seen in non-randomized epidemiological studies, where various biases may exist.

Peer-review

This is a meta-analysis of 58 studies that address the issue of environmental tobacco smoke and the development of heart disease. Overall, the authors found an association between exposure and heart disease risk.

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SYSTEMATIC REVIEWS

Statin use and risk of cancer: An overview of meta-analyses

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Abstract

AIM

To conduct an overview of meta-analyses to critically appraise the evidence and present a comprehensive evaluation of the association between statin use and risk of site specific cancers.

METHODS

MEDLINE, EMBASE, the Cochrane Database of Systematic Reviews and Web of Science databases were searched from inception until 31st May 2016. The electronic database search was supplemented by a hand search in PROSPERO and relevant journals which are not indexed in above databases. Meta-analyses that examined the association between statin use and risk of site specific cancers were included. Two reviewers independently screened the literature, abstracted data, and assessed study quality using the Assessment of Multiple Systematic Reviews (AMSTAR) tool.

RESULTS

Overall, 38 meta-analyses covered 13 site specific cancers were included. More than half (68%) of the meta-analyses were moderate in quality with an AMSTAR score 4-7 out of a possible 11. Based on current evidence from meta-analyses, use of statin decreases the risk of certain cancers, such as colorectal (8%-12%), gastric (27%-44%), hematological (19%), liver (37%-42%), oesophageal (14%-28%), ovarian (21%) and prostate cancer (7%). On the other side, evidence from meta-analyses also suggests that there is no association between statin use and risk of bladder, breast, endometrial, kidney, lung, pancreatic and skin cancers.

CONCLUSION

This overview of meta-analyses with variable quality has been shown that the statins may have a potential role in cancer chemoprevention and reduce the risk of some site specific cancers, but not all.

Key words: Statin; Cancer; Meta-analysis

Core tip: Statins are one of the most commonly prescribed pharmaceutical agents worldwide and atorvastatin remained the largest source of spending in the class. In recent years, emerging experimental evidence suggests that statins may have a potential role in cancer chemoprevention. However, a large number of randomized controlled trials and observational studies published to examine the association between statin use and risk of site specific cancers were given conflicting results. This overview of meta-analyses with variable quality has been shown that the statins may have a potential role in cancer chemoprevention and reduce the risk of colorectal (8%-12%), gastric (27%-44%), hematological (19%), liver (37%-42%), oesophageal (14%-28%), ovarian (21%) and prostate cancer (7%).

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INTRODUCTION

Statins (HMG-CoA reductase inhibitors) are a class of drugs that reduce serum cholesterol levels by inhibiting HMG-CoA reductase, a rate-limiting enzyme in the mevalonate synthesis pathway[1]. They are commonly used in the management and prevention of cardiovascular diseases. Statins are one of the most commonly prescribed pharmaceutical agents worldwide and atorvastatin remained the largest source of spending in the class[2]. With the effect of recommendations for primary prevention with statins by the recent American College of Cardiology/American Heart Association guidelines on the assessment of cardiovascular risk and on the treatment of blood cholesterol, more than a Billion people are expected take statins[3]. Cancers are among the foremost causes of morbidity and mortality worldwide. There were approximately 14 million new cancer cases and 8.2 million cancer related deaths in 2012. Over the next 2 decades, the number of new cancer cases are expected to rise by about 70%[4].

Apart from reduction in cholesterol level and cardiovascular mortality due to substantially increased use of statins during past three decades[5], there is a long-lasting debate on the potential association between statin use and the risk of cancer. In recent years, emerging experimental evidence suggests that statins may have a potential role in cancer chemoprevention[6-8]. It has been proven that statins activates several mechanisms to cancer cell death. Statins induce cell apoptosis by influencing the expression/activity of proteins involved in cell cycle such as cyclins, cyclin-dependent kinases (CDK), and/or inhibitors of CDK. Statins may inhibit cell cycle progression by both extrinsic and intrinsic pathways. By inhibiting isoprenoid synthesis, statins may lead to changes in molecular pathways dependent on the epidermal growth factor receptor. Also, statins may weakens the cell membrane by inhibiting cholesterol synthesis[9]. A large number of randomized controlled trials (RCTs) and observational studies published to examine the association between statin use and risk of site specific cancers were given conflicting results[10]. Many researchers conducted meta-analyses to provide more reliable findings on this association.

In spite of the fact that the meta-analysis show up at the highest level of the evidence in the evidence based practice, comparative data across different domains are often lacking. Overviews are a relatively new approach to generate evidence from several systematic reviews/meta-analyses and become popular in generating the evidence in health care[11].

Therefore, the objective of this overview is to summarize and critically appraise the evidence of relevant meta-analyses to evaluate the association between statin use and risk of site specific cancers.

MATERIALS AND METHODS

Protocol

A protocol for our overview of meta-analyses were drafted using the Cochrane Handbook for overviews of reviews[10]. The drafted protocol was circulated to subject experts and methodologists for feedback purpose. Based on the feedback, the protocol was revised and final version published in PROSPERO International prospective register of systematic reviews (Registration Number: CRD42014013160) (Supplementary Table).

Literature search

A comprehensive literature search was performed in MEDLINE, EMBASE, the Cochrane Database of Systematic Reviews and Web of Science from inception to 31st May 2016 to identify the relevant studies. The search strategy included both medical subject headings (MeSH) and free text terms related to statin and cancer. "Hydroxymethylglutaryl-CoA Reductase Inhibitors and Neoplasms" were the MeSH terms used for statin and cancer, respectively. "Statin(s) or HMG-CoA reductase inhibitor(s) or lipid-lowering agent(s) or atorvastatin or pravastatin or fluvastatin or lovastatin or cerivastatin or mevastatin or rivastatin or rosuvastatin or simvastatin and cancer(s) or neoplasm(s) or malignancy(ies)" were the free text terms used for search strategy. Search strategies were limited to systematic reviews and meta-analyses focused on human participants. In addition, specific journals and cross references of relevant studies were searched manually to capture relevant systematic reviews/meta-analyses and also PROSPERO database was searched to identify completed, unpublished systematic reviews/meta-analyses[12].

Screening

Two authors (KU and CSS) were independently involved

in title/abstract based and full text based screening to capture all relevant articles using a predefined inclusion and exclusion criteria. Disagreements were resolved by discussion and a third author (RKM) was approached whenever required.

Eligibility criteria

We included meta-analyses (didn't find any systematic reviews without meta-analysis) that focused on risk of getting site specific cancers among statin users. Meta-analyses conducted by using RCTs and/or observational studies and published at any point in time were included. Meta-analyses focused on total cancer (*i.e.*, the aggregate of all malignancies) were excluded as all original studies included in these meta-analyses were also included in meta-analyses on site specific cancers with some additional studies. Meta-analyses conducted to identify the effect of statin use on management or prognosis of cancer and also at risk of recurrence of cancer were excluded.

Data abstraction

To abstract the relevant data from each included study, specific data abstraction form was drafted, pilot-tested by all authors independently on a random sample of five articles and same were revised after this exercise, as necessary. After finalizing the data abstraction form, two authors (KU and CSS) have analysed all articles independently to capture relevant data. Discrepancies were resolved by discussion and third author (RKM) was approached whenever required. The following information was captured from each study: (1) first author's last name, year of publication, and country where the study conducted; (2) search methods followed, number of studies identified, type of study designs included, and criteria for study selection and data extraction; (3) methods followed to check the quality of individual studies and to identify the heterogeneity and publication bias; (4) number of subjects and cancer cases involved, outcomes of quality, heterogeneity and publication bias tests, and pooled RR estimates with 95%CIs for primary outcome, secondary outcome and subgroup analyses; and (5) conclusions and if any limitations of the study.

Quality appraisal

Risk of bias assessment of included studies was performed by using the Assessment of Multiple Systematic Reviews (AMSTAR) tool[13]. AMSTAR is highly reliable and validated tool assesses the degree to which review methods avoided bias by evaluating the methods against 11 distinct criteria[14]. Quality rating was as follows: A score of 8-11 is high quality; 4-7 is moderate quality and 3 or lower is low quality. Each included meta-analysis was appraised for quality by two authors independently (KU and RKM) and conflicts were resolved by discussion or the involvement of a third author (CSS).

Data synthesis

The present work was performed as per Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Checklist S1).

RESULTS

Search results

The literature search resulted in 830 titles and abstracts, of which 766 were excluded for not fulfilling the eligibility criteria. Of the 59 full-text articles retrieved and screened in duplicate, 27 were excluded for reasons depicted in Figure 1. Resulted 32 full-text meta-analyses[15-46] in addition to five relevant conference abstracts[47-51] and one relevant full-text published in World Journal of Meta-analysis[52] were included in this overview. We didn't find any completed, unpublished systematic reviews/meta-analyses on this topic in PROSPERO.

Study characteristics

A total of 38 included meta-analyses covered 13 site specific cancers as an outcome for statin use. Majorly seven meta-analyses published on colorectal cancer[18-20,47-50], followed by gastric (4)[21-24], liver (4)[29-31,52], esophageal (4)[35-37,51], skin (4)[43-46], lung (3)[32-34], prostate (3)[40-42], breast (2)[16,17], hematological (2)[26,27], pancreatic cancers (2)[38,39] and each on bladder[15], gynecological[25] and kidney cancers[28]. The characteristics of the included meta-analyses are presented in Table 1.

All included meta-analyses published between 2005 and 2014; majority [25 (66%)] were published in and after 2012. The first authors of the meta-analyses predominantly based in China (15 studies) followed by Greece (7), United States (7), Canada (2), India (2), United Kingdom (2), and each in Australia, Italy and Japan. Except two studies[42,49] where the information on databases searched not available, remaining 36 (95%) studies searched MEDLINE for relevant studies, followed by EMBASE [22 studies (58%)], Web of Science/Web of Knowledge/Science Citation Index [20 studies (53%)] and Cochrane Library [15 studies (39%)]. Out of 38 included studies, 22 (58%) were included both RCTs and observational studies, nine (24%) studies included only RCTs and five (13%) studies included only observational studies, and information not available for remaining two studies[42,51]. For the assessment of heterogeneity, 34 studies used both Cochrane Q test and I^2 test, and information not available for remaining four studies[28,47-49]. Majority [34 (89%)] of the studies assessed publication bias either by using Begg and Mazumdar adjusted rank correlation test and the Egger regression asymmetry test or funnel plot. Two studies[35,36] not assessed publication bias and information not available for remaining two studies[21,29].

Quality appraisal results

More than half [26 (68%)] of the meta-analyses were deemed moderate quality with an AMSTAR score 4-7 out of a possible 11. Only six (16%) studies were found to be high quality with score $\geqslant$ 8, among these one study was a Cochrane systematic review with the highest quality

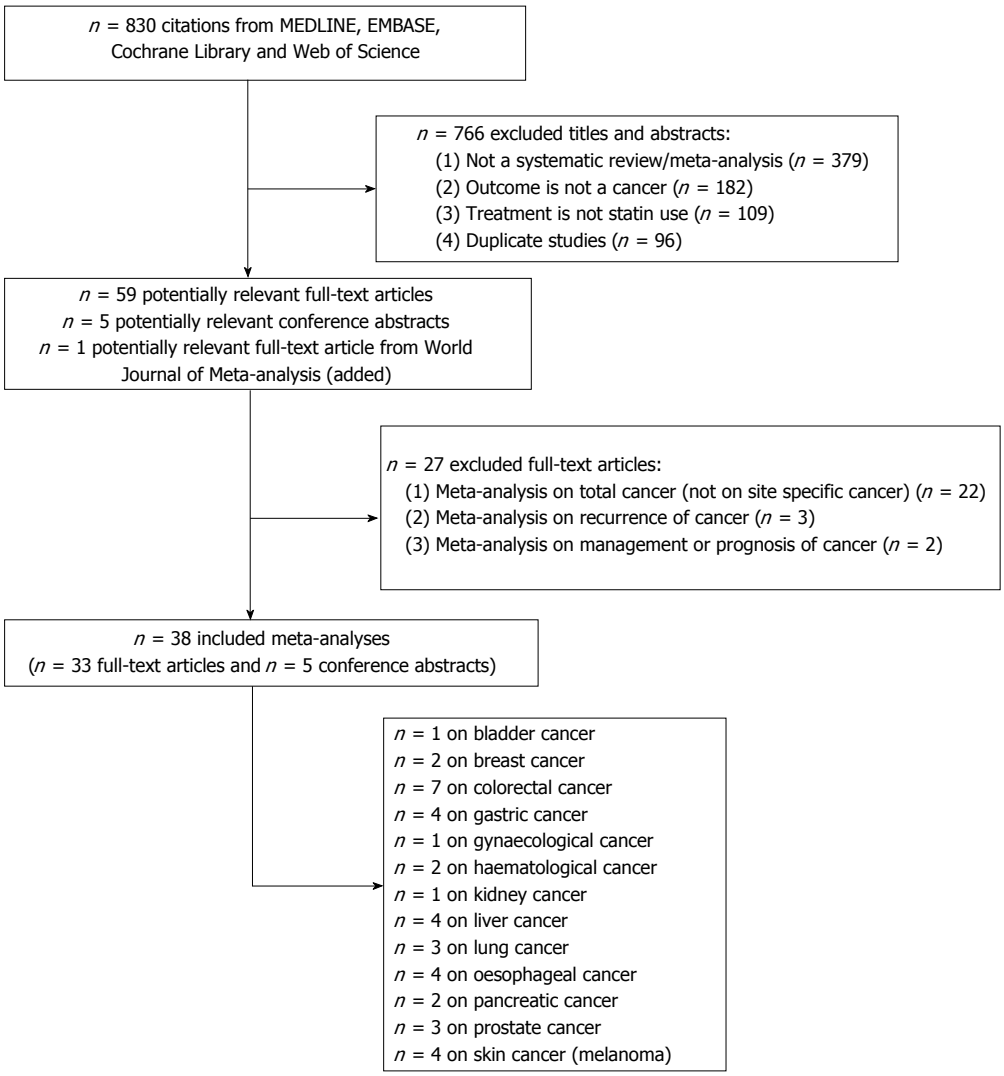

Figure 1 Study flow.

score 10[43]. One study[21] found to be low quality with the score 0 and five studies[47-51] were not having sufficient information to calculate AMSTAR quality score. Majority of meta-analyses were degraded due to lack of "a priori" design, not searched for gray literature, not provided a list of excluded studies from screening of potentially relevant full-text articles and not used any scale to assess the scientific quality of the included studies in formulating conclusions.

Outcome results

The pooled relative risk with 95%CI of the primary outcome of all included studies is shown in forest plot (Figure 2) and it is depicted with sub-group analysis based on cancer type.

Statin use and risk of bladder cancer: Only one meta-analysis[15] was conducted to identify the risk of bladder cancer among statin users. There was no association found between statin use and risk of bladder cancer and the result was same even after subgroup analysis of study design and for long-term statin use.

Statin use and risk of breast cancer: A meta-analysis published by Bonovas *et al*[16] in 2005 to estimate the association between use of statin and risk of breast cancer by including seven RCTs and nine observational studies. There was no association found between statin use and risk of breast cancer with no heterogeneity among studies. The association becomes same even after subgroup analysis of study design. In 2012, Undela *et al*[17] updated this meta-analysis by including 15 more observational studies published after previous meta-analysis. This study also gives an almost similar conclusion, though there was a heterogeneity identified among studies. Additionally, this updated meta-analysis found 47% reduced risk of recurrence of breast cancer among statin users, but no association between long-term statin use and risk of breast cancer.

Statin use and risk of colorectal cancer: Seven meta-analyses (3 full-text[18-20] and 4 conference abstracts[47-50]) published on this association between 2007 and 2014. Almost all the studies included both RCTs and observational studies published between 1995

Table 1 Characteristics of the included meta-analyses

First author, Year[a] (Country)[b]	Databases searched (Period)[c]	Studies included (*n*)	Studies published between (Years)	Total subjects involved	Total cancer cases	Heterogeneity presents?	Pooled RR (95%CI) for primary outcome (*n*)[d]	Pooled RR (95%CI) for secondary outcome (*n*)	Pooled RR (95%CI) for Sub-group analysis[e]	AMSTAR score
Bladder cancer										
Zhang X, 2013 (China)[15]	MEDLINE, EMBASE and Ovid (January 1966 to October 2012)	RCTs (3), Cohort (5) and CC (5)	2001-2012	1266268	2511	Yes (q = 0.001, I^2 = 62.6%)	1.07 (0.95-1.21) (↔)	LSU = 1.21 (0.92-1.51) (8) (↔)	RCTs = 0.83 (0.63-1.10) (↔); Cohort = 1.11 (0.91-1.35) (↔); CC = 1.12 (0.98-1.28) (↔)	7
Breast cancer										
Bonovas S, 2005 (Greece)[16]	MEDLINE (1966 to March2005) and SCI (1970 to March2005)	RCTs (7), Cohort (4) and CC (5)	1993-2005	327238	24599	No (q = 0.12, I^2 = 30%)	1.03 (0.93-1.14) (↔)	None	RCTs = 1.04 (0.81-1.33) (↔) ; Cohort = 1.06 (0.92-1.21) (↔); CC = 0.99 (0.83-1.18) (↔)	7
Undela K, 2012 (India)[17]	PubMed (1966 to January 2012) and ASCO, AACR conference abstracts	Cohort (13) and CC (11)	1993-2011	2440988	76759	Yes (q < 0.001, I^2 = 57%)	0.99 (0.94-1.04) (↔)	LSU = 1.03 (0.96-1.11) (10) (↔); Recurrence 0.53 (0.37-0.76) (2) (↓)	Cohort = 1.01 (0.98-1.04) (↔); CC = 0.95 (0.84-1.08) (↔)	5
Colorectal cancer										
Bonovas S, 2007 (Greece)[18]	MEDLINE (1966 to December 2006) and WS (1970 to December 2006)	RCTs (6), Cohort (3) and CC (9)	2000-2007	1527721	38734	No (q = 0.35, I^2 = 7%)	0.92 (0.90-0.95) (↓)	None	RCTs = 0.95 (0.81-1.11) (↔); Cohort = 0.96 (0.84-1.11) (↔); CC = 0.92 (0.89-0.95) (↓)	7
Bardou M, 2010 (Canada)[f][47]	EMBASE, MEDLINE, CENTRAL, and ISI WK	RCTs (11)	NA	95984	1659	No (NA)	0.94 (0.85-1.04) (↔)	None	NA	--
Bardou M, 2010 (Canada)[f][48]	EMBASE, MEDLINE, CENTRAL, and ISI WK	Cohort (8) and CC (13)	NA	1606234	NA	No (NA)	0.92 (0.87-0.98) (↓)	None	Cohort = 0.89 (0.75-1.05) (↔); CC = 0.92 (0.90-0.94) (↓)	--
Ditah I, 2010 (United States)[f][49]	NA	RCTs (6), Cohort (6) and CC (12)	Up to Jun 2010	> 1.7 million	NA	NA	0.89 (0.85-0.94) (↓)	None	RCTs = 0.95 (0.80-1.13) (↔); Cohort = 0.89 (0.85-0.94) (↓); CC = 0.86 (0.79-0.94) (↓)	--
Samadder NJ, 2010 (United States)[f][50]	MEDLINE, EMBASE, WS and abstracts of national GI conferences (up to October 2009)	Cohort (5) and CC (17)	NA	2.5 million	NA	Yes (q < 0.01, I^2 = 47%)	0.88 (0.84-0.93) (↓)	Rectal = 0.81 (0.62-1.05) (8) (↓)	Cohort = 0.88 (0.84-0.93) (↓); CC = 0.90 (0.86-0.94) (↓)	--
Liu Y, 2014 (China)[19]	PubMed, EMBASE, WS, Cochrane Library (up to 30th July 2013)	RCTs (11), Cohort (13) and CC (18)	1995-2013	7908674	519317	Yes (q < 0.001, I^2 = 66.5%)	0.90 (0.86-0.95) (↓)	LSU = 0.96 (0.88-1.04) (20) (↔)	RCTs = 0.96, (0.85-1.08) (↔); Cohort = 0.93 (0.87-0.99) (↓); CC = 0.84 (0.76-0.93) (↓)	7
Lytras T, 2014 (Greece)[20]	MEDLINE (1966 to July 2013)	RCTs (8), Cohort (13) and CC (19)	1996-2013	8224019	130992	Yes (q < 0.001, I^2 = 71%)	0.91 (0.87-0.96) (↓)	None	RCTs = 0.90 (0.78-1.04) (↔); Cohort = 0.91 (0.83-1.00) (↓); CC = 0.92 (0.87-0.98) (↓)	0034
Gastric cancer										
Shimoyama S, 2011 (Japan)[21]	PubMed Central (1993 to 2008)	RCTs (6)	1994-2007	37851	284	No (q = 0.42, I^2 = 0.0%)	1.37 (0.57-3.25) (3) (↔)	UGIC = 1.20 (0.94-1.53) (3) (↔)	None	0
Singh PP, 2013 (United States)[22]	PubMed (1966 to 1st December 2012), EMBASE (1988 to	RCTs (*post-hoc* analyses) (3),	2004-2012	5459975	5581	Yes (q < 0.01, I^2 = 89%)	0.68 (0.51-0.91) (↓)	None	RCTs = 0.83 (0.66-1.05) (↔); OSs = 0.65 (0.45-0.93) (↓)	8

	1st December 2012) and WS (1993 to 1st December 2012)	Cohort (1) and CC (7)							
Wu X, 2013 (China)[23]	PubMed and EMBASE (up to March 2013)	RCTs (3), Cohort (3) and CC (5)	2004-2013	1136918	7611	Yes (q < 0.001, I^2 = 88.9%)	0.73 (0.58-0.93) (↓)	None	RCTs = 0.84 (0.61-1.14) (↔); Cohort = 0.87 (0.77-0.99) (↓); CC = 0.56 (0.31-1.01) (↔)	6
Ma Z, 2014 (China)[24]	Cochrane CRCT (Issue 12, 2012), PubMed, EMBASE, ISI WK, CNKI, CBML, CSJFD and Wanfang Database	CC (6)	2004-2013	60793	5993	Yes (q < 0.001, I^2 = 97%)	0.56 (0.35-0.90) (↓)	None	None	6
Gynaecologic cancer										
Liu Y, 2014 (China)[25]	PubMed, EMBASE and Cochrane Library (up to 10th February 2014)	RCTs (4), Cohort (5) and CC (5)	2000-2013	928721	12904	Yes (q = 0.016, I^2 = 42.3%)	0.89 (0.78-1.01) (↔)	Endometrial = 0.90 (0.75-1.07) (10) (↔); Ovarian = 0.79 (0.64-0.98) (5) (↓)	RCTs = 0.97 (0.62-1.50) (↔); Cohort = 0.97 (0.87-1.09) (↔); CC = 0.61 (0.40-0.91) (↓)	7
Haematological cancer										
Bonovas S, 2007 (Greece)[26]	MEDLINE (1966 to December 2006) and WS (1970 to December 2006)	RCTs (6), Cohort (1) and CC (7)	1996-2006	412053	5619	Yes (q < 0.001, I^2 = 71%)	0.85 (0.64-1.12) (↔)	None	RCTs = 0.92 (0.72-1.16) (↔); OSs = 0.83 (0.53-1.29) (↔)	5
Yi X, 2014 (China)[27]	PubMed, EMBASE and Cochrane CRCT (January 1966 to July 2013)	RCTs (6), Cohort (4), CC (10)	1996-2012	1139584	15297	Yes (q < 0.001, I^2 = 59%)	0.81 (0.70-0.92) (↓)	None	RCTs = 0.92 (0.77-1.09) (↔); OSs = 0.79 (0.67-0.93) (↓)	4
Kidney cancer										
Zhang X, 2014 (China)[28]	MEDLINE, EMBASE and Cochrane Library (January 1966 to October 2012)	RCTs (2), Cohort (5) and CC (5)	2001- 2012	3143236	2829	Yes (q < 0.001, I^2 = 87.8%)	0.92 (0.71- 1.19) (↔)	LSU = 1.01 (0.83-1.22) (6) (↔)	RCTs = 1.01 (0.57-1.79) (↔); Cohort = 1.07 (0.96-1.20) (↔); CC = 0.74 (0.45-1.23) (↔)	5
Liver cancer										
Pradelli D, 2013 (Italy)[29]	MEDLINE (up to March 2012)	Cohort (3) and CC (2)	NA	NA	2574	Yes (q < 0.001, I^2 = 65%)	0.58 (0.46-0.74) (↓)	LSU = 0.66 (0.55-0.80) (↓)	None	6
Singh S, 2013 (United States)[30]	MEDLINE (1966 to 25th May 2012), EMBASE (1988 to 25th May 2012) and WS (1993 to 25th May 2012)	RCTs (3), Cohort (4) and CC (3)	2005-2012	1459417	4298	Yes (q = 0.08, I^2 = 58%)	0.63 (0.52-0.76) (↓)	None	RCTs = 0.95 (0.62-1.45) (↔); OSs = 0.60 (0.49-0.73) (↓)	8
Zhang H, 2013 (China)[52]	MEDLINE, EMBASE and Cochrane Library (January 1966 to March2013)	Cohort (4) and CC (3)	2005-2013	4725593	9785	Yes (q = 0.004, I^2 = 68.6%)	0.61 (0.49-0.76) (↓)	None	Cohort = 0.62 (0.3-0.89) (↓); CC = 0.63 (0.49-0.82) (↓)	5
Shi M, 2014 (China)[31]	PubMed, BIOSIS Previews, WS, EMBASE, EBSCO and Cochrane Library (from inception to 5th March 2014)	RCT (IPD analysis) (1), Cohort (5) and CC (6)	2005-2013	5640313	35756	Yes (q < 0.001, I^2 = 65%)	0.58 (0.51-0.67) (↓)	HCDS = 0.53 (0.36-0.79) (6) (↓)	RCT = 1.06 (0.66-1.71) (↔); Cohort = 0.51 (0.44-0.58) (↓); CC = 0.63 (0.54-0.73) (↓)	6

Lung cancer									
Deng Z, 2013 (China)[g][32]	MEDLINE, EMBASE and WS (from inception to September 2013)	RCTs (8) Cohort (7) and CC (8)	1998-2012	2795302	38024	OSs = Yes (q < 0.001, I^2 = 94.9%) RCTs = No (q = 0.483, I^2 = 0.0%)	1.03 (0.96-1.11) (↔)	None	RCTs = 0.95 (0.85-1.06) (↔); Cohort = 0.94 (0.82-1.07) (↔); CC = 0.85 (0.62-1.16) (↔)	6
Tan M, 2013 (China)[33]	PubMed (up to March 2012)	RCTs (5), Cohort (7) and CC (7)	1998-2011	5009404	38013	Yes (q < 0.001, I^2 = 93.3%)	0.89 (0.77-1.03) (↔)	LSU = 0.81 (0.42-1.56) (↔)	RCTs = 0.91 (0.76-1.09) (↔); Cohort = 0.94 (0.82-1.07) (↔); CC = 0.82 (0.57-1.16) (↔)	6
Wang J, 2013 (China)[34]	MEDLINE, EMBASE and Cochrane Library (January 1966 to November 2012)	RCTs (5), Cohort (8) and CC (7)	1998-2012	4980009	37560	Yes (q < 0.001, I^2 = 93.6%)	0.89 (0.78-1.02) (↔)	LSU = 0.80 (0.39-1.64) (6) (↔)	RCTs = 0.92 (0.79-1.06) (↔); Cohort = 0.93 (0.82-1.06) (↔); CC = 0.81 (0.57-1.16) (↔)	8
Oesophageal cancer									
Alexandre L, 2012 (United Kingdom)[35]	PubMed, EMBASE and Cochrane CRCT (up to December 2011)	Cohort (3)	2004-2011	35214	9310	No (q = 0.88, I^2 = 0.0%)	0.86 (0.78-0.94) (↓)	In patients with BE = 0.53 (0.36-0.78) (↓)	None	7
Andrici J, 2013 (Australia)[f][51]	MEDLINE, PubMed, EMBASE and Current Contents Connect (up to 29th October 2012)	7 studies	NA	NA	NA	No (q = 0.31, I^2 = 15%)	0.75 (0.67-0.84) (↓)	In patients with BE = 0.56 (0.41-0.76) (↓); LSU = 0.45 (0.31-0.67) (↓)	Prospective = 0.75 (0.67-0.86) (↓); Retrospective = 0.68 (0.54-0.86) (↓)	--
Beales ILP, 2013 (United Kingdom)[36]	The PubMed, EMBASE, Cochrane Library, WS, Wiley Interscience and Google Scholar (up to 1st February 2013)	Cohort (5) and CC (6)	2004-2012	6454353	371203	No (q = 0.42, I^2 = 0.0%)	0.81 (0.75-0.88) (6) (↓)	In patients with BE = 0.56 (0.41-0.76) (4) (↓)	None	6
Singh S, 2013 (United States)[37]	MEDLINE (1980 to 31st Aug 2012), EMBASE (1988 to 31st Aug 2012) and WS (1993 to 31st August 2012)	RCT (*Post hoc* analyses) (1), Cohort (5) and CC (7)	2004-2012	1132969	9285	Yes (q < 0.01, I^2 = 74%)	0.72 (0.60-0.86) (↓)	In patients with BE = 0.59 (0.45-0.78) (5) (↓); LSU = 0.44 (0.24-0.78) (2) (↓)	RCT = 0.98 (0.69-1.40) (↔); Cohort = 0.79 (0.62-1.02) (↔); CC = 0.66 (0.51-0.85) (↓)	8
Pancreatic cancer									
Bonovas S, 2008 (Greece)[38]	MEDLINE, Scopus, Google Scholar, and SCI (up to December 2007) and AACO and AACR conference abstracts	RCTs (3), Cohort (4) and CC (5)	1993-2007	914601	3521	Yes (q < 0.001, I^2 = 86%)	0.88 (0.63-1.23) (↔)	None	RCTs = 0.99 (0.44-2.21) (↔); Cohort = 1.11 (0.95-1.29) (↔); CC = 0.70 (0.37-1.33) (↔)	7
Cui X, 2012 (China)[39]	MEDLINE, BIOSIS Previews and Cochrane Library (from inception to 30th August 2011)	RCTs (3), Cohort (5) and CC (8)	2001-2011	1692863	7807	Yes (q < 0.001, I^2 = 81%)	0.89 (0.74-1.07) (↔)	LSU = 0.97 (0.76-1.23) (5) (↔)	RCTs = 0.99 (0.44-2.21) (↔); Cohort = 1.05 (0.93-1.19) (↔); CC = 0.74 (0.51-1.07) (↔)	8
Prostate cancer									
Bonovas S, 2008 (Greece)[40]	MEDLINE (1966 to November 2007) and WK-SCI Expanded	RCTs (6), Cohort (6) and CC (7)	1993-2007	> 884871	62372	Yes (q < 0.001, I^2 = 98%)	0.95 (0.73-1.23) (↔)	LSU = 0.93 (0.77-1.13) (6) (↔); APC = 0.77 (0.64-0.93) (5) (↓)	RCTs = 1.06 (0.93-1.20) (↔); Cohort = 0.98 (0.89-1.09) (↔); CC = 0.74 (0.46-1.20) (↔)	5

	(1970 to November 2007)									
Bansal D, 2012 (India)[41]	PubMed (up to February 2012)	Cohort (15) and Case-control (12)	1993-2011	1893571	56847	Yes (q < 0.001, I^2 = 82%)	0.93 (0.87-0.99) (↓)	LSU = 0.94 (0.84-1.05) (11) (↔); APC = 0.80 (0.70-0.90) (7) (↓)	Cohort = 0.93 (0.87-1.01) (↔); CC = 0.87 (0.72-1.05) (↔)	7
Zhang Y, 2013 (China)[42]	NA	7 studies	2005-2010	NA	NA	NA	1.19 (1.01-1.40) (↑)	NA	NA	6
Skin cancer (Melanoma)										
Dellavalle R, 2005 (United States)[43]	Cochrane Skin Group Specialised Register (up to February 2003) and CENTRAL (Issue 1, 2005), MEDLINE (up to March 2003), EMBASE (up to September 2003), CANCERLIT (up to October 2002), WS (up to May 2003)	RCTs (7)	1993-1998	31198	126	No (q = 0.23, I^2 = 29%)	0.90 (0.56-1.44) (↔)	Lovastatin = 0.52 (0.27-0.99) (1) (↓)	None	10
Freeman SR, 2006 (United States)[44]	MEDLINE (from January 1966 to March 2003), EMBASE (from January 1980 to September 2003), The Cochrane CRCT (up to March2003), CANCERLIT (from January 1975 to October 2002), and WS - SCI (from January 1970 to May 2003)	RCTs (12)	1993-2003	39426	127	No (q = 0.31, I^2 = 16.6%)	0.87 (0.61-1.23) (↔)	Lovastatin = 0.52 (0.27-0.99) (1) (↓)	None	6
Bonovas S, 2010 (Greece)[45]	MEDLINE, Scopus, Google Scholar and SCI Expanded (up to June 2009)	RCTs (16)	1994-2006	62568	165	No (q = 0.25, I^2 = 22%)	0.92 (0.67-1.26) (↔)	None	None	6
Li X, 2014 (China)[46]	PubMed (up to 2013) anwd WS (1985-2013)	RCTs (17), Cohort (4) and CC (7)	1995-2013	517887	11787	Yes (q < 0.07, I^2 = 33.8%)	0.94 (0.85-1.04) (24) (↔); NMSC = 1.03 (0.90-1.19) (14) (↔)	None	None	4

[a]Publication year; [b]Country of study conducted; [c]Period of literature search; [d]*n* mentioned only where the number of studies differ from studies included; [e]Though many subgroup analyses conducted in individual studies, here we focused mainly on subgroup analysis based on study design due to space constraint; [f]Conference abstracts; [g]Conducted among elderly people. APC: Advanced prostate cancer; BE: Barrett's oesophagus; CBML: China BioMedical Literature; CC: Case-control; CNKI: Chinese National Knowledge Infrastructure; CRCT: Central Register of Controlled Trials; CSJFD: Chinese Scientific Journal Full-text Database; GI: Gastro-intestinal; HCDS: Higher cumulative dosage of statin; I^2: I^2 test value; IPD: Individual patient data; ISI WK: Institute for Scientific Information Web of knowledge (Former term for Web of science); LSU: Long-term statin use; *n*: Number of studies; NA: Not available; NMSC: Non-melanoma skin cancer; Oss: Observational studies; q: Cochran Q test P value; RCTs: Randomized controlled trials; RR: Relative risk; SCI: Science citation index; UGIC: Upper gastrointestinal cancer; WS: Web of science; ↔: Non-significant association; ↓: Statistically significantly decreased risk; ↑: Statistically significantly increased risk.

and 2013 and identified heterogeneity among studies. Except the study by Bardou *et al*[48] (which included only RCTs), remaining all the studies found 8%-12% reduced risk of colorectal cancer among statin users. However, a modest reduction in risk or an effect may be associated with higher doses of statins[18]. Based on the subgroup analyses by two meta-analyses[19,20] published in 2014 (which are full-text and included a maximum number of observational studies), risk reduction was 7%-9% among cohort studies and 8%-16% among case-control studies. Studies included RCTs reported no association between use of statin and risk of colorectal cancer alone for RCTs. One study[50] found there was a 19% reduction in the risk of rectal cancer among statin users. Another study[19] found no association between long-term statin use and risk of colorectal cancer.

Statin use and risk of gastric cancer: Four meta-analyses[21-24] published between 2011 and 2014 to identify the risk of gastric cancer among statin users. Except the study conducted by Shimoyama *et al*[21] (published in 2011, searched only PubMed Central and included only RCTs), remaining all studies suggested that the statin use reduces the risk of gastric cancer by 27%-44%, though they identified the heterogeneity among studies. In subgroup analysis, observational studies were found to identify this reduced risk, but not RCTs.

Statin use and risk of gynecological cancer: A meta-analysis[25] published recently to identify the association between statin use and risk of gynecological cancer. The study included both RCTs and observational studies published between 2000 and 2013 on this association. It didn't find any association between statin use and risk of gynecological cancer. On subgroup analysis, the association remains same for RCTs and cohort studies, but case-control studies alone show 39% decreased risk of gynecological cancer among statin users. On secondary analysis using available studies, there was no association found between statin use and risk of endometrial cancer, but decreased (21%) risk of ovarian cancer.

Statin use and risk of hematological cancer: A meta-analysis published by Bonovas *et al*[26] in 2007 to estimate the association between statin use and risk of hematological cancer by including six RCTs and eight observational studies. No association identified between statin use and risk of hematological cancer. The association found to be same even after subgroup analysis of study design. In 2014, Yi *et al*[27] updated this meta-analysis by including six more observational studies published after Bonovas *et al*[26] meta-analysis and gave contrast results by finding 19% decreased risk of hematological cancer among statin users. On subgroup analysis, this association remains same for observational studies, but not for RCTs.

Statin use and risk of kidney cancer: Only one meta-analysis[28] published to estimate the effect of statin use on kidney cancer by including two RCTs and 10 observational studies published between 2001 and 2012. This study found no association between statin use and risk of kidney cancer with heterogeneity among studies. On subgroup and secondary analysis the association remains same among RCTs, cohort and case control studies and also for long-term statin use.

Statin use and risk of liver cancer: Four meta-analyses[29-31,52] published in 2013 and 2014 regarding statin use and risk of liver cancer. All studies included observational studies but different in number and only two studies[30,31] included RCTs. All studies found significant heterogeneity among the studies included and shown 37%-42% decreased risk of liver cancer among statin users. This chemoprotective association is more pronounced in the Asian population, where viral hepatitis is the most important risk factor for liver cancer[30]. On subgroup analysis by study design, the risk remains similar (37%-49% decreased risk) among observational studies but not for RCTs.

Statin use and risk of lung cancer: Three meta-analyses[32-34] published in 2013 including almost similar number of RCTs and observational studies to identify the association between statin use and risk of lung cancer. All the three found significant heterogeneity among studies and no association between statin use and risk of lung cancer. On subgroup and secondary analysis the association remains same among RCTs, cohort and case control studies and also for long-term statin use.

Statin use and risk of oesophageal cancer: Four meta-analyses (3 full-text[35-37] and 1 conference abstract[51]) published in 2012 and 2013 on the association. Only observational studies contributed to the analysis in all studies except the Singh *et al*[37] study also included one RCT (*post hoc* analysis). Results were consistent among all studies with 14%-28% decreased risk of esophageal cancer among statin users. On subgroup analysis, only case-control studies found with 44% decreased risk of esophageal cancer but not cohort studies and RCT. By using available studies, all the meta-analyses conducted secondary analysis on the risk of Barrett's esophagus among statin users and found 41%-47% decreased risk. Two studies[37,51] also identified 55% decreased risk of esophageal cancer among long-term statin users.

Statin use and risk of pancreatic cancer: A meta-analysis published by Bonovas *et al*[38] in 2008 to estimate the co-relation between statin use and risk of pancreatic cancer by including three RCTs and nine observational studies. There was no relationship between statin use and risk of pancreatic cancer with heterogeneity among studies. The association found to be same even after subgroup analysis of study design. In

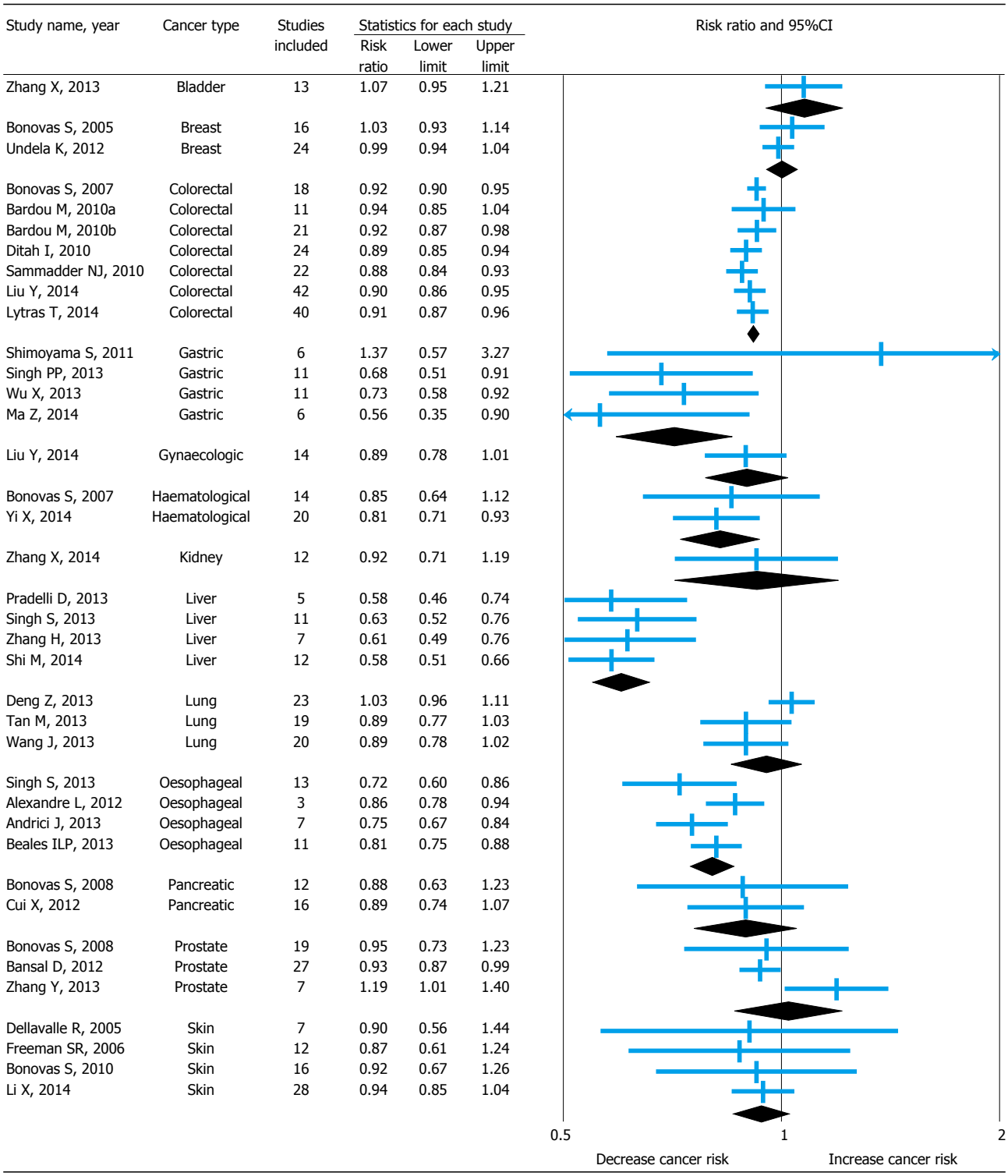

Figure 2 Forest plot of pooled relative risk with 95%CI of primary outcome from all included studies.

2012, Cui *et al*[39] updated this meta-analysis by including four more observational studies published after previous meta-analysis. This study also gives an almost similar conclusion. Additionally, this updated meta-analysis reported no association between long-term statin use and risk of pancreatic cancer.

Statin use and risk of prostate cancer: A meta-analysis published by Bonovas *et al*[40] in 2008 to estimate the association between statin use and risk of prostate cancer by including six RCTs and 13 observational studies. No association identified between statin use and risk of prostate cancer. The association remains same even after subgroup analysis of study design and also for long-term statin use. In 2012, Bansal *et al*[41] updated this meta-analysis by including

14 more observational studies published after Bonovas *et al*[40] meta-analysis and gave contrast results by finding small (7%) but significant decreased risk of prostate cancer among statin users. But in a subgroup analysis of study design, no association observed between cohort and case-control studies alone. Both the studies also tried to identify the risk of advanced prostate cancer among statin users and found 23%-30% decreased risk. On the other hand, a study conducted by Zhang *et al*[42] in 2013 by including only seven studies published after Bonovas *et al*[40] meta-analysis and found a 19% increased risk of prostate cancer among statin users.

Statin use and risk of skin cancer (melanoma): Four meta-analyses[43-46] conducted on this association, including one Cochrane systematic review published in 2005. All studies included only RCTs except the study by Li *et al*[46] also included 11 observational studies. All the studies found no association between statin use and risk of melanoma and also the association found to be same for non-melanoma skin cancer by Li *et al*[46] study. Interestingly, one RCT[43,44] suggested that the lovastatin can decrease the risk of melanoma by 48%.

DISCUSSION

Meta-analytic evidence of association between statin use and risk of site specific cancers was piling since last decade. This overview of 38 meta-analyses covered 13 site specific cancers revealed that the statin use may reduce the risk of certain types of cancers like colorectal (8%-12%), gastric (27%-44%), hematological (19%), liver (37%-42%), esophageal (14%-28%), ovarian (21%) and prostate (7%). On the other hand, some evidence also suggests that there is no association between statin use and risk of bladder, breast, endometrial, kidney, lung, pancreatic and skin cancers. On secondary analysis, few meta-analyses suggested that statin use can also reduce the risk of rectal cancer (19%), advanced prostate cancer (23%-30%), Barret's esophagus (47%) and also reduce the risk of recurrence of breast cancer (47%).

In this review, we tried to identify the change in the risk of cancer among different types, doses and duration of statin use with the available information. Some of the meta-analyses categorized statins according to whether they were lipophilic (simvastatin, lovastatin, fluvastatin, and atorvastin) or hydrophilic (pravastatin and rosuvastatin) and conducted subgroup analysis. The studies didn't find any statistically significant association between lipophilic or hydrophilic statins and risk of colorectal cancer[18,20], haematological cancer[26], lung cancer[33], pancreatic cancer[38,39] and skin cancer[45]. In contrast, one meta-analysis showed an association between lipophilic statin use and colorectal cancer risk (RR = 0.88, 95%CI: 0.85-0.93) and a null association between hydrophilic statin use and colorectal cancer risk (RR = 0.88, 95%CI: 0.76-1.02)[19]; and another meta-analysis found a significant decrease in liver cancer risk for both lipophilic statins (RR = 0.57, 95%CI: 0.50-0.65) and hydrophilic statins (RR = 0.59, 95%CI: 0.41-0.84). The same study also revealed that higher cumulative dose of statin use, defined as statin use over 180 cumulative defined daily doses or 0.5 years (cumulative duration), showed a trend towards more risk reduction of liver cancer (RR = 0.53, 95%CI: 0.36-0.79)[31]. Some of the studies also conducted secondary analysis to identify the association between long-term statin use (usually $\geqslant$ 5 years) and risk of cancer, and identified reduced risk of oesophageal cancer (55%)[37,51], ovarian cancer (52%)[25], but not for bladder[15], breast[17], colorectal[19], endometrial[25], kidney[28], Lung[33], pancreatic[39] and prostate cancers[40,41].

Recently published overview to identify the role of statin use in cancer prevention and modifying cancer-related outcomes also come out with similar conclusions[53]. However, this study suffers with some limitations in methodology and not covered few cancer types. Moreover, a recent meta-analysis of long-term efficacy and safety of statin treatment confirmed that statin treatment did not increase the incidence of cancer and deaths from cancers[54]. Despite the examinations on statins consequences for tumor cells have proceeded from the mid 1990s, the exact mechanism that could clarify the anticancer effect of statins still unclear. Different types, dose and route of administration of statins being used, type/stage of tumors and time of exposure to statins may impact the mechanisms that lead to cell-cycle arrest and induction of apoptosis. One review observed that statins may decrease the cholesterol levels, leads to further changes in cell flagging[9].

According to recent laboratory studies, statins seems to have chemo-preventive affect against cancer at various sites. Evidence suggests that statins are selectively localized to the liver, and only < 5% dose reaches the systemic circulation. This low systemic availability uncertians chemo-protective nature of statin[15,16].

We have made efforts to minimize the risk of bias in every step of this overview. However, this overview has few limitations. First, glitches in the nature of the primary data included in 38 meta-analyses; RCTs have not been adequately powered to detect potentially small differences in cancer risk due to the small number of cancer cases as it was not the primary outcome of these trials and the observational data may have suffered some common limitations of pharmacoepidemiological studies. Secondly, as most of the findings come from observational studies, there may be a chance of presenting "healthy-user bias" for part of the beneficial effects of statins.

Statins are a promising group of drugs in cancer treatment because of their ability to reduce both cholesterol and isoprenoid levels. Meta-analyses of variable quality showed that the statins may have a potential role in cancer chemoprevention and reduce the risk of certain site specific cancers, but not all. Until a definitive benefit is demonstrated by randomized controlled trials, statins cannot be recommended either for cancer prevention or

for modifying cancer-related outcomes.

COMMENTS

Background

In recent years, emerging experimental evidence suggests that statins may have a potential role in cancer chemoprevention. However, a large number of randomized controlled trials and observational studies published to examine the association between statin use and risk of site specific cancers were given conflicting results.

Research frontiers

The objective of this overview is to summarize and critically appraise the evidence of relevant meta-analyses and present a comprehensive evaluation of the association between statin use and risk of site specific cancers.

Innovations and breakthroughs

This overview of 38 meta-analyses covered 13 site specific cancers revealed that the statin use may reduce the risk of certain types of cancers like colorectal (8%-12%), gastric (27%-44%), hematological (19%), liver (37%-42%), esophageal (14%-28%), ovarian (21%) and prostate cancer (7%). On the other hand, some evidence also suggests that there is no association between statin use and risk of bladder, breast, endometrial, kidney, lung, pancreatic and skin cancers. On secondary analysis, few meta-analyses suggested that statin use can also reduce the risk of rectal cancer (19%), advanced prostate cancer (23%-30%), Barret's esophagus (47%) and also reduce the risk of recurrence of breast cancer (47%).

Applications

Statins are a promising group of drugs in cancer treatment because of their ability to reduce both cholesterol and isoprenoid levels. Meta-analyses of variable quality showed that the statins may have a potential role in cancer chemoprevention and reduce the risk of certain site specific cancers, but not all. Until a definitive benefit is demonstrated by randomized controlled trials, statins cannot be recommended either for cancer prevention or for modifying cancer-related outcomes.

Peer-review

These authors made a comprehensive review of meta-analyses on statin use and risk of cancer. They also made tables and figures, which make readers easy to catch the study methods, strength and results from each meta-analysis. It will be informative for readers interested in this topic.

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META-ANALYSIS

Is there a difference between 19G core biopsy needle and 22G core biopsy needle in diagnosing the correct etiology? - A meta-analysis and systematic review

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Abstract

AIM

To compare the accuracy of endoscopic ultrasonography (EUS) 19G core biopsies and 22G core biopsies in diagnosing the correct etiology for a solid mass.

METHODS

Articles were searched in Medline, PubMed, and Ovid journals. Pooling was conducted by both fixed and random effects models.

RESULTS

Initial search identified 4460 reference articles for 19G and 22G, of these 670 relevant articles were selected and reviewed. Data was extracted from 6 studies for 19G (n = 289) and 16 studies for 22G (n = 592) which met the inclusion criteria. EUS 19G core biopsies had a pooled sensitivity of 91.6% (95%CI: 87.1-95.0) and pooled specificity of 95.9% (95%CI: 88.6-99.2), whereas EUS 22G had a pooled sensitivity of 83.3% (95%CI: 79.7-86.6) and pooled specificity of 64.3% (95%CI: 54.7-73.1). The positive likelihood ratio of EUS 19G core biopsies was 9.08 (95%CI: 1.12-73.66) and EUS 22G core biopsies was 1.99 (95%CI: 1.09-3.66).

The negative likelihood ratio of EUS 19G core biopsies was 0.12 (95%CI: 0.07-0.24) and EUS 22G core biopsies was 0.25 (95%CI: 0.14-0.41). The diagnostic odds ratio was 84.74 (95%CI: 18.31-392.26) for 19G core biopsies and 10.55 (95% CI: 3.29-33.87) for 22G needles.

CONCLUSION

EUS 19G core biopsies have an excellent diagnostic value and seem to be better than EUS 22G biopsies in detecting the correct etiology for a solid mass.

Key words: Endoscopic ultrasound guided fine needle aspiration; Solid mass lesions; Endoscopic ultrasound; Pancreatic mass; Pancreatic cytology; Core biopsies; 19G procore needle; Meta-analysis; Systematic review; 22G procore needle

Core tip: Management of pancreatic solid mass lesions relies greatly on accuracy of diagnosis of these lesions. Procore fine needle biopsy needles have been found to have a diagnostic accuracy comparable to, if not better than the standard needles in diagnosing the intestinal and extra-intestinal mass lesions. Amongst the Procore needles, the 19G and 22G Procore needles have both been shown to obtain good quality core tissue samples but both have unique characteristics of their own. This meta-analysis compares the feasibility and accuracy of 19G and 22G Procore needles in determining the diagnosis of solid mass lesions.

Kandula M, Bechtold ML, Verma K, Aulakh BS, Taneja D, Puli SR. Is there a difference between 19G core biopsy needle and 22G core biopsy needle in diagnosing the correct etiology? - A meta-analysis and systematic review. *World J Meta-Anal* 2017; 5(2): 54-62 Available from: URL: http://www.wjgnet.com/2308-3840/full/v5/i2/54.htm DOI: http://dx.doi.org/10.13105/wjma.v5.i2.54

INTRODUCTION

Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) is the recommended procedure for the sampling of solid mass lesions within the gastrointestinal tract and extra-intestinal organs, especially pancreatic mass lesions[1-4]. It has been reported from previous studies that EUS-FNA has high diagnostic accuracy (78%-95%)[5,6], sensitivity (64%-95%) and specificity (75%-100%)[6,7] for cytological diagnosis. To make an accurate diagnosis though, histological studies are essential in addition to cytological studies. Although cytological study can detect cellular findings like anisonucleosis and nuclear enlargement that suggest malignancy, inflammation in the tissue causes regenerative and reactive changes that make it hard to distinguish it from well differentiated neoplasia based on cytological study alone. Moreover, there are certain neoplasms like lymphomas and stromal tumors that would require tissue architecture and cell morphology for accurate pathological assessment and this is not possible without obtaining histological samples[8-10]. Other factors that influence the diagnostic accuracy of EUS-FNA include the availability of an onsite cytopathologist to render a diagnosis, experience of the endosonographer, location of the lesion, the method of preparation and the type and size of the needle used to obtain the sample[11-14].

Currently, there are three needle sizes (19G, 22G and 25G) that are commercially available, of which 22G is probably the most widely used. Theoretically, it is difficult to obtain histological samples with smaller needles. Hence, the trucut biopsy needle (Cook Medical, Bloomington, IN, United States) was developed with 19G needles[15]. EUS-trucut needle biopsy (EUS-TNB) technique was more accurate than FNA for neoplasms requiring histological analysis, but the 19G caliber posed certain difficulties. It was difficult to maneuver the needle owing to its rigidity, and the mechanical friction of the firing mechanism limited its use in evaluating pancreatic head masses and duodenal lesions where a transduodenal approach was required[8].

The Procore EUS-fine needle biopsy (EUS-FNB) needle, a newer generation, with reverse beveled technology was developed to improve quality of core tissue samples for histologic analysis. These needles (Procore, Cook Medical, Winston-Salem, NC, United States) available in different sizes were shown to have promising results. The histologic samples obtained by the 19G procore needle had a diagnostic accuracy of more than 90% as shown in a large prospective study done in Europe[16]. There were still some technical problems encountered with the 19G Procore when performing transduodenal passes. Hence the same FNB device was developed in the 22G caliber. In several other studies, the 22G Procore needle was found to require lower number of passes to achieve the same contributive sample rate as the FNA needles[17-19].

There have been a lot of studies comparing the Procore FNB needles with standard FNA and TNB needles. These studies have established that the feasibility, yield and accuracy of the Procore needles in diagnosing intestinal, extra-intestinal mass lesions as well as peri-intestinal lymphadenopathy is comparable, if not better than the standard needles. We conducted a meta-analysis from the relevant studies done so far, and reviewed the literature to determine if there was a difference in the diagnostic accuracy of 19G Procore *vs* 22G Procore biopsy needles in the evaluation of solid mass lesions.

MATERIALS AND METHODS

Study selection criteria

Only EUS 19G and 22G core biopsy studies on solid mass lesions confirmed by surgery or appropriate follow-up were selected. Only studies from which a 2 × 2 table

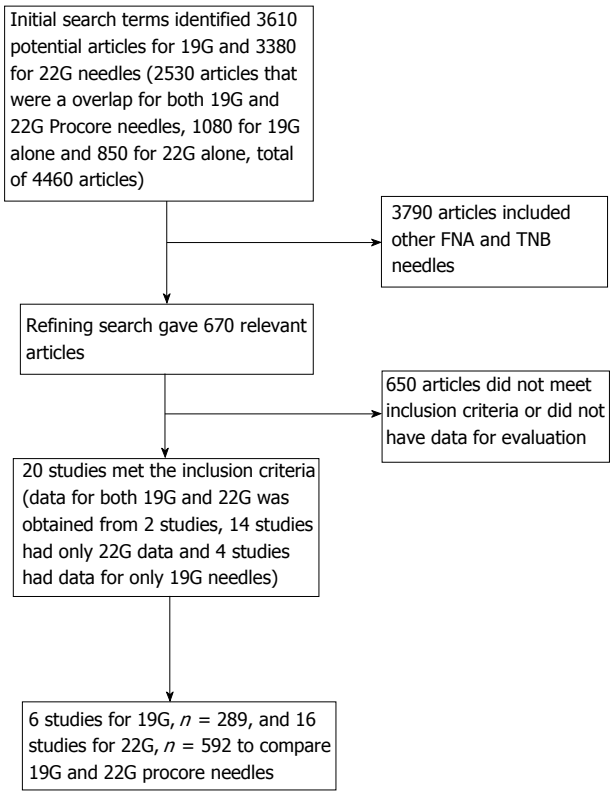

Figure 1 Flow chart showing search results and study selection. FNA: Fine needle aspiration; TNB: Trucut needle biopsy.

could be constructed for true positive, false negative, false positive and true negative values were included.

Data collection and extraction

Articles were searched in MEDLINE, PubMed, Ovid journals, Cumulative Index for Nursing and Allied Health Literature, ACP journal club, DARE, International Pharmaceutical Abstracts, old MEDLINE, MEDLINE nonindexed citations, OVID Healthstar, and Cochrane Controlled Trials Registry. Search included articles of all languages from the year 1946 to present. The search terms used were EUS-FNA, ultrasound, endosonography, solid mass lesions, pancreatic mass, pancreatic cytology, core biopsies, 19G procore needle, 22G needle, surgery, histopathology, sensitivity, specificity, positive predictive value, and negative predictive value. Data included in the meta-analysis was obtained by intention to treat analysis of the original data. Two plus two tables were constructed with the data extracted from each study. Two authors independently searched and extracted the data into an abstraction form. No additional data was obtained from the authors. Any differences were resolved by mutual agreement.

Quality of studies

Clinical trial with a control arm can be assessed for the quality of the study. A number of criteria have been used to assess this quality of a study (*e.g.,* randomization, selection bias of the arms in the study, concealment of allocation, and blinding of outcome)[20,21]. There is no consensus on how to assess studies without a control arm. Hence, these criteria do not apply to studies without a control arm[21]. Therefore, for this meta-analysis and systematic review, studies were selected based on completeness of data and inclusion criteria.

Statistical analysis

Meta-analysis for the accuracy of EUS guided 19G core biopsies and 22G core biopsies in diagnosing solid mass lesions was performed by calculating pooled estimates of sensitivity, specificity, likelihood ratios, and diagnostic odds ratios. Pooling was conducted using both Mantel-Haenszel Method (fixed effects model) and DerSimonian Laird Method (random effects model). The confidence intervals were calculated using the F Distribution Method[22]. Forrest plots were drawn to show the point estimates in each study in relation to the summary pooled estimate. The width of the point estimates in the Forrest plots indicates the assigned weight to that study. For 0 value cells, a 0.5 was added as described by Cox[23]. The heterogeneity of the sensitivities and specificities were tested by applying the likelihood ratio test[24]. The heterogeneity of likelihood ratios and diagnostic odds ratios were tested using Cochran's *Q* test based upon inverse variance weights[25]. Heterogeneity among studies was also tested by using summary receiver operating characteristic (SROC) curves. SROC curves were used to calculate the area under the curve (AUC). The effect of publication and selection bias on the summary estimates was tested by Egger bias indicator[26] and Begg-Mazumdar bias indicator[27]. Also, funnel plots were constructed to evaluate potential publication bias using the standard error and diagnostic odds ratio[28,29].

RESULTS

Initial search identified 3610 reference articles for 19G core biopsies and 3380 reference articles for 22G core biopsies (4460 total as there was an overlap of the articles), of these, 670 relevant articles were selected and reviewed. Six studies (n = 289) for 19G core biopsies and 16 studies (n = 592) for 22G core biopsies which met the inclusion criteria were included in this analysis. Figure 1 shows the search results and Table 1 shows the characteristics for EUS studies included in this meta-analysis. Of the 20 studies included in this analysis, 12 were published as full-text articles and 8 were abstracts in peer reviewed journals. The pooled estimates given are estimates calculated by the fixed and random effects model.

Accuracy of EUS guided 19G core biopsies to diagnose solid mass lesions

Pooled sensitivity of EUS 19G core biopsies in diagnosing solid mass lesions was 91.6% (95%CI: 87.1%-95.0%). 19G Procore needle had a pooled specificity of 95.9% (95%CI: 88.6%-99.2%). Forrest plot in Figure 2 shows

Table 1 Basic characteristics of the studies

Ref.	Type of article/study	Needle type	Number of biopsies	Type of lesion	Accurate diagnoses (TP and TN)
Irions *et al*[40], 2011	Abstract	22G	6	Pancreatic adenocarcinoma, esophageal SCC	4
Barresi *et al*[44], 2014	Full article	22G	60	Pancreatic lesions	36
Alatawi *et al*[17], 2015	Full article	22G	50	Pancreatic lesions	48
Vanbiervliet *et al*[33], 2014	Full article	22G	80	Adenocarcinoma, metastatic lung cancer	67
Ganc *et al*[19], 2014	Full article	22G	15	Pancreatic mass lesions	8
Ramay *et al*[48], 2013	Abstract	22G	24	Perigastric, peripancreatic subcarinal, mediastinal lymph nodes	24
Larghi *et al*[43], 2011	Full article	22G	61	Adenocarcinoma, neuroendocrine tumors, lymphoma	54
Strand *et al*[34], 2014	Full article	22G	28	Solid pancreatic neoplasms	7
Bang *et al*[32], 2012	Full article	22G	28	Pancreatic masses	25
Ganc *et al*[19], 2014	Abstract	22G	30	Pancreatic masses	28
Krishnamurthy *et al*[45], 2013	Abstract	22G	37	Adenocarcinoma, neuroendocrine tumors	24
Komanduri *et al*	Abstract	22G	10	Pancreatic lesions	10
Kim *et al*	Full article	22G	12	GI stromal tumors, pancreatic masses, lymphoma	9
Ramay *et al*[48], 2013	Abstract	22G	40	Pancreatic lesions	40
Park *et al*[47], 2012	Abstract	22G	43	Solid pancreatic lesions	32
Fabbri *et al*[46], 2015	Full article	22G	68	Solid pancreatic lesions, pancreatic cystic lesions	56
Petrone *et al*[39], 2012	Abstract	19G	49	Pancreatic mass, submucosal lesions, mediastinal mass	46
Iglesias-García *et al*[41], 2014	Full article	19G	114	Pancreatic tumors, mediastinal lymphadenopathy, intraabdominal masses	106
Komanduri *et al*	Abstract	19G	10	Pancreatic lesions	10
Lovacheva *et al*[35], 2013	Abstract	19G	23	Mediastinal lymph nodes	19
Iglesias-García *et al*[41], 2014	Full article	19G	87	Pancreatic tumors, mediastinal lymphadenopathy, intraabdominal masses	83
Irions *et al*[40], 2011	Abstract	19G	6	Pancreatic adenocarcinoma, GIST, benign lymph nodes	4

TP: True positives; TN: True negatives; SCC: Squamous cell carcinoma; GI: Gastrointestinal; GIST: Gastrointestinal stromal tumors.

the sensitivity and specificity of 19G core biopsies to diagnose solid mass lesions. The positive likelihood ratio was 9.07 (95%CI: 1.12-73.65) and negative likelihood ratio was 0.12 (95%CI: 0.06-0.24). The diagnostic odds ratio, the odds of having the correct histologic etiology of a mass in positive as compared to negative EUS-FNB studies was 84.7 (95%CI: 18.3-392.2). All the pooled estimates calculated by fixed and random effect models were similar. SROC curves showed an area under the curve of 0.95. Figure 3 shows the SROC curves for EUS 19G core biopsies to diagnose solid mass lesions. The p for chi-squared heterogeneity for all the pooled accuracy estimates was > 0.10.

Accuracy of EUS 22G core biopsies to diagnose solid mass lesions

Pooled sensitivity of EUS 22G core biopsies in diagnosing solid mass lesions was 83.3% (95%CI: 79.7%-86.6%). 22G Procore needle had a pooled specificity of 64.3% (95%CI: 54.7%-73.1%). The positive likelihood ratio was 1.99 (95%CI: 1.09%-3.66%) and negative likelihood ratio was 0.25 (95%CI: 0.14%-0.41%). The diagnostic odds ratio, the odds of having the correct histologic etiology of a mass in positive as compared to negative EUS-FNB studies was 10.55 (95%CI: 3.28%-33.87%). All the pooled estimates calculated by fixed and random effect models were similar. SROC curves showed an area under the curve of 0.95. The *P* for χ^2 heterogeneity for all the pooled accuracy estimates was > 0.10.

Bias estimates

The publication bias calculated by Begg-Mazumdar bias indicator gave a Kendall's tau *b* value of -0.2, *P* = 0.21 and Egger bias indicator gave a value of -0.56 (95%CI: -2.28 to 1.16, *P* = 0.50). Funnel plots in Figure 4 show no effect of publication bias on the pooled estimates calculated for 19G or 22G core biopsies.

DISCUSSION

The Procore needles with reverse bevel technology for EUS-FNB are a recent development in the EUS-platform for maximizing acquisition of core tissue specimens for histopathological analysis. The 19G Procore needle was initially developed to overcome the limitations encountered with EUS-TNB, like rigidity of the 19G caliber needle as well as the mechanical friction of the firing mechanism produced by the torqued endoscope[8]. The same device was developed in the 22G platform because of the difficulties encountered during transduodenal passes with the 19G needle (the needle had to be advanced out of the scope in the stomach before reaching the duodenum)[30]. Obtaining core biopsy specimens would allow for detailed analysis of preserved tissue architecture and also provide the opportunity to immunostain the tissue, thus increasing diagnostic accuracy. It has also been shown to be not inferior to rapid onsite cytological examination, which is known to be a significant factor in decreasing the number of inadequate diagnoses, thus also playing a role in economical cost saving[31,32]. The 19G and 22G Procore needles have been studied significantly as to their feasibility and yield in the sampling of solid pancreatic lesions and all these studies have shown that they are comparable to the standard

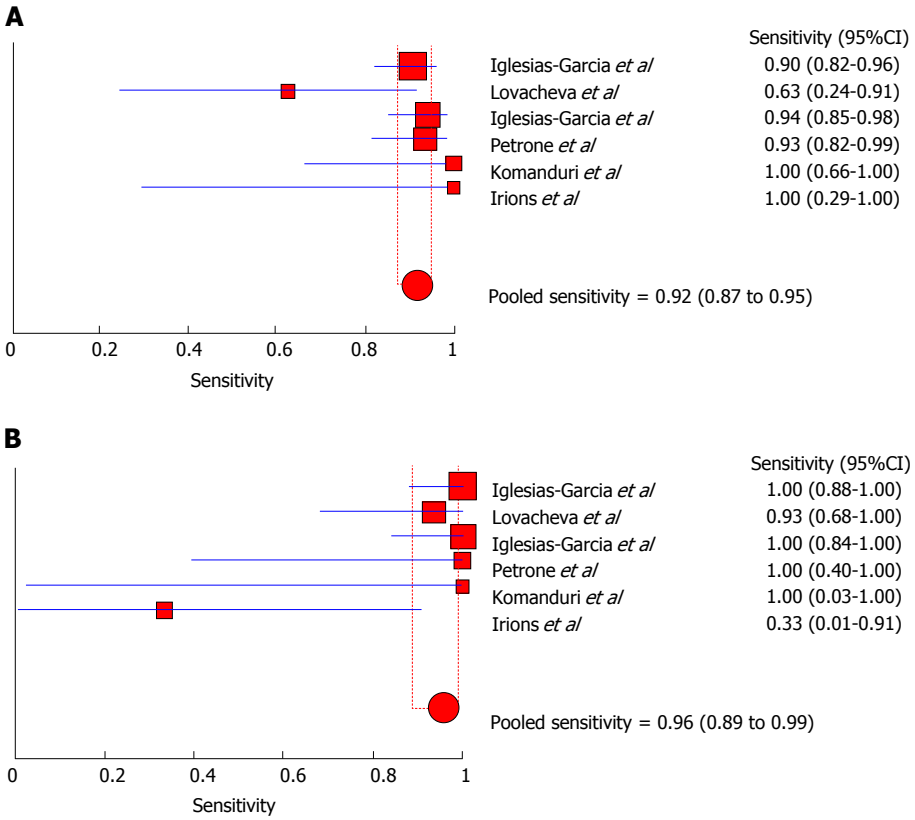

Figure 2 Forest plot showing sensitivity (A) and specificity (B) of 19G Procore needle.

FNA needles[31-34]. Our meta-analysis showed that of these two Procore needles, the 19G needle is superior to the 22G needle in core histology yield and diagnostic accuracy.

In the study by Iglesias-Garcia *et al*[16], EUS-FNB by 19G Procore needle of 114 lesions were evaluated for sample quality for histological evaluation, and over-all diagnostic accuracy compared with a standard diagnosis. It was found that the 19G Procore needle offered the possibility of obtaining a core sample for histological evaluation with a diagnostic accuracy of over 85%. It reached an accuracy of 92.9% for the detection of malignancy[16]. Lovacheva *et al*[35] confirmed that 19G Procore needle had a high diagnostic yield when it came to malignancies and histological diagnosis, although there was no significant difference to FNA for cytology in benign diseases. This is much better than the EUS-biopsy with the quick-core needle where the overall accuracy ranged between 61% and 84%[36-38]. Although transduodenal passes were difficult with the 19G Procore needle, it was still better than the Quick-Core needle where the sample quality was significantly affected for lesions that needed to be punctured from the duodenum. Petrone *et al*[39] had even better results where the needle provided adequate histological sample in 98% of the cases with an overall accuracy reaching 94% with regard to the final gold standard diagnosis. Irions *et al*[40] studied both the 19G and 22G Procore needles and determined that samples could be obtained safely and with high yield using either of them. Core samples in this study were obtained with more than one pass in 80% of the lesions. In another recent study by Iglesias-García *et al*[41] with the 19G Procore needle, there were no complications related to the procedure in their 87 patients and it was determined to be as safe as the standard FNA needle. Moreover, this study showed that a single pass of the needle obtained the same results as multiple passes in previous studies by Yasuda *et al*[42] and Larghi *et al*[43] done with the standard needle, as well as other recent studies with the Quick-Core needle. This may be because of the reverse bevel technology in the Procore needle that cuts the tissue in to and fro movements during a single needle pass and thus obtains an adequate core tissue specimen.

Bang *et al*[32] did a study in 2012 to compare 22G FNA and FNB needles and found no significant difference in the yield or quality of the histologic specimens in these groups. They did not find any difference in the median number of passes required to establish an on-site diagnosis. The rate of optimal specimens in this study was 80% as compared to 92.9% reported with the 19G needle in the Iglesias-Garcia study. Over-all, the quality of specimens obtained by the small caliber 22G needle was unsatisfactory for histologic analysis, though this could also be because there were passes that were performed for onsite analysis before specimens were collected for cell block. On the safety front, the 22G FNB needle was similar to the FNA needle and comparable to the 19G needle, with only a couple of minor complications[32]. Barresi *et al*[44] followed this up and studied the feasibility

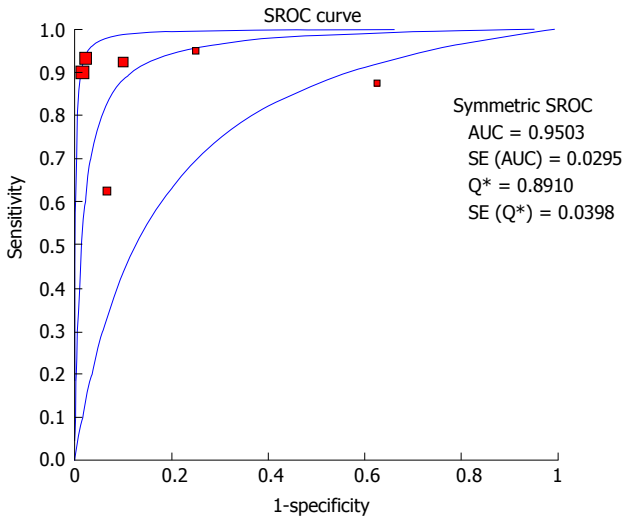

Figure 3 Summary receiver operating characteristic curves for endoscopic ultrasonography 19G core biopsies to diagnose solid lesions. SROC: Summary receiver operating characteristic; AUC: Area under the curve.

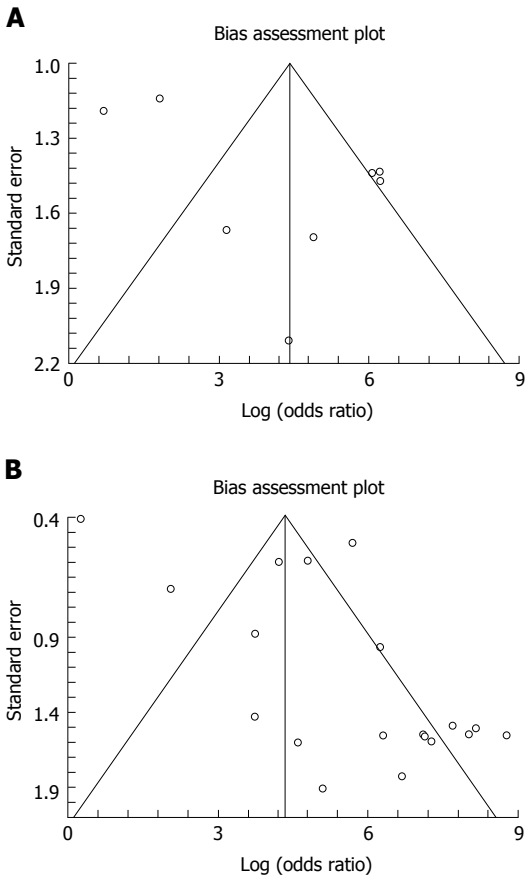

Figure 4 Bias assessment plot for 19G (A) and 22G (B) Procore needle.

and diagnostic yield of 22G Procore needle for EUS-FNA and biopsy of pancreatic cystic lesions. In a subgroup analysis of malignant lesions and lesions with a solid component, the adequacy for cyto-histological diagnosis of the samples obtained by 22G FNB needle was found to be 100% and 94.4% respectively, which is superior to conventional standard FNA needles[44]. Some studies looked at different aspects of FNB needle sampling, like stromal fragments in the sample allowing for a more precise histologic diagnosis, or FNB needles making the procedure quicker, and lower number of needle passes required with Procore needles when compared to standard needles[19,45-48]. There were several other studies done previously that showed that there was no improvement in diagnostic yield with FNB as compared to FNA needles. Strand et al did a study that did not show a significant advantage of using FNB over FNA in terms of being a core biopsy needle although it was comparable in terms of providing material for cytology[34]. However, this was a small study and there were also concerns about technical quality of the procedures. Vanbiervliet *et al*[33] compared the standard and core 22G needle and showed that the diagnostic accuracy was comparable for solid pancreatic lesions although each patient had two passes with the standard needle and one pass with the core needle, thus biasing the study. Alatawi *et al*[17] compared 22G FNA and FNB needles in 100 patients and concluded that despite similar diagnostic accuracy, FNB needles required lower number of needle passes and yielded samples of higher histological quality, thus mitigating previous studies on the limited contribution of FNB needles in pancreatic cancer work up.

From the above discussion, it is clear that Procore needles, both 19 gauge and 22 gauge, with reverse bevel technology has been very promising in obtaining samples for the diagnosis of solid mass lesions. In this pooled analysis, it has been shown that the 19G procore needle is better at obtaining samples for diagnosing solid mass lesions than 22G Procore needle. The sensitivity of the 19G needle is 91.6% as compared to 83.3% for the 22G. The difference in specificity is even higher with the 19G having 95.9% specificity while the 22G has a specificity of only 64.3% when it came to the adequacy of specimens and diagnostic accuracy with that histologic sample for solid mass lesions. Further studies are required to determine the factors that may have influenced the relatively low specificity of 22G Procore needle seen in this pooled analysis, which may include the differences in sample yield and method of obtaining the sample. Diagnostic odds ratio is defined as the odds of having the correct histologic etiology of the mass in positive as compared to negative EUS-FNB studies. To diagnose the histologic etiology of a solid mass lesion in the intestinal and extra-intestinal organs, the EUS-FNB using the 19G Procore needle had a very high diagnostic odds ratio (approximately 84 times) as compared to the 22G Procore needle (approximately 10 times). For example, if a core biopsy of solid pancreatic mass is done using a 19G Procore needle, the odds of having the correct histologic diagnosis is around 84 times as compared to only 10 times with the 22G needle. The positive likelihood ratio of a test is a gauge of how well the test identifies a disease state. Higher the positive likelihood ratio, the better the test performs in identifying the true disease status. On the other hand, a negative

likelihood ratio of a test is a gauge of how well the test performs in excluding a disease state. The lower the negative likelihood ratio, the better the test performs in excluding a disease. For diagnosing a solid mass lesion, EUS-FNB using a 19G Procore needle had a higher positive likelihood ratio than the 22G needle but the negative likelihood ratio was low for both of them. This indicates that the 19G Procore needle performs better in ruling in a diagnosis than the 22G needle though both of them fared fairly low in excluding a diagnosis.

In our study, the 19G Procore was found to be superior in almost every aspect. One limitation that this needle had was that the authors in these studies notably reported failures when it came to transduodenal passes with the 19G Procore needle. The FNB needle had to be advanced out of the echoendoscope while in the stomach before the scope could be passed into the duodenum[16,41]. This difficulty was not present with the 22G Procore needle where the FNB needle exited the sheath with relative ease in all the patients in the study by Bang *et al*[32]. Another limitation is that there are several factors influencing the diagnostic accuracy that include experience and expertise of the endosonographers and pathologists, as well as size and location of the lesion. Some of the studies had on-site pathologists and others did not and this may affect the difference in the diagnostic accuracy between the 19G and 22G core biopsies depending on whether they used them or not. When comparing diagnostic yield based on number of needle passes, comparing FNA and FNB needles in the same patient, although makes a study more statistically significant, would be difficult as subsequent needle passes would follow the same pathway as the first one and some studies[17,18] compared them in different patients to overcome this bias. The number of studies from which data was extracted was not equal for 19G (6 studies) and 22G (16 studies) as there were not as many studies done on the 19G yet, with only two studies that directly compared them, and this may have affected the results.

Heterogeneity among different studies was determined by drawing SROC curves and finding the AUC, since different studies might use slightly different criteria for staging. An AUC of 1 for any test indicates that the test is excellent. SROC curves for 19G Procore needle showed that the value for AUC was very close to 1, indicating that this needle has an excellent diagnostic value in detecting the correct histologic etiology of a solid mass lesion.

Studies with statistically significant results tend to be published and cited. Smaller studies may show larger treatment effects due to fewer case-mix differences (*e.g.,* patients with only early or late disease) than larger trials. This bias can be estimated by bias indicators and construction of funnel plots. This publication and selection bias may affect the summary estimates. Also, bias among studies can affect the shape of the funnel plot. In this meta-analysis and systematic review, bias calculations using Egger bias indicator[26] and Begg-Mazumdar bias indicator[27] showed no statistically significant bias. Furthermore, analysis using funnel plots showed no significant publication among the studies included in this analysis.

In conclusion, EUS 19G core biopsies have an excellent diagnostic value and seem to be superior to the EUS 22G biopsies in detecting the correct etiology for a solid mass lesion. The specificity and sensitivity are both higher for the 19G Procore needle when compared to the 22G Procore needle. Though the 22G may be easier to maneuver for lesions requiring transduodenal passes, the overall diagnostic accuracy is greater for 19G. In conclusion, 19G needles may be strongly considered over 22G needles when evaluating solid mass lesions. Further randomized controlled trials comparing the two needles directly are required for more definitive conclusions.

COMMENTS

Background

Procore fine needle biopsy needles have been found to have a diagnostic accuracy comparable to, if not better than the standard needles in diagnosing intestinal and extra-intestinal mass lesions. This is a meta-analysis and systematic review comparing the 19G and 22G core biopsy needles in making the correct etiologic diagnosis.

Research frontiers

Management of pancreatic solid mass lesions relies greatly on accuracy of diagnosis of these lesions. Research has been directed towards the various fine biopsy needles used in the diagnosis which will in turn affect the management and prognosis in a patient.

Innovations and breakthroughs

In the present study, the authors investigated the outcomes of two commonly used Procore needles in the diagnosis of solid mass lesions. This is the first meta-analysis that compares 19G procore and 22G procore needles with regards to their overall accuracy and efficacy.

Applications

This study gives information about both procore needles and their outcomes with solid mass lesions, thus helping the endoscopist in choosing the appropriate needle for their specific procedure.

Peer-review

This is an interesting paper and is worth to be published.

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META-ANALYSIS

Mucin expression and the pancreas: A systematic review and meta-analysis

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Abstract

AIM

To assess mucin expression in pancreatic premalignant and malignant states, and to establish its role as a prognostic marker.

METHODS

English Medical literature searches were conducted for "mucin" and "pancreas". Observational studies were included. Meta-analysis was performed by using Comprehensive meta-analysis software. Pooled odds ratios and 95%CIs were calculated.

RESULTS

Out of 949 eligible papers we found 20 according to the inclusion criteria, including 4262 patients, published till May 31, 2016. Mucin expression increased in pancreatic lesions with OR 10.206 (95%CI: 4.781-21.781, $P < 0.0001$). Measure of heterogeneity was high: Q = 296.973, df (Q) = 55.00, I^2 = 81.48%. We found a significant increase in the expression of MUC2, MUC4 and MUC5AC, 13.39, 118.43 and 13.91 times respectively, in pancreatic lesion in comparison with normal pancreatic tissue, and decreased expression of MUC5B.

CONCLUSION

Mucin expression may serve as prognostic marker for transformation of intraductal papillary mucinous neoplasms to ductal adenocarcinoma, for aggressiveness of the pancreatic tumor, and as targets for potential therapy.

Key words: Mucin; Pancreas; Pancreatic cancer; Gene expression

Core tip: There is a higher mucin expression in intraductal papillary mucinous neoplasms (IPMN) and ductal pancreatic cancer. Mucin expression may be a bad prognostic factor. MUC2, MUC4, MUC5AC and probably MUC1, are expressed in IPMN advanced to ductal adenocarcinoma. These mucins are also bad prognostic factors for ductal adenocarcinoma.

and meta-analysis. *World J Meta-Anal* 2017; 5(2): 63-70 Available from: URL: http://www.wjgnet.com/2308-3840/full/v5/i2/63.htm DOI: http://dx.doi.org/10.13105/wjma.v5.i2.63

INTRODUCTION

Mucins are high-molecular-weight glycoproteins, heavily glycosylated, synthesized and secreted by all mucosal surfaces of the human body and have an important role in healthy state and malignant diseases[1-3]. Change in mucins synthesis and secretion may be primary event or secondary to carcinogenesis or inflammation.

There are 21 known mucin genes in the human genome, encode for 2 types of mucins: Secreted and membrane-bound[4]. Membrane-bound mucins are involved in cell signaling and have a role in cellular processes such as growth, immune modulation, motility and adhesion.

Pancreatic carcinogenesis is associated with genetic and epigenetic changes, than may affect MUCs (mucin genes). MUCs may be expressed *de novo* during carcinogenesis. Mucins have potential value for diagnosis and follow-up of pancreatic neoplasms and for therapeutic interventions[5]. Mucin expression patterns may serve as a criterion for classification of intraductal papillary mucinous neoplasms (IPMN).

Several studies looked at mucins expression, comparing pancreatic lesions with normal pancreatic tissue. MUC1, membrane-bound mucin, is expressed in normal pancreatic tissue, but there is no detectable MUC2, MUC4 and MUC5AC[6,7].

Secretion of MUC1 is associated with adenocarcinoma and high grade dysplasia in pancreatic intraepithelial neoplasia (PanIN)[8-10]. MUC1 is rarely expressed in IPMN.

Positive expression of MUC2 in IPMN (intestinal type) indicates progression to carcinoma with secretion of MUC1[8,11]. Absence of MUC2 expression (gastric type) implies benign phenotype. MUC1 is rarely expressed in mucinous cyst in one study, while in another study mucinous cysts were found positive for MUC1/DF3[12,13].

MUC4 secretion correlates to the severity of dysplasia in PanIN and a poor prognosis in patients with adenocarcinomas, but results are somewhat inconsistent in different studies[14-17]. Expression of MUC4 in pancreatic cancer cell line was associated with increased proliferation, motility, adhesion, aggregation and metastasis[18].

The 2015 American Gastroenterological Association guidelines define 3 high-risk features of pancreatic cyst for developing cancer: Cyst size > 3 cm, dilated pancreatic duct and mural nodule[19]. There are no characteristics of mucin expression in the cyst fluid or the epithelial lining, as a marker for carcinogenesis.

The aim of this metaanalysis and systematic review is to assess the knowledge about mucin expression in pancreatic premalignant and malignant states, and to understand the possible role of mucin expressions as prognostic markers.

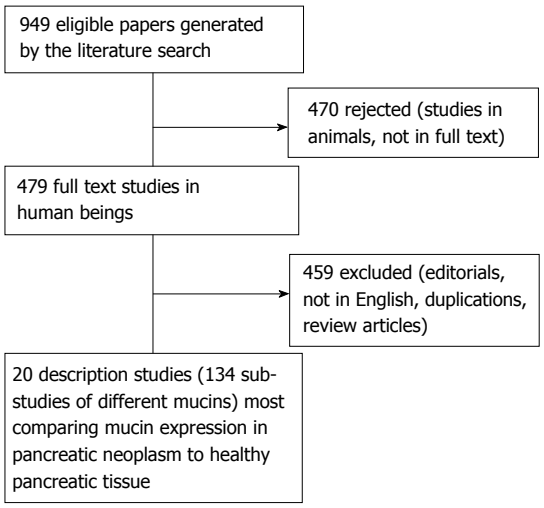


Figure 1 Flow chart of the articles identified for the meta-analysis.

MATERIALS AND METHODS

Search strategy

Searches were conducted for "mucin" and "pancreas" through May 31, 2016, using MEDLINE, PubMed, Scopus, EMBASE, and CENTRAL. Hand searches of articles references were also performed. Only fully published human studies in English were included (Figure 1).

Study selection

Observational studies about mucin expression in pancreatic tissue of cysts and adenocarcinoma were included. PRISMA guidelines for systematic reviews were strictly followed.

Data extraction

Author, country, year of publication, number of patients, and the number of positive staining were extracted. Data was stratified according to lesions (ductal adenocarcinoma, IPMN, mucinous cyst) and according to the mucin expressed (Table 1).

Statistical analysis

Metaanalysis was performed by using Comprehensive metaanalysis software (Version 3, Biostat Inc., Englewood, NJ, United States). Pooled odds ratios and 95%CIs were calculated for mucin expression in pre-malignant and malignant pancreatic lesions. In all methods used (IMH, ISH or RT-PCR) OR represents quantitatively the number of patients with higher expression.

Heterogeneity was evaluated by Cochran *Q*-test, and considered to be present when *Q*-test $P < 0.10$. I^2 statistic was used to measure the proportion of inconsistency. We calculated publication bias using funnel plot of standard error by log odds ratio. Even distribution of the studies denied significant publication bias.

RESULTS

Out of 949 eligible papers we found 20 according to

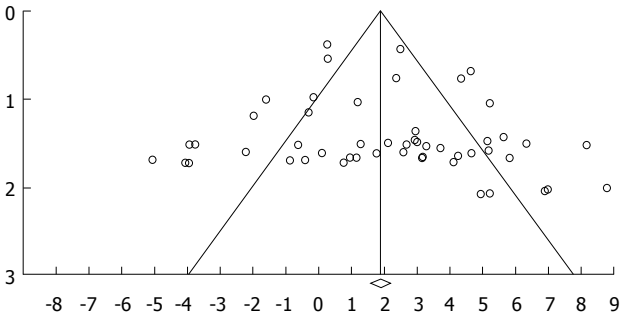


Figure 2 Funnel plot for publication bias.

Table 1 Summary of mucin expression in pancreatic lesions

Mucin gene	OR of mucin expression	*P*
MUC1	3.64	0.09
MUC2	13.39	0.05
MUC3	14.33	0.08
MUC4	118.43	< 0.001
MUC5AC	13.91	< 0.001
MUC5B	0.08	< 0.001
MUC6	0.52	0.41
MUC7	0	NA
Total mucin	9.99-21.72	< 0.001

OR: Odds ratio; NA: Not applicable.

the inclusion criteria, including 4262 patients, published till May 31, 2016 from 4 countries (Japan 10, United States 9, France 1, Norway 1)[6,8,9,11-15,20-32] (Figure 1). There are 134 sub-studies (stratifying data according to mucin types and lesions). In 104 sub-studies immunohistochemistry (IMH) has been used, in 20 sub-studies RT-PCR for RNA, and in10 histochemistry. Eleven studies and 84 sub-studies had also results of normal pancreatic tissue for comparison with the neoplastic lesion. Ductal adenocarcinoma was examined in 14 studies and 60 sub-studies (2206 cases); IPMN was examined in 12 studies and 46 sub-studies (1691 cases). There were 365 cases of mucinous or colloid carcinoma, mucinous cystic neoplasm, hyperplastic pancreatic lesion, chronic pancreatitis and pseudo cysts. Funnel plot denies a significant publication bias (Figure 2).

In the random-effect model, mucin expression was significantly higher in pancreatic lesions than in normal pancreatic tissue with OR 10.206 (95%CI: 4.781-21.781, $P < 0.0001$) (Figure 3). Measure of heterogeneity was high, demonstrated in the included studies: Q = 296.973, df (Q) = 55.00, I^2= 81.48%. OR for mucin expression in pancreatic ductal adenocarcinoma and IPMN was 9.99 with 95%CI: 3.68-27.15, $P < 0.001$, and 21.72 with 95%CI: 4.01-117.55, $P < 0.001$, respectively (Figure 4). OR for expression in pancreatic lesion of MUC1- 4, MUC5AC, MUC5B, MUC6 and MUC7, was 3.64 with 95%CI: 0.80-16.49, $P = 0.09$; 13.39 with 95%CI: 1.03-173.43, $P = 0.05$; 14.33 with 95%CI: 0.742-95.97, $P = 0.08$; 118.43 with 95%CI: 19.39-723.48, $P < 0.001$; 13.91 with 95%CI: 2.35-82.14, $P < 0.001$; 0.08 with 95%CI: 0.02-0.36, $P < 0.001$; 0.52 with 95%CI: 0.11-2.47, $P = 0.41$; respectively (Figure 5). MUC7 was never expressed in pancreatic lesion or normal tissue (Table 1).

Studies description

Yamada *et al*[20] using histochemical methods compared the mucin expression between malignant and benign tumors of the pancreas. They found significant higher expression of sialomucin (> 50% of glands) in malignant tumors and higher expression of neutral mucin (> 50% of glands) in benign tumors. Osako *et al*[21] demonstrated a significant contrast between expression of mammary type mucin and intestinal type mucin in carcinomas and intraductal papillary tumor. The oncogenic mucin antigens, Tn and sialyl Tn (STn), were expressed in malignant and premalignant states but not in normal pancreatic mucosa. Incomplete glycosylation of mucins that results in expression of T, Tn, and sialyl-Tn antigens in pancreatic adenocarcinoma was described by Terada *et al*[13,32]. They found increased expression of Tn antigen and STn antigen in comparison with normal pancreatic tissue, but the same expression of *MUC1* and T antigen. Similar findings were described for IPMN, which support the sequence of events from IPMN to adenocarcinoma. Yonezawa *et al*[8] found higher expression of *MUC1* in ductal adenocarcinoma than IPMN, and lower expression of *MUC2*. Invasive growth areas of IPMN had *MUC1* expression similar to adenocarcinoma. The same group demonstrated up regulation of *MUC5AC* mRNA in IPMN cases with a favorable prognosis, whereas such expression was not found in ductal adenocarcinoma cases with a poor prognosis[22].

Andrianifahanana *et al*[23] described a significant higher *MUC4* expression in adenocarcinoma tissue than in chronic pancreatitis or normal pancreatic tissue. Lüttges *et al*[9] found expression of *MUC2* in all IPMN and mucinous carcinoma cases of the pancreas but in only one of 35 of ductular adenocarcinoma cases. *MUC1* expression was only demonstrated in ductular adenocarcinoma tissue. The same group also found strong expression of *MUC5AC* and *MUC2* in mucinous cystic neoplasms of the pancreas, but no such expression of *MUC1* and *MUC6*[12]. Kim *et al*[24] found a significant higher expression of *MUC1, MUC5AC*, Md2, STn antigen and sulpho Lewis a antigen in ductal adenocarcinoma of the pancreas than in normal pancreatic tissue. Swartz *et al*[14] found higher expression of *MUC4* in invasive ductal adenocarcinoma of the pancreas than in PanIN. Expression was not demonstrated in normal pancreatic tissue. Nakamura *et al*[11] described 2 kinds of IPMN, according to *MUC2* expression with higher invasive property for *MUC2* positive than negative tumors. Terris *et al*[25] found increased expression of *MUC5AC* and *MUC2* in IPMN, similar to colloid carcinoma, and different from ductal adenocarcinoma where *MUC1* expression was increased. Horinouchi *et al*[6] found higher expression of *MUC1* and *MUC5AC* in ductal adenocarcinoma than in IPMN. *MUC2* was only expressed in IPMN of "dark phenotype". Saitou *et al*[15] found a

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Osaka M	DF3 IMH ductal adenocarcinoma	Japan	1993	19.00	1.07	338.67	2.00	0.05
Osaka M	DF4 IMH IPMN	Japan	1993	0.02	0.00	0.47	-2.46	0.01
Osaka M	MRP IMH IPMN	Japan	1993	1001.00	18.00	55675.78	3.37	0.00
Osaka M	STn antigen IMH ductal adenocarcinoma	Japan	1993	6643.00	128.67	342958.99	4.37	0.00
Osaka M	STn antigen IPMN	Japan	1993	1001.00	18.00	55675.78	3.37	0.00
Osaka M	T antigen IMH ductal adenocarcinoma	Japan	1993	1.34	0.46	3.90	0.54	0.59
Osaka M	T antigen IMH IPMN	Japan	1993	3.67	0.19	71.54	0.86	0.39
Osaka M	Tn antigen IMH ductal adenocarcinoma	Japan	1993	6643.00	128.67	342958.99	4.37	0.00
Osaka M	Tn antigen IMH IPMN	Japan	1993	1001.00	18.00	55675.78	3.37	0.00
Terada T	MUC1 IMH SP IPMN	Japan	1996	0.11	0.00	2.60	-1.37	0.17
Terada T	STn antigen IMH SP ductal adenocarcinoma	Japan	1996	143.00	2.42	8467.01	2.38	0.02
Terada T	STn antigen IMH SP IPMN	Japan	1996	24.20	0.93	629.32	1.92	0.06
Terada T	Tn antigen IMH SP ductal adenocarcinoma	Japan	1996	143.00	2.42	8467.01	2.38	0.02
Terada T	Tn antigen IMH SP IPMN	Japan	1996	13.44	0.58	314.29	1.62	0.11
Yonezawa S	MUC2 RT-PCR IPMN	Japan	1999	172.62	9.56	3118.40	3.49	0.00
Yonezawa S	MUC5AC RT-PCR ductal adenocarcinoma	Japan	1999	20.52	1.10	382.54	2.02	0.04
Yonezawa S	MUC5AC RT-PCR IPMN	Japan	1999	569.44	29.39	11033.41	4.20	0.00
Andrianifanana M	MUC4 RT-PCR ductal adenocarcinoma	United States	2001	41.67	1.96	887.56	2.39	0.02
Andrianifanana M	MUC5AC RT-PCR chronic pancreatitis	United States	2001	0.42	0.01	11.92	-0.51	0.61
Andrianifanana M	MUC5AC RT-PCR ductal adenocarcinoma	United States	2001	0.01	0.00	0.18	-2.98	0.00
Andrianifanana M	MUC5B RT-PCR chronic pancreatitis	United States	2001	0.42	0.01	11.92	-0.51	0.61
Kim GE	MUC1 IMH ductal adenocarcinoma	United States	2002	12.33	5.24	29.02	5.75	0.00
Kim GE	MUC2 IMH ductal adenocarcinoma	United States	2002	8.42	0.44	159.89	1.42	0.16
Kim GE	MUC5AC IMH ductal adenocarcinoma	United States	2002	104.50	27.30	400.02	6.79	0.00
Kim GE	MUC6 IMH ductal adenocarcinoma	United States	2002	1.32	0.62	2.79	0.71	0.48
Kim GE	Nd2 IMH ductal adenocarcinoma	United States	2002	77.78	17.18	352.02	5.65	0.00
Kim GE	Sialyl Tn antigen IMH ductal adenocarcinoma	United States	2002	186.20	23.73	1460.83	4.97	0.00
Kim GE	Sulpho Lewis a IMH ductal adenocarcinoma	United States	2002	10.86	2.41	48.98	3.10	0.00
Swartz MJ	MUC4 IMH ductal adenocarcinoma	United States	2002	3591.86	180.43	71505.47	5.36	0.00
Swartz MJ	MUC4 IMH PanIN	United States	2002	287.13	17.19	4796.26	3.94	0.00
Terris B	MUC1 IMH colloid carcinoma	France	2002	2.20	0.07	64.90	0.46	0.65
Terris B	MUC1 IMH ductal adenocarcinoma	France	2002	62.33	2.13	1822.63	2.40	0.02
Terris B	MUC1 IMH IPMN	France	2002	1.12	0.05	26.83	0.07	0.94
Terris B	MUC2 IMH colloid carcinoma	France	2002	187.00	3.21	10884.81	2.52	0.01
Terris B	MUC2 IMH IPMN	France	2002	107.80	4.51	2576.92	2.89	0.00
Terris B	MUC3 IMH IPMN	France	2002	14.83	0.74	295.97	1.77	0.08
Terris B	MUC4 IMH ductal adenocarcinoma	France	2002	5.92	0.25	141.48	1.10	0.27
Terris B	MUC4 IMH IPMN	France	2002	27.13	1.33	554.23	2.14	0.03
Terris B	MUC5AC IMH colloid carcinoma	France	2002	23.80	0.89	633.53	1.89	0.06
Terris B	MUC5AC IMH ductal adenocarcinoma	France	2002	0.75	0.08	7.21	-0.25	0.80
Terris B	MUC5AC IMH IPMN	France	2002	71.40	2.81	1814.71	2.59	0.01
Terris B	MUC5B IMH ductal adenocarcinoma	France	2002	0.02	0.00	0.52	-2.34	0.02
Terris B	MUC5B IMH colloid carcinoma	France	2002	0.02	0.00	0.59	-2.27	0.02
Terris B	MUC5B IMH IPMN	France	2002	0.14	0.01	1.46	-1.64	0.10
Terris B	MUC6 IMH IPMN	France	2002	0.67	0.02	18.84	-0.23	0.82
Kanno A	MUC4 IHC IPMN	Japan	2006	341.00	12.90	9015.65	3.49	0.00
Kanno A	MUC5AC IHC hyperplastic tissue	Japan	2006	3.19	0.12	84.43	0.69	0.49
Kanno A	MUC5AC IHC IPMN	Japan	2006	1089.00	20.37	58214.67	3.44	0.00
Giorgadze TA	MUC1 IMH EUS-S ductal adenocarcinoma	United States	2006	3.33	0.44	25.39	1.16	0.25
Giorgadze TA	MUC2 IMH EUS-S ductal adenocarcinoma	United States	2006	0.20	0.03	1.47	-1.58	0.11
Giorgadze TA	MUC2 IMH SP ductal adenocarcinoma	United States	2006	2.64	0.10	69.88	0.58	0.56
Giorgadze TA	MUC5AC IMH EUS-S ductal adenocarcinoma	United States	2006	19.33	1.33	281.60	2.17	0.03
Giorgadze TA	MUC5AC IMH SP ductal adenocarcinoma	United States	2006	179.80	7.94	4070.43	3.26	0.00
Giorgadze TA	MUC6AC IMH EUS-S ductal adenocarcinoma	United States	2006	0.87	0.13	6.03	-0.14	0.89
Giorgadze TA	MUC6AC IMH SP ductal adenocarcinoma	United States	2006	0.02	0.00	0.38	-2.59	0.01
Streppel MM	MUC16 IMH PanIN	United States	2012	0.54	0.03	10.84	-0.40	0.69
				10.21	4.78	21.79	6.00	0.00

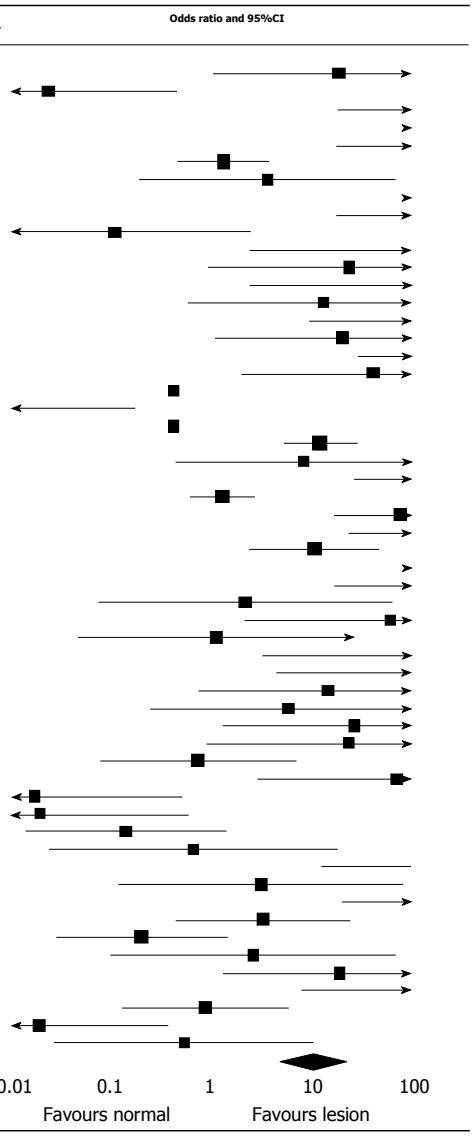
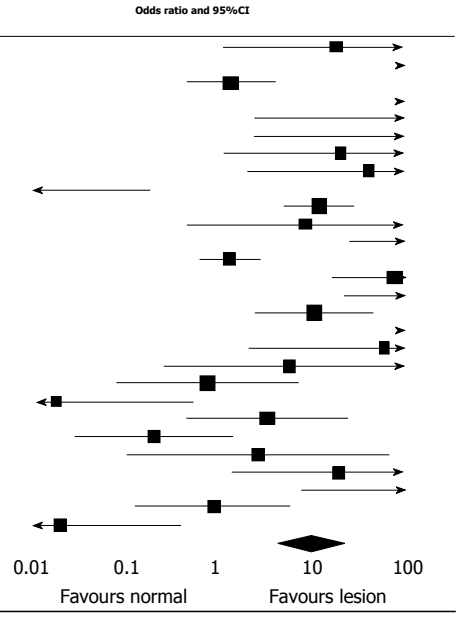


Figure 3 Metaanalysis of mucin expression in pancreatic lesions (20 studies, 134 sub-studies). PanIN: Pancreatic intraepithelial neoplasia; IPMN: Intraductal papillary mucinous neoplasms; STn: Sialyl Tn.

positive correlation between the strength of *MUC4* expression in ductal adenocarcinoma of the pancreas and aggressive behavior. Such a correlation could not be demonstrated for *MUC1*. Kanno *et al*[26] found *MUC4* and *MUC5AC* expression in adenoma and IPMN but not in normal or hyperplastic pancreatic tissue. Giorgadze *et al*[27] reviewed pancreatic 56 EUS-FNA specimens and 26 pancreatectomy specimens for expression profiles of *MUC1, MUC2, MUC5AC* and *MUC6*. *MUC5AC* expression was significantly higher in adenocarcinoma than in normal tissue both in EUS-FNA specimens and surgical specimens. Westgaard *et al*[28] found that in adenocarcinoma *MUC1* and *MUC4* expression was associated with a poor prognosis. Gonzalez Obeso *et al*[29] used alcian blue and mucicarmine stains in 11 pseudo cysts and 42 IPMNs or mucinous cysts aspirates. They could not demonstrate a significant difference in mucin staining between the various types of cysts. Streppel *et al*[30] found *MUC16* (CA125) expression in 81.5% of 200 pancreatic adenocarcinoma tissues, in comparison with none of 29 IPMN cases and in 2% of normal pancreatic tissues. Kitazono *et al*[31] looked at the expression rates of *MUC4* in intestinal-type IPMNs and gastric-type IPMNs using monoclonal antibodies 8G7 and 1G8. The expression rate of *MUC4* in the intestinal-type IPMNs was higher than in the gastric-type IPMNs. Maker *et al*[33] examined 40 cases of pancreatic IPMN comparing mucin expression in cases with high risk IPMN (with high grade dysplasia or carcinoma) and cases with low risk IPMN (with low grade dysplasia). They found a significant increase in *MUC2* and *MUC4* expression (10.0 ± 3.0 ng/mL and 20.6 ± 10.6 ng/mL *vs* 4.4 ± 1.2 ng/mL and 4.5 ± 1.4 ng/mL, $P = 0.03$, respectively). No change was demonstrated for *MUC1* and *MUC5AC*. This study is not included in the metaanalysis since numerical data is absent and only means of mucin expression are given.

A

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Osaka M	DF3 IMH ductal adenocarcinoma	Japan	1993	19.00	1.07	338.67	2.00	0.05
Osaka M	STn antigen IMH ductal adenocarcinoma	Japan	1993	6643.00	128.67	342958.99	4.37	0.00
Osaka M	T antigen IMH ductal adenocarcinoma	Japan	1993	1.34	0.46	3.90	0.54	0.59
Osaka M	Tn antigen IMH ductal adenocarcinoma	Japan	1993	6643.00	128.67	342958.99	4.37	0.00
Terada T	STn antigen IMH SP ductal adenocarcinoma	Japan	1996	143.00	2.42	8467.01	2.38	0.02
Terada T	Tn antigen IMH SP ductal adenocarcinoma	Japan	1996	143.00	2.42	8467.01	2.38	0.02
Yonezawa S	MUC5AC RT-PCR ductal adenocarcinoma	Japan	1999	20.52	1.10	382.54	2.02	0.04
Andrianifanana M	MUC4 RT-PCR ductal adenocarcinoma	United States	2001	41.67	1.96	887.56	2.39	0.02
Andrianifanana M	MUC5AC RT-PCR ductal adenocarcinoma	United States	2001	0.01	0.00	0.18	-2.98	0.00
Kim GE	MUC1 IMH ductal adenocarcinoma	United States	2002	12.33	5.24	29.02	5.75	0.00
Kim GE	MUC2 IMH ductal adenocarcinoma	United States	2002	8.42	0.44	159.89	1.42	0.16
Kim GE	MUC5AC IMH ductal adenocarcinoma	United States	2002	104.50	27.30	400.02	6.79	0.00
Kim GE	MUC6 IMH ductal adenocarcinoma	United States	2002	1.32	0.62	2.79	0.71	0.48
Kim GE	Nd2 IMH ductal adenocarcinoma	United States	2002	77.78	17.18	352.02	5.65	0.00
Kim GE	Sialyl Tn antigen IMH ductal adenocarcinoma	United States	2002	186.20	23.73	1460.83	4.97	0.00
Kim GE	Sulpho Lewis a IMH ductal adenocarcinoma	United States	2002	10.86	2.41	48.98	3.10	0.00
Swartz MJ	MUC4 IMH ductal adenocarcinoma	United States	2002	3591.86	180.43	71505.47	5.36	0.00
Terris B	MUC1 IMH ductal adenocarcinoma	France	2002	62.33	2.13	1822.63	2.40	0.02
Terris B	MUC4 IMH ductal adenocarcinoma	France	2002	5.92	0.25	141.48	1.10	0.27
Terris B	MUC5AC IMH ductal adenocarcinoma	France	2002	0.75	0.08	7.21	-0.25	0.80
Terris B	MUC5B IMH ductal adenocarcinoma	France	2002	0.02	0.00	0.52	-2.34	0.02
Giorgadze TA	MUC1 IMH EUS-S ductal adenocarcinoma	United States	2006	3.33	0.44	25.39	1.16	0.25
Giorgadze TA	MUC2 IMH EUS-S ductal adenocarcinoma	United States	2006	0.20	0.03	1.47	-1.58	0.11
Giorgadze TA	MUC2 IMH SP ductal adenocarcinoma	United States	2006	2.64	0.10	69.88	0.58	0.56
Giorgadze TA	MUC5AC IMH EUS-S ductal adenocarcinoma	United States	2006	19.33	1.33	281.60	2.17	0.03
Giorgadze TA	MUC5AC IMH SP ductal adenocarcinoma	United States	2006	179.80	7.94	4070.43	3.26	0.00
Giorgadze TA	MUC6AC IMH EUS-S ductal adenocarcinoma	United States	2006	0.87	0.13	6.03	-0.14	0.89
Giorgadze TA	MUC6AC IMH SP ductal adenocarcinoma	United States	2006	0.02	0.00	0.38	-2.59	0.01
				9.99	3.68	27.15	4.51	0.00



B

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Osaka M	DF3 IMH IPMN	Japan	1993	0.02	0.00	0.47	-2.46	0.01
Osaka M	MRP IMH IPMN	Japan	1993	1001.00	18.00	55675.78	3.37	0.00
Osaka M	STn antigen IMH IPMN	Japan	1993	1001.00	18.00	55675.78	3.37	0.00
Osaka M	T antigen IMH IPMN	Japan	1993	3.67	0.19	71.54	0.86	0.39
Osaka M	Tn antigen IMH IPMN	Japan	1993	1001.00	18.00	55675.78	3.37	0.00
Yonezawa S	MUC2 RT-PCR IPMN	Japan	1999	172.62	9.56	3118.40	3.49	0.00
Yonezawa S	MUC5AC RT-PCR IPMN	Japan	1999	569.44	29.39	11033.41	4.20	0.00
Terris B	MUC1 IMH IPMN	France	2002	1.12	0.05	26.83	0.07	0.94
Terris B	MUC2 IMH IPMN	France	2002	107.80	4.51	2576.92	2.89	0.00
Terris B	MUC3 IMH IPMN	France	2002	14.83	0.74	295.97	1.77	0.08
Terris B	MUC4 IMH IPMN	France	2002	27.13	1.33	554.23	2.14	0.03
Terris B	MUC5AC IMH IPMN	France	2002	71.40	2.81	1814.71	2.59	0.01
Terris B	MUC5B IMH IPMN	France	2002	0.14	0.01	1.46	-1.64	0.10
Terris B	MUC6 IMH IPMN	France	2002	0.67	0.02	18.84	-0.23	0.82
Kanno A	MUC4 IHC IPMN	Japan	2006	341.00	12.90	9015.65	3.49	0.00
Kanno A	MUC5AC IHC IPMN	Japan	2006	1089.00	20.37	58214.67	3.44	0.00
Streppel MM	MUC16 IMH PanIN	United States	2012	0.54	0.03	10.84	-0.40	0.69
				21.72	4.01	117.55	3.57	0.00

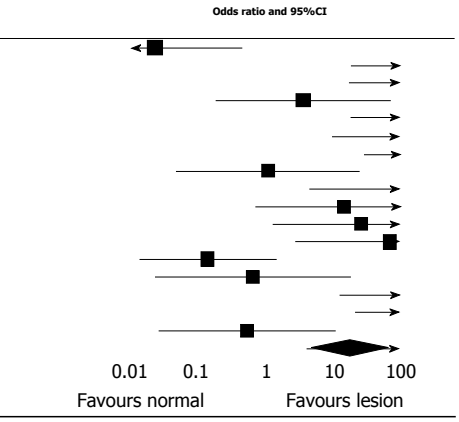


Figure 4 Meta-analysis of mucin expression in pancreatic lesions, sub-studies of different lesions. A: Ductal adenocarcinoma; B: Intraductal papillary mucinous neoplasm (20 studies, 102 sub-studies). PanIN: Pancreatic intraepithelial neoplasia; IPMN: Intraductal papillary mucinous neoplasms; STn: Sialyl Tn.

DISCUSSION

Mucin is an important component of the mucus layers protecting epithelial surfaces of the respiratory, digestive, urinary and reproductive organs, and as such was studied intensively. The role of mucin in exocrine/endocrine gland such as the pancreas is less understood. Most of the studies about pancreatic mucin expression involved malignant transformation and characteristics of pancreatic cysts. In Table 1 we summarized the knowledge about mucin expression in the pancreas, including the findings of our metaanalysis.

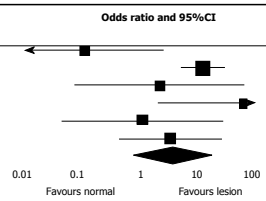
In our metaanalysis we found a significant increase in the expression of *MUC2*, *MUC4*, and *MUC5AC*, 13.39, 118.43 and 13.91 times respectively, in pancreatic lesion in comparison with normal pancreatic tissue (Table 1), and decreased expression of MUC5B. The results for *MUC1*, *MUC3*, *MUC6*, Tn and STn were not statistically significant.

Exploring individual studies some different and inconsistent finding are presented, but it is obvious that higher malignant behavior of IPMN and transfer into ductal adenocarcinoma is characterized by increased expression of *MUC2*, *MUC4* and *MUC5AC*[9,12-15,20,21,23,26-28,33]. The expression of these mucins in the ductal adenocarcinoma implied a bad prognosis. *MUC1* expression, even though did not reach significance in the metaanalysis, was also a marker for bad prognosis in ductal adenocarcinoma[8,24,28].

IPMN could be originates from the pancreatic main duct, or side-branches, being of gastric type (*MUC5AC* is expressed in dark cells) or of intestinal type (*MUC2* is expressed in clear cells). Gastric IPMNs are *MUC1* and *MUC2* negative, usually located in the branch small ducts, and rarely develop into cancer. Intestinal IPMNs are *MUC1* negative but *MUC2* positive. However, when they transform into cancer, the *MUC1* becomes positive. They are mostly located in the main duct. *MUC4* expression in IPMNs may help to distinguish intestinal

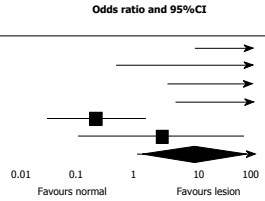
A

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Terada T	MUC1 IMH SP IPMN	Japan	1996	0.11	0.00	2.60	-1.37	0.17
Kim GE	MUC1 IMH ductal adenocarcinoma	United States	2002	12.33	5.24	29.02	5.75	0.00
Terris B	MUC1 IMH colloid carcinoma	France	2002	2.20	0.07	64.90	0.46	0.65
Terris B	MUC1 IMH ductal adenocarcinoma	France	2002	62.33	2.13	1822.63	2.40	0.02
Terris B	MUC1 IMH IPMN	France	2002	1.12	0.05	26.83	0.07	0.94
Giorgadze TA	MUC1 IMH EUS-S ductal adenocarcinoma	United States	2006	3.33	0.44	25.39	1.16	0.25
				3.64	0.80	16.49	1.68	0.09



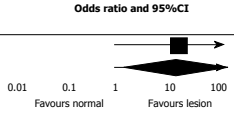
B

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Yonezawa S	MUC2 RT-PCR IPMN	Japan	1999	172.62	9.56	3118.40	3.49	0.00
Kim GE	MUC2 IMH ductal adenocarcinoma	United States	2002	8.42	0.44	159.89	1.42	0.16
Terris B	MUC2 IMH colloid carcinoma	France	2002	187.00	3.21	10884.81	2.52	0.01
Terris B	MUC2 IMH IPMN	France	2002	107.80	4.51	2576.92	2.89	0.00
Giorgadze TA	MUC2 IMH EUS-S ductal adenocarcinoma	United States	2006	0.20	0.03	1.47	-1.58	0.11
Giorgadze TA	MUC2 IMH SP ductal adenocarcinoma	United States	2006	2.64	0.10	69.88	0.58	0.56
				13.39	1.03	173.43	1.98	0.05



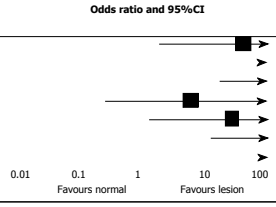
C

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Terris B	MUC3 IMH IPMN	France	2002	14.83	0.74	295.97	1.77	0.08
				14.83	0.74	295.97	1.77	0.08



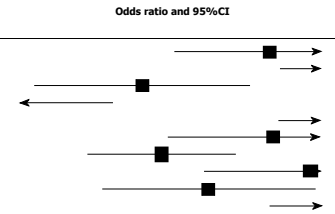
D

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Andrianifanana M	MUC4 RT-PCR ductal adenocarcinoma	United States	2001	41.67	1.96	887.56	2.39	0.02
Swartz MJ	MUC4 IMH ductal adenocarcinoma	United States	2002	3591.86	180.43	71505.47	5.36	0.00
Swartz MJ	MUC4 IMH PanIN	United States	2002	287.13	17.19	4796.26	3.94	0.00
Terris B	MUC4 IMH ductal adenocarcinoma	France	2002	5.92	0.25	141.48	1.10	0.27
Terris B	MUC4 IMH IPMN	France	2002	27.13	1.33	554.23	2.14	0.03
Kanno A	MUC4 IHC IPMN	Japan	2006	341.00	12.90	9015.65	3.49	0.00
				118.43	19.39	723.48	5.17	0.00



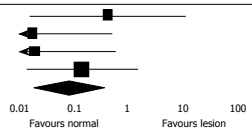
E

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Yonezawa S	MUC5AC RT-PCR ductal adenocarcinoma	Japan	1999	20.52	1.10	382.54	2.02	0.04
Yonezawa S	MUC5AC RT-PCR IPMN	Japan	1999	569.44	29.39	11033.41	4.20	0.00
Andrianifanana M	MUC5AC RT-PCR chronic pancreatitis	United States	2001	0.42	0.01	11.92	-0.51	0.61
Andrianifanana M	MUC5AC RT-PCR ductal adenocarcinoma	United States	2001	0.01	0.00	0.18	-2.98	0.00
Kim GE	MUC5AC IMH ductal adenocarcinoma	United States	2002	104.50	27.30	400.02	6.79	0.00
Terris B	MUC5AC IMH colloid carcinoma	France	2002	23.80	0.89	633.53	1.89	0.06
Terris B	MUC5AC IMH ductal adenocarcinoma	France	2002	0.75	0.08	7.21	-0.25	0.80
Terris B	MUC5AC IMH IPMN	France	2002	71.40	2.81	1814.71	2.59	0.01
Kanno A	MUC5AC IHC hyperplastic tissue	Japan	2006	3.19	0.12	84.43	0.69	0.49
Kanno A	MUC5AC IHC IPMN	Japan	2006	1089.00	20.37	58214.67	3.44	0.00
Giorgadze TA	MUC5AC IMH EUS-S ductal adenocarcinoma	United States	2006	19.33	1.33	281.60	2.17	0.03
Giorgadze TA	MUC5AC IMH SP ductal adenocarcinoma	United States	2006	179.80	7.94	4070.43	3.26	0.00
				13.91	2.35	82.14	2.91	0.00



F

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Andrianifanana M	MUC5B RT-PCR chronic pancreatitis	United States	2001	0.42	0.01	11.92	-0.51	0.61
Terris B	MUC5B IMH ductal adenocarcinoma	France	2002	0.02	0.00	0.52	-2.34	0.02
Terris B	MUC5B IMH colloid carcinoma	France	2002	0.02	0.00	0.59	-2.27	0.02
Terris B	MUC5B IMH IPMN	France	2002	0.14	0.01	1.46	-1.64	0.10
				0.08	0.02	0.36	-3.31	0.00



G

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Kim GE	MUC6 IMH ductal adenocarcinoma	United States	2002	1.32	0.62	2.79	0.71	0.48
Terris B	MUC6 IMH IPMN	France	2002	0.67	0.02	18.84	-0.23	0.82
Giorgadze TA	MUC6AC IMH EUS-S ductal adenocarcinoma	United States	2006	0.87	0.13	6.03	-0.14	0.89
Giorgadze TA	MUC6AC IMH SP ductal adenocarcinoma	United States	2006	0.02	0.00	0.38	-2.59	0.01
				0.52	0.11	2.47	-0.83	0.41

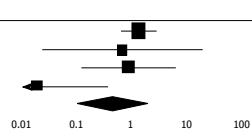


Figure 5 Meta-analysis of mucin expression in pancreatic lesions, sub-studies of different mucins. A: MUC1; B: MUC2; C: MUC3; D: MUC4; E: MUC5AC; F: MUC5B; G: MUC6 (17 studies, 104 sub-studies). PanIN: Pancreatic intraepithelial neoplasia; IPMN: Intraductal papillary mucinous neoplasms; STn: Sialyl Tn.

IPMNs from the safer gastric-type IPMNs.

Our meta-analysis has some limitations, since the methods of mucin expression measurement, and the definition of the pancreatic lesion may be inaccurate. There is heterogeneity regarding detection of mucin expression and disease classification. In some studies

immunohistochemistry was used for protein detection and in other RT-PCR or *in situ* hybridization for RNA detection. The definition of pancreatic mucinous cyst and side-branch or main-duct IPMN (Previously IPMT) also was changed during the last decade, and the results of different mucins expression in different lesions should be taken with caution. Also PanIN (pancreatic intra epithelial neoplasia), the pancreatic gland equivalent of adenomatous change or dysplasia, has been never studied in the context or mucin genes expression.

In conclusion, expression of *MUC2*, *MUC4*, *MUC5AC* and probably *MUC1*, may serve as prognostic marker for transformation of IPMN to ductal adenocarcinoma, for aggressiveness of the pancreatic tumor, and as targets for potential therapy. Further studies are needed to establish these observations.

COMMENTS

Background

Pancreatic carcinogenesis is associated with genetic and epigenetic changes, that may affect mucin genes. Certain mucins are expressed during carcinogenesis, while specific patterns have been recognized in pre-malignant and malignant lesions.

Research frontiers

Assessing mucin expression in pancreatic premalignant and malignant states, and to establish its role as a prognostic marker.

Innovations and breakthroughs

Mucin expression was higher in pancreatic lesions than in healthy pancreatic tissue: OR 10.206 (95%CI: 4.781-21.781, $P < 0.0001$).

Applications

The author found a significant increase in the expression of *MUC2*, *MUC4* and *MUC5AC*, 13.39, 118.43 and 13.91 times respectively, in pancreatic lesion in comparison with normal pancreatic tissue, and decrease expression of MUC5B.

Peer-review

This is a very good paper, with a large amount of interesting data and work. The analysis is conducted respecting the protocols of meta-analysis.

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