Original Article

The detection rate of methylene blue combined with another tracer in sentinel lymph node biopsy of early-stage breast cancer: a systematic review and network meta-analysis

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Background: Methylene blue (MB) alone or combined with 99mtechnetium-labeled sulphur colloid (Tc99m) or indocyanine green (ICG) is widely used for sentinel lymph node biopsy (SLNB) of early-stage breast cancer in developing countries and regions. However, studies investigating the effectiveness of MB combined with another tracer have produced heterogeneous results. The purpose of this network meta-analysis (NMA) was to evaluate the detection rate of MB alone, MB + Tc99m, and MB + ICG, and to examine the differences between the 3 methods.

Methods: We conducted a comprehensive electronic literature search on the PubMed, Embase, Web of Science, CNKI, and Wanfang Data databases from inception to October 2021. The meta-analysis included 7,498 patients in 49 studies. The risk of bias for each study was independently assessed as low, moderate, or high using criteria adapted from the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool. Fixed- and random-effects models were used to calculate pooled estimates. Mixed-comparison analysis using random-effects models. We assessed statistical heterogeneity by I2 statistics and evaluated publication bias using Begg's test.

Results: The identification rate (IR), false-negative rate (FNR), sensitivity (SEN), and accuracy rate (AR) using MB + Tc99m were 96%, 7%, 93%, and 96%, respectively; the IR, FNR, SEN, and AR using MB + ICG were 97%, 7%, 93%, and 97%, respectively. The NMA found that IR and AR between MB + ICG and MB + Tc99m was OR =1.37 (95% CI: 0.41–4.20) and OR =1.33 (95% CI: 0.56–3.32), respectively.

Discussion: Our results are similar to those of most previous studies, and meta-analysis showed that the MB + Tc99m or MB + ICG mapping methods can be used to obtain higher IR and lower FNR than MB alone. Our NMA showed no statistical significance between MB + Tc99m and MB + ICG with IR and AR. Both MB + Tc99m and MB + ICG can be used as effective mapping methods in SLNB of early-stage breast cancer to improve the detection rate.

Keywords: Breast cancer; indocyanine green (ICG); methylene blue (MB); sentinel lymph node biopsy (SLNB); 99mtechnetium-labeled sulphur colloid (Tc99m); meta-analysis

Submitted Jul 09, 2021. Accepted for publication Nov 05, 2021.
doi: 10.21037/tcr-21-1239
View this article at: https://dx.doi.org/10.21037/tcr-21-1239

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Introduction

Breast cancer is the most common malignant tumor occurring in women worldwide. In China, the incidence of breast cancer increases every year (1). The eighth edition of the American Joint Committee on Cancer (AJCC)'s Cancer Staging Manual provides comprehensive advice on the staging, prognosis, and treatment of cancer, and is considered more accurate than traditional anatomical staging (2). Axillary lymph node (ALN) status is an important factor in breast cancer staging and prognosis; therefore, the accurate evaluation of ALN status is essential for the formulation an appropriate treatment plan. Sentinel lymph node biopsy (SLNB) has become the standard staging scheme for patients with cN_0 early-stage breast cancer (3,4). Improving the identification rate (IR), sensitivity (SEN), and accuracy rate (AR) of SLNB while simultaneously reducing the false-negative rate (FNR) is a critical concern for surgeons, and the selection of tracers is key to the success of SLNB.

Common tracers for SLNB include blue dye, radioisotope, and fluorescence, or blue dye combined with radioisotope or fluorescence. The combination of radioisotope and blue dyes, such as patent blue or isosulfan blue, is considered the standard mapping method worldwide. Meanwhile, the combined use of indocyanine green (ICG) and blue dye has gradually become more frequent in clinical practice as a means of improving detection rates (5). However, hospitals in many developing countries, including China, have limited access to patent blue or isosulfan blue and are unable to provide the personnel and equipment required for radioisotope use. Consequently, with regard to the selection of blue dye, the 2021 Chinese Society of Breast Surgery (CSBrS) practice guidelines recommend blue dye alone or fluorescence alone as class IA, radioisotope alone or the combination of radioisotope and blue dye as class IB (6).

Researchers have sought to improve the detection rate of SLNB by combining different tracers. In previous studies, methylene blue (MB) combined with ^{99m}technetium-labeled sulphur colloid (MB + Tc99m) and MB combined with ICG (MB + ICG) showed certain advantages over MB alone. However, as these studies were small in scale and technically heterogeneous, they did not provide clear results. The detection rates of MB alone, MB + Tc99m, and MB + ICG are therefore uncertain. We thus sought to evaluate the detection rates of these 3 methods and to examine their differences using a network meta-analysis (NMA).

We present the following article in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting checklist (available at https://dx.doi.org/10.21037/tcr-21-1239), and our protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY; registration no. INPLASY202150107).

Methods

Literature retrieval strategy

This meta-analysis was reported and screened according to the PRISMA Guidelines (7). We conducted a comprehensive electronic literature search on the PubMed, Embase, Web of Science, CNKI, and Wanfang Data databases from inception to October 2021. The following search terms from the Medical Subject Headings (MeSH) vocabulary were used: "Breast Neoplasms", "Methylene Blue", and "Sentinel Lymph Node Biopsy". The Chinese databases were searched with the equivalent Chinese keywords to those from the English databases. In addition, the reference lists of previous reviews were also reviewed for plausible articles. Letters, editorials, case reports, and reviews were excluded from the study. We did not attempt to obtain any unpublished research. Any disagreements were resolved through discussion.

Eligibility criteria

Inclusion criteria

The inclusion criteria for literature were the following: all patients examined were diagnosed with early-stage breast cancer by cytology or histopathology; at least 1 group in the study underwent MB alone, MB + ICG, or MB + Tc99m as a mapping method for SLNB; some or all of the IR, SEN, AR, and FNR indicators could be extracted or calculated from the study; the study was a cohort study or case–control study; and the study publication language was English or Chinese.

Exclusion criteria

Studies that included clinical node-positive patients (cN+), distant metastasis, or surgical contraindications for SLNB were excluded. Patients who received neoadjuvant chemotherapy or radiotherapy before SLNB were also excluded. Studies that used other blue dyes, such as patent blue or isosulfan blue, were excluded. In terms of outcomes, studies that lacked available data were excluded. Studies that

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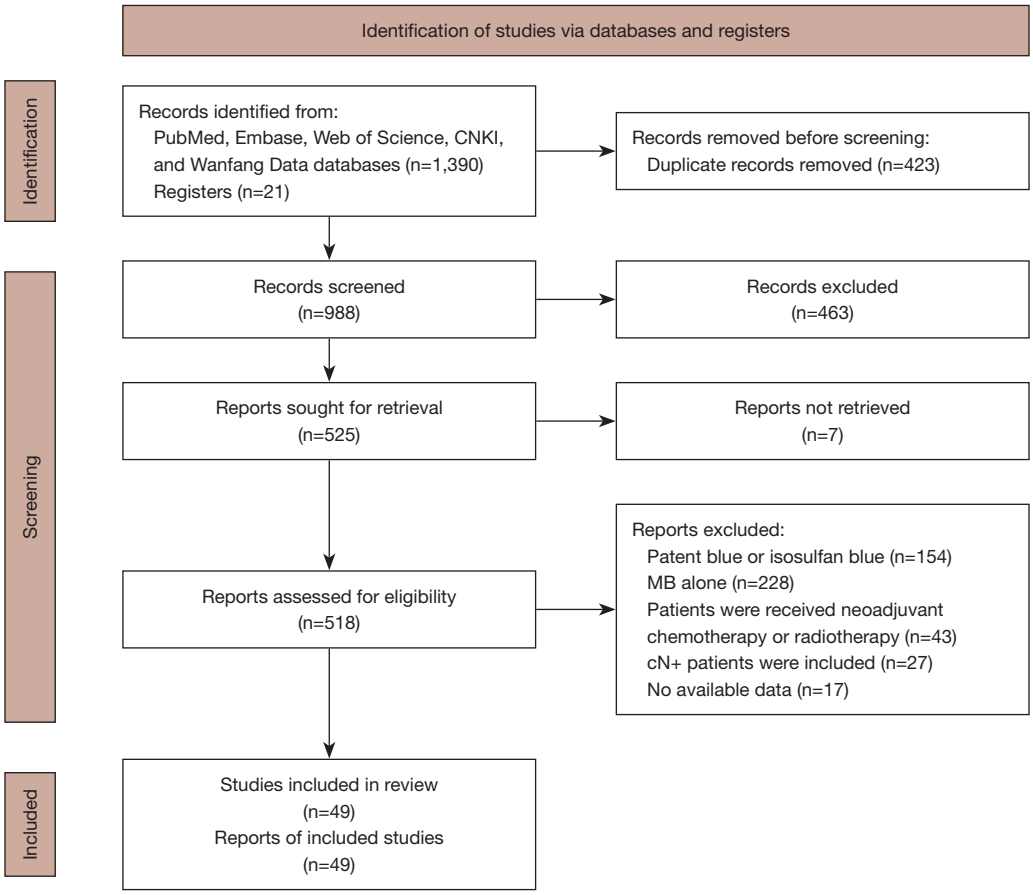

Figure 1 The flowchart of study selection for this meta-analysis.

consisted of letters, editorials, case reports, or reviews were excluded. For studies with overlapping patients or repeated reports, only studies with the largest number of patients were included.

The retrieval strategy is shown in *Figure 1*.

Selection process and data collection process

The data were extracted by 2 independent reviewers (HJL and MSS) and verified for accuracy by 2 other reviewers. Any disagreements were resolved through discussion. Summaries of study characteristics included the first author, publication year, study origin, the age of the patients, the tumor stage, the mapping method for SLNB, and the number of patients enrolled.

Risk of bias assessment

The risk of bias in the studies was assessed with Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2), a standardized tool for evaluating the quality of diagnostic accuracy studies (8). QUADAS-2 contains 4 domains for assessing the risk of bias: patient selection, index test, reference standard, and flow and timing. Signaling questions (yes/no/unclear) are used to assess the risk of bias in each domain. If the answers to all signaling questions in a domain are yes, then the risk of bias can be judged as low. If any signaling question is answered no, then the potential for bias exists. Review authors must then use the guidelines developed in phase 2 to judge the risk of bias. The "unclear" answer is used when insufficient data are reported to allow a judgment. The first 3 domains, patient selection, index test, and reference standard, are further assessed in terms of the applicability of the study to the research question. All studies in this meta-analysis were independently analyzed by 2 independent reviewers (HJL and MSS). The questions adopted in our review are listed in Table S1 and the outcome in our review are listed in Table S2.

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Statistical analysis

In this study, IR was defined as the number of patients for whom SLNs were successfully identified divided by the total number of patients who underwent SLNB. AR was defined as the proportion of people whose ALN status were correctly predicted by SLNB. The results of each successfully identified SLN were further classified as true positive (TP), true negative (TN), or false negative (FN). We then evaluated 2 diagnostic parameters: FNR [FN/(FN + TP)] and sensitivity [TP/(TP + FN)].

The R meta4diag package version 3.6.3 (https://www.r-project.org) was used to perform the pooled analyses of FNR and SEN, which were considered to be diagnostic parameters in this study. The pooled analyses of IR and AR, which were single proportions, were conducted using the "metaprop" function in the R meta package. The logit transformation was implemented to calculate overall proportions. The method of inverse variance was conducted for the pooling of individual studies. The inconsistency statistic (I^2) was used to evaluate the heterogeneity among the studies. The random-effects model was adopted if I^2 was >50%; otherwise, the fixed effects model was used. Potential publication bias was determined by a funnel plots and assessed using Begg's test.

Mixed-comparison analysis using random-effects models, i.e., the NMA was conducted for comparison of IR and AR across the tracers. The NMA was carried out with a random-effects model of the Bayesian framework analysis using the "GeMTC" R package, which includes the software JAGS 4.3.0. Odds ratios (OR) and their 95% CI were applied for the comparisons of IR and AR between the 3 mapping methods.

In this study, all statistical tests were 2 sided, and P values of less than 0.05 were deemed significant.

Results

Basic characteristics of included studies

Our meta-analysis included 7,498 patients in 49 studies published between inception and 2021, of which 43 studies were from China, 4 from India, and 1 each from Turkey and Italy. At least 1 group of patients in 26 studies were subjected to MB + Tc99m in SLNB, and at least 1 group of patients in 35 studies were subjected to MB + ICG in SLNB. *Table 1* lists the basic characteristics of 49 studies, while the retrieval strategy is shown in *Figure 1*.

Identification Rate

IR with MB + Tc99m

Twenty-two studies reported the IR, and with low heterogeneity (I^2=19%, $P_{heterogeneity}$=0.21). A fixed-effects model was used to estimate the IR with MB + Tc99m, with a result of 96% (95% CI: 95–97%; *Figure 2A*).

IR with MB + ICG

Twenty-eight studies reported the IR, and with low heterogeneity (I^2=0%, $P_{heterogeneity}$=0.74). A fixed-effects model was used to estimate the IR with MB + ICG, with a result of 97% (95% CI: 97–98%; *Figure 2B*).

False-negative rate

Seventeen studies reported FNR that ranged from 0% to 14%. The summary estimates of FNR with MB + Tc99m were 7% (95% CI: 5–10%; *Figure 3A*). Eight studies reported FNR that ranged from 3% to 11%. The summary estimates of FNR with MB + ICG were 7% (95% CI: 4–10%; *Figure 3B*).

Sensitivity

Seventeen studies reported SEN that ranged from 86% to 96%. The summary estimates of SEN with MB + Tc99m were 93% (95% CI: 90–95%; *Figure 4A*). Eight studies reported SEN that ranged from 89% to 97%. The summary estimates of SEN with MB + ICG were 93% (95% CI: 90–96%; *Figure 4B*).

Accuracy

Using the random-effects model to estimate the AR with MB + Tc99m produced a result of 96% (95% CI: 94–97%, I^2=0%; $P_{heterogeneity}$ =0.86; *Figure 5A*). Using the fixed-effects model to estimate the AR with MB + ICG produced a result of 97% (95% CI: 96–98%; I^2=0%, $P_{heterogeneity}$ =0.88; *Figure 5B*).

Network meta-analysis

We wanted to simultaneously assess and compare the detection rate among the tracer methods of MB, MB + Tc99m, and MB + ICG. However, studies directly comparing MB + Tc99m and MB + ICG are scarce. We found that 31 of the 49 studies included at least 2 groups

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Table 1 Characteristics of the Included Studies

No.	Study	Year	Origin	Age [year, range]	Tumor stage	The mapping method	No. of patients
1	Tang *et al.* (9)	2005	China	45[a] [29–65]	T_{1-2}	MB + Tc99m	83
						MB	38
2	Zhao *et al.* (10)	2005	China	NR	T_{1-3}	MB + Tc99m	38
3	Lu *et al.* (11)	2006	China	48.7 [32–73]	T_{1-2}	MB + Tc99m	120
4	D'Eredita *et al.* (12)	2006	Italy	57 [27–87]	T_{1-2}	MB + Tc99m	40
				57.6 [40–78]		MB	40
5	Liu *et al.* (13)	2007	China	50±10	T_{1-2}	MB + Tc99m	60
				52±12		MB	104
6	Lin *et al.* (14)	2007	China	44±15.8 [33–74]	T_{1-2}	MB + Tc99m	112
7	Somashekhar *et al.* (15)	2008	India	52 [24–82]	T_{1-2}	MB + Tc99m	100
8	Wang *et al.* (16)	2009	China	52 [34–78]	T_{1-2}	MB + Tc99m	37
						MB	34
9	Chen *et al.* (17)	2009	China	46 [32–58]	T_{1-2}	MB + Tc99m	13
						MB	7
10	Yang *et al.* (18)	2010	China	45 [24–73]	T_{1-2}	MB + Tc99m	109
11	Liu *et al.* (19)	2010	China	52.7 [36–75]	T_{1-2}	MB + Tc99m	36
12	Chen *et al.* (20)	2011	China	31–72	T_{1-2}	MB + Tc99m	31
13	Coskun *et al.* (21)	2012	Turkey	49.8 [27–74]	$T_{(NR)}$	MB + Tc99m	47
						MB	53
14	Lu *et al.* (22)	2012	China	45[a] [26–76]	T_{1-2}	MB + Tc99m	65
15	Tian *et al.* (23)	2012	China	48[a] [19–85]	T_{1-2}	MB + Tc99m	199
						MB	199
16	Cao *et al.* (24)	2014	China	52[a] [29–81]	T_{1-2}	MB + ICG	107
						MB	107
17	Zhang *et al.* (25)	2015	China	45.6±8.5 [27–68]	T_{1-2}	MB + Tc99m	40
18	Ji *et al.* (26)	2015	China	53.00±11.2 [28–71]	T_{1-3}	MB + ICG	65
19	Lei *et al.* (27)	2015	China	22–80	T_{1-2}	MB + Tc99m	195
20	Yuan *et al.* (28)	2016	China	48 [22–77]	T_{1-2}	MB + Tc99m	52
						MB + ICG	52
21	Zhang *et al.* (29)	2016	China	NR	T_{1-2}	MB + ICG	131
						MB	145
22	Liu *et al.* (30)	2016	China	50.21±8.73	T_{1-2}	MB + ICG	62
				49.73±9.60		MB	62

Table 1 (*continued*)

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Table 1 (*continued*)

No.	Study	Year	Origin	Age [year, range]	Tumor stage	The mapping method	No. of patients
23	Cui *et al.* (31)	2016	China	49.58±6.39 [28–71]	T_{1-2}	MB + ICG	100
				50.11±6.80 [26–75]	T_{1-2}	MB	100
24	Tang *et al.* (32)	2016	China	45.6±12.9	$T_{(NR)}$	MB + ICG	95
				46.2+15.9	$T_{(NR)}$	MB	65
25	Zhang *et al.* (33)	2016	China	NR	T_{1-2}	MB + ICG	131
						MB	145
26	Guo *et al.* (5)	2017	China	52 [33–74]	T_{1-2}	MB + ICG	198
						MB	198
27	Ji *et al.* (34)	2017	China	53±11.2	T_{1-3}	MB + ICG	65
28	Heng *et al.* (35)	2017	China	NR	T_{1-2}	MB + ICG	46
						MB	74
29	Sun *et al.* (36)	2017	China	NR	$T_{(NR)}$	MB + ICG	85
						MB	85
30	Yuan *et al.* (37)	2019	China	52.6±10.8	T_{1-3}	MB + ICG	245
						MB	38
31	Agarwal *et al.* (38)	2018	India	NR	$T_{(NR)}$	MB + Tc99m	78
32	Shen *et al.* (39)	2018	China	47.8±10.8	T_{1-2}	MB + ICG	374
				47.2±9.7		MB	149
33	Li *et al.* (40)	2018	China	54.3+1.6	T_{1-2}	MB + ICG	85
				54.1±1.8		MB	85
34	Zhang *et al.* (41)	2018	China	47.52±5.78	T_{1-3}	MB + ICG	136
				48.52±6.30		MB	132
35	Lei *et al.* (42)	2015	China	63.7 [61–69]	T_{1-2}	MB + ICG	63
36	Gupta *et al.* (43)	2020	India	54.5 [53.5±11.05]	T_{1-2}	MB + Tc99m	30
				53.5 [56.6±11.26]		MB	30
37	Qin *et al.* (44)	2019	China	NR	T_{1-3}	MB + ICG	60
						MB	60
38	Zhou *et al.* (45)	2019	China	46.9±15	T_{1-3}	MB + ICG	316
39	Zhu *et al.* (46)	2019	China	46.3	T_{1-2}	MB + ICG	105
				48.3		MB	101
40	Zhu *et al.* (47)	2019	China	46.2±15.9 [33–74]	$T_{(NR)}$	MB + ICG	95
				45.6±12.9 [32–75]		MB	65
41	Zhao *et al.* (48)	2019	China	47.53±5.45	$T_{(NR)}$	MB + ICG	86
				48.02±5.27		MB	86

Table 1 (*continued*)

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Table 1 (*continued*)

No.	Study	Year	Origin	Age [year, range]	Tumor stage	The mapping method	No. of patients
42	Liu *et al.* (49)	2019	China	52.5±17.5	T_{1-2}	MB + ICG	70
				52.3±17.4		MB	70
43	Zhou *et al.* (50)	2019	China	52.8 [27–78]	$T_{(NR)}$	MB + ICG	140
						MB	140
44	Gong *et al.* (51)	2019	China	NR	T_{1-2}	MB + Tc99m	43
45	Huang *et al.* (52)	2019	China	20–70	T_{1-2}	MB + ICG	20
46	Bai *et al.* (53)	2020	China	52.4±9.8	T_{1-2}	MB + ICG	57
				53.1±8.4		MB	57
47	Huang *et al.* (54)	2020	China	51.8±5.2	T_{1-2}	MB + ICG	50
				50.6±4.9		MB	50
48	Zhang *et al.* (55)	2021	China	30–77 [M46.5]	T_{1-2}	MB + ICG	197
						MB	218
49	Fang *et al.* (56)	2021	China	NR	T_{1-2}	MB + Tc99m	92
						MB	92

[a], Median. NR, no record; MB, methylene blue; ICG, indocyanine green; Tc99m, 99m Technetium-labeled Sulphur Colloid.

of patients who used MB alone and MB + Tc99m or MB + ICG. We therefore conducted an NMA, in pairwise comparison: if MB participated in the comparison, then MB was taken as the reference; otherwise, MB + Tc99m was taken as the reference.

Mixed-comparison analysis using random-effects models was conducted for comparison of IR and AR across three tracers. Compared with MB alone, MB + Tc99m (OR, 4.66; 95% CI: 2.19–10.08) and MB + ICG (OR, 6.17; 95% CI: 4.02–10.29) contributed to higher IR. No statistical significance was found in comparison between MB + Tc99m and MB + ICG (OR, 1.33; 95% CI: 0.56–3.32). With regard to AR, significant difference was only observed between MB and MB + ICG (OR, 2.89; 95% CI: 1.51–5.75), indicating a higher AR when using MB + ICG as the tracer. No significant difference was found in comparison between MB and MB + Tc99m (OR, 2.12; 95% CI: 0.84–5.81), or between MB + Tc99m and MB + ICG (OR, 1.37; 95% CI: 0.41–4.20).

Table 2 gives the estimated mean difference in accuracy rate (top right) and identification rate (bottom left) between each combination of mapping methods obtained from mixed-comparison models.

Quality assessment of included studies and publication bias

QUADAS-2 was used to assess the quality of each study, and these results are listed in Table S2. All the studies had a high risk of patient selection bias, as they had a case–control design. Some studies had a high risk or an unclear risk of flow and timing bias, mainly due to the advancement of surgical treatment methods for breast cancer and not all patients having received axillary lymph node dissection (ALND). All other risks were rated as low.

Since the number of articles that each research indicator was reported were varied, we used IR, which was reported in the highest number of studies, to evaluate publication bias. The left and right sides in the IR funnel plot are nearly symmetrical, which suggests that there was a low possibility of publication bias (*Figure 6A*,*6B*). The Begg's test values of IR using MB + Tc99m and MB + ICG were P=0.17 and P=0.04, respectively, which suggests that there also was a low possibility of publication bias of MB + ICG.

Discussion

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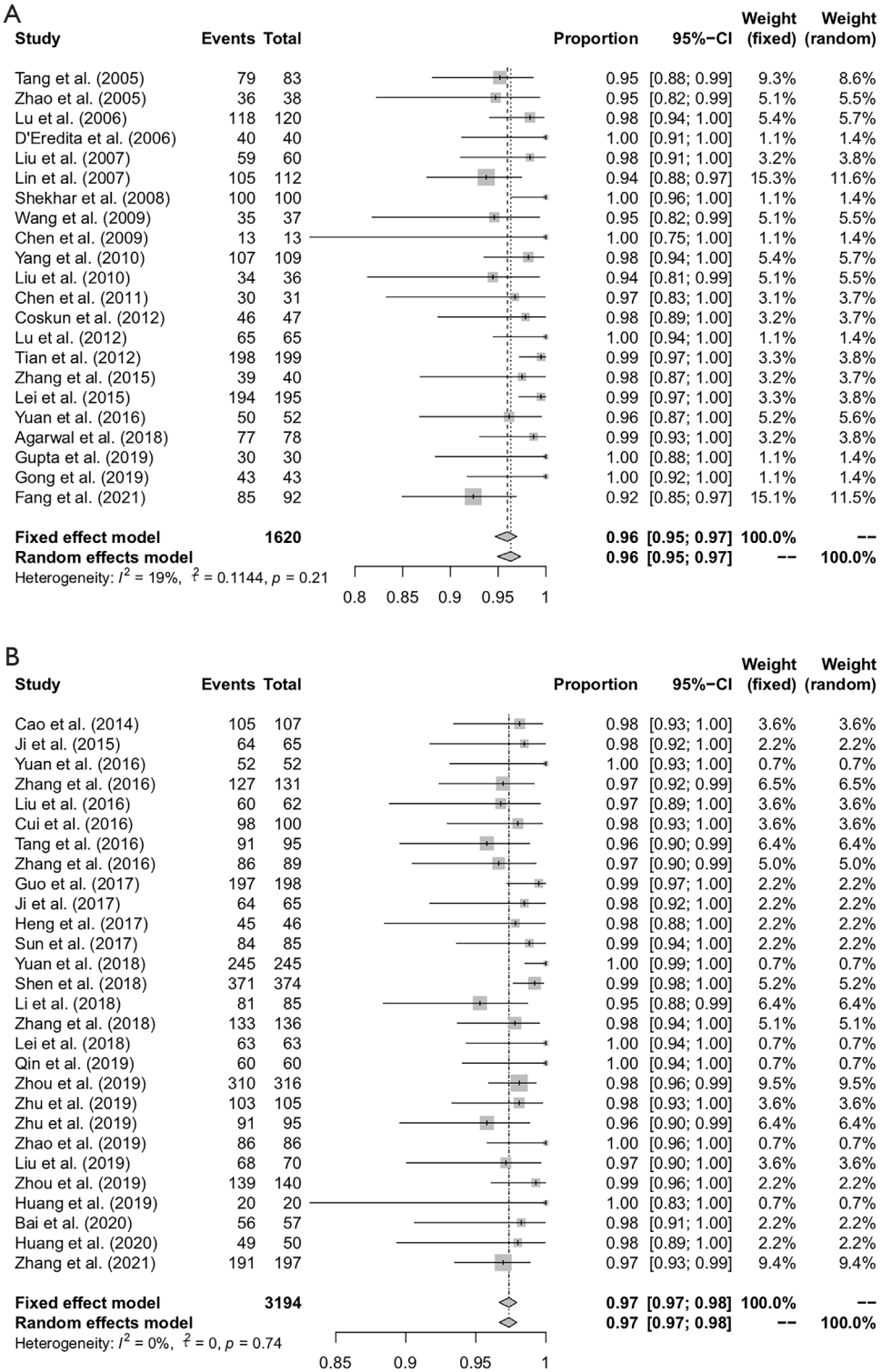

Figure 2 The identification rate of MB + Tc99m and MB + ICG. (A) A fixed-effects model was used to estimate the IR with MB + Tc99m. (B) A fixed-effects model was used to estimate the IR with MB + ICG. MB, methylene blue; Tc99m, 99m technetium-labeled sulphur colloid; ICG, indocyanine green; IR, identification rate.

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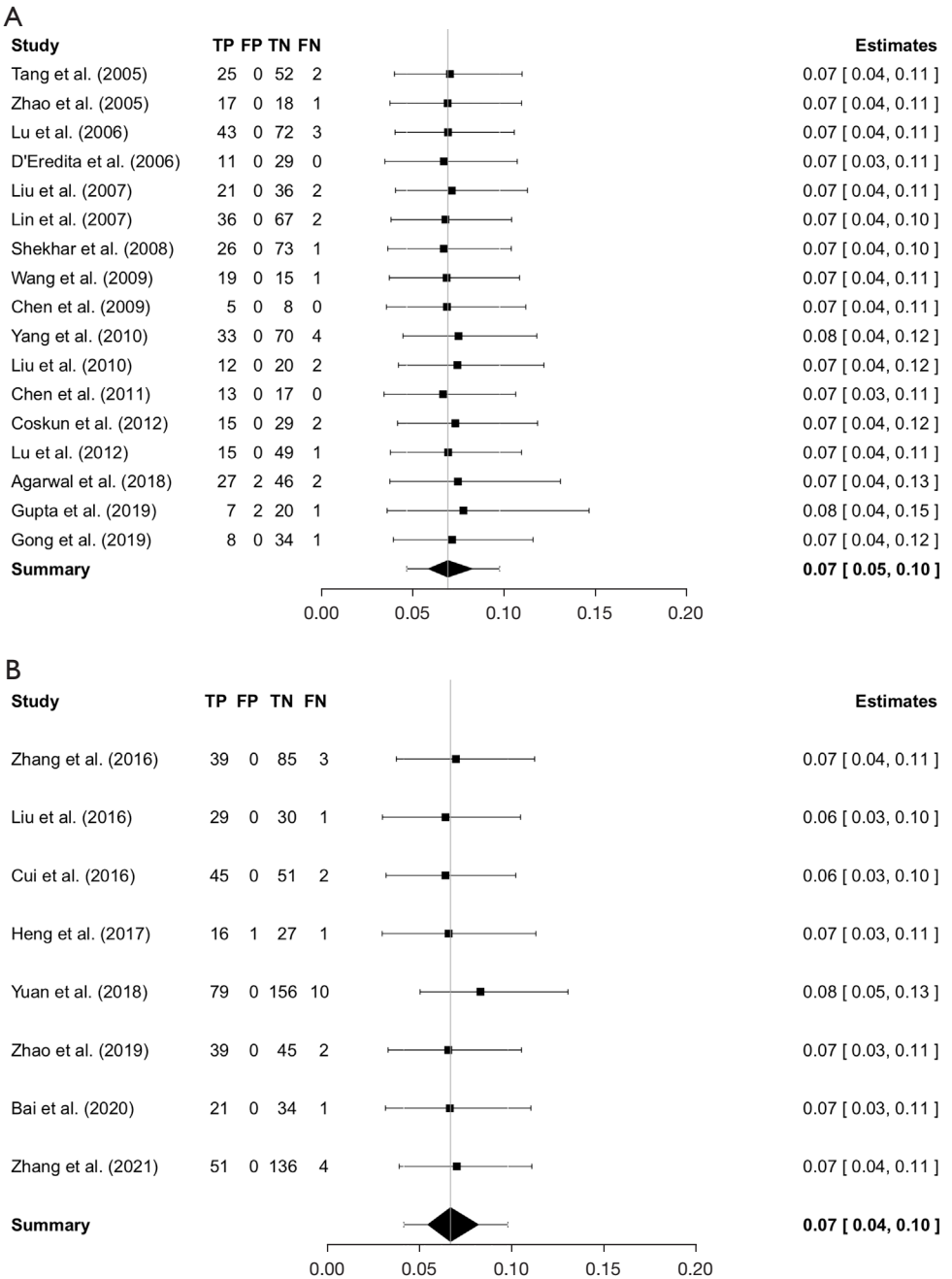

Figure 3 The false-negative rate of MB + Tc99m and MB + ICG. (A) The summary estimates of the false-negative rate with MB + Tc99m. (B) The summary estimates of the false-negative rate with MB + ICG. MB, methylene blue; Tc99m, 99m technetium-labeled sulphur colloid; ICG, indocyanine green; TP, true positive; FP, false positive; FN, false negative; TN, true negative.

and other guidelines agree that SLNB should be the standard method used for ALN staging in cN_0 early-stage breast cancer, and that patients who are SLNB negative can be exempted from ALND (4,6,57-60). As a common tracer for SLNB, blue dye has been used widely in clinical practice, either alone or in combination with other tracers. The standard blue dyes, patent blue or isosulphan blue, combined with radioisotope tracers is the preferred trace method recommended by the American Society of Clinical Oncology (ASCO) for SLNB (61). However, for reasons

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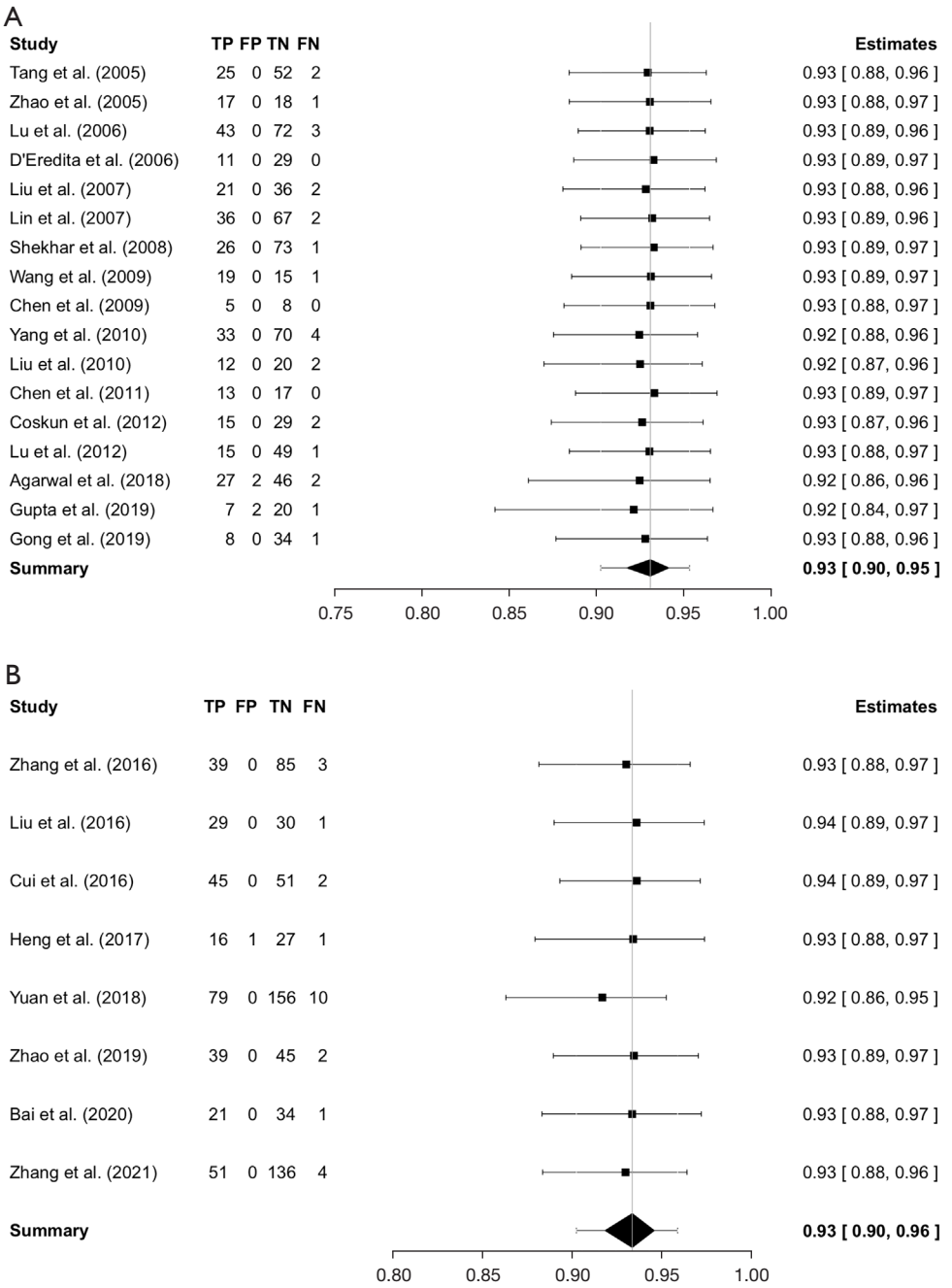

Figure 4 The Sensitivity of MB + Tc99m and MB + ICG. (A) The summary estimates of sensitivity with MB + Tc99m. (B) The summary estimates of sensitivity with MB + ICG. MB, methylene blue; Tc99m, 99m technetium-labeled sulphur colloid; ICG, indocyanine green; TP, true positive; FP, false positive; FN, false negative; TN, true negative.

involving the availability of drugs and health economics, these two standard tracers cannot be used clinically in many developing countries, including China.

Studies have demonstrated that the IR and FNR of MB used as a substitute for blue dye in SLNB show no clinical or statistical differences when compared with isosulphan blue (62). A meta-analysis of 18 studies from 2000 to 2017 found that when MB alone was used, the IR was 91% and

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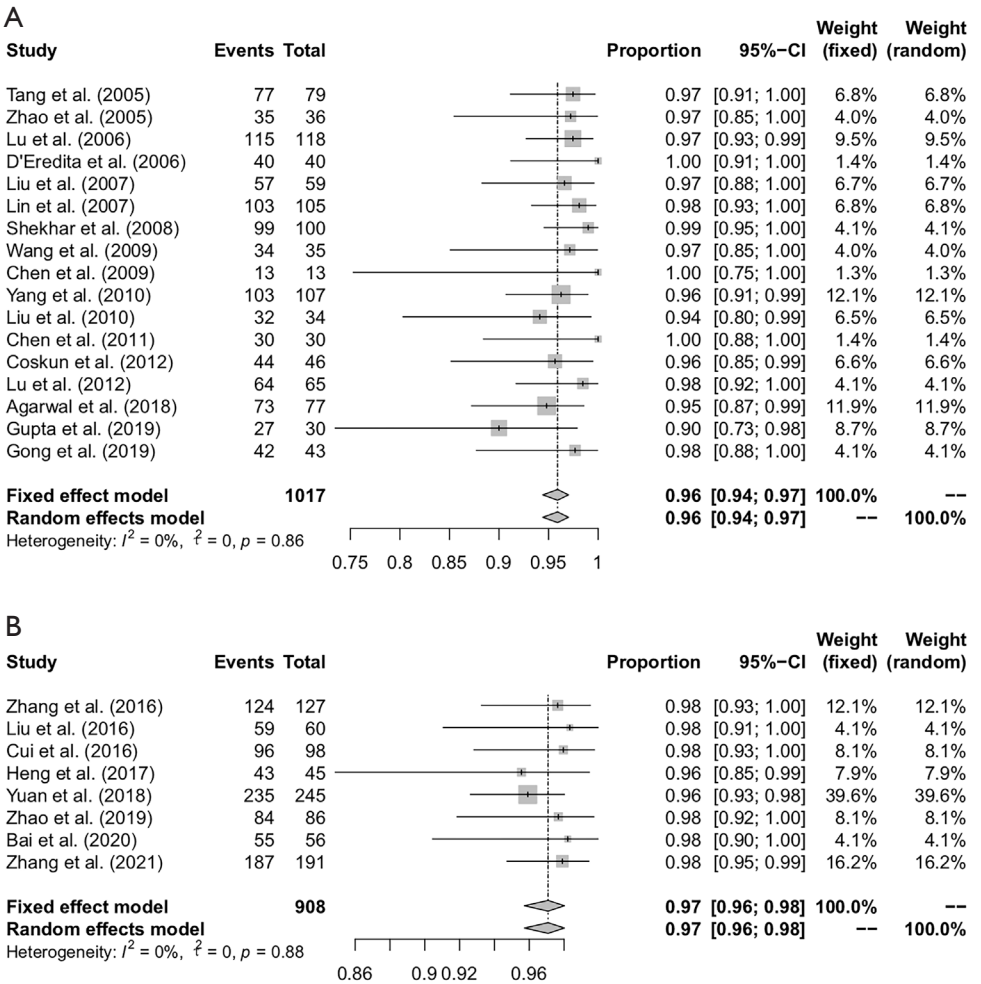

Figure 5 The Accuracy of MB + Tc99m and MB + ICG. (A) A random-effects model was used to estimate the AR with MB + Tc99m, with a result of 96% (95% CI: 94–97%; I2=0%). (B) A fixed-effects model was used to estimate the AR with MB + ICG, with a result of 97% (95% CI: 96–98%, I2=0%) of MB + ICG. MB, methylene blue; Tc99m, 99m technetium-labeled sulphur colloid; ICG, indocyanine green; AR, accuracy rate.

Table 2 Mixed-comparison analysis for comparison of accuracy rate (top right) and identification rate (bottom left) across the three tracers

Mapping method	MB	MB + Tc99m	MB + ICG
MB	–	2.12 (0.84–5.81)	2.89 (1.51–5.75)
MB + Tc99m	4.66 (2.19–10.08)	–	1.37 (0.41–4.20)
MB + ICG	6.17 (4.02–10.29)	1.33 (0.56–3.32)	–

Above the leading diagonal are the estimates of the mean difference in accuracy rate (95% CI), and below the leading diagonal are the estimates of the mean difference in identification rate. Results are presented as odds ratios and 95% confidence intervals. MB, methylene blue; Tc99m, 99m technetium-labeled sulphur colloid; ICG, indocyanine green.

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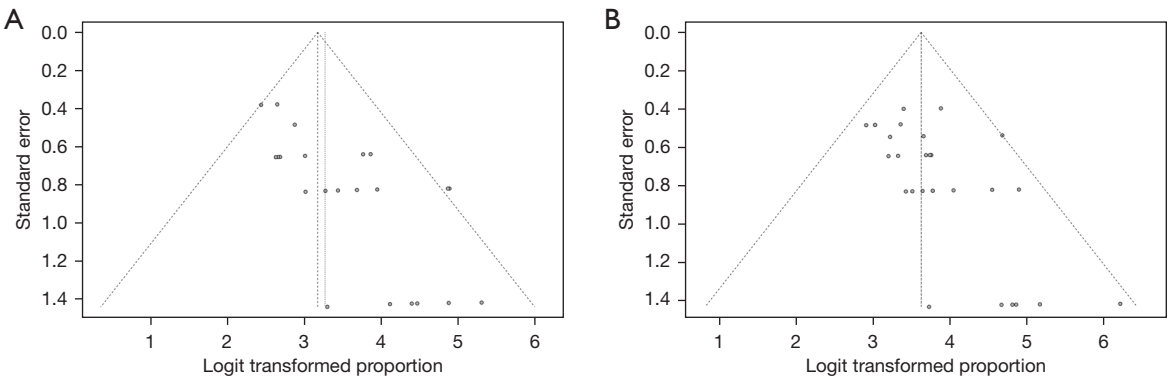

Figure 6 Funnel plot used to assess the effects of publication bias on the IR of MB + Tc99m or MB + ICG. (A) The funnel plot of IR of MB + Tc99m. (B) The funnel plot of IR of MB + ICG. IR, identification rate; MB, methylene blue; Tc99m, 99m technetium-labeled sulphur colloid; ICG, indocyanine green.

the FNR was 13%, with a FNR of <10% reported in the past 5 years. These rates conform to the recommended standards of the American Society of Breast Surgeons (ASBrS) (63). MB alone is therefore a safe and effective alternative to standard blue dyes in the clinical practice of SLNB.

To further improve IR and reduce FNR, MB has been combined with other tracers, including radioisotopes and fluorescent tracers, which are now used widely in clinical practice. Previous systematic reviews and meta-analyses have investigated the use of MB, ICG, and Tc99m in SLNB. Wang *et al.* (64) conducted a meta-analysis of 15 studies from China. The results showed that the detection rate, number of detections, sensitivity, and specificity of MB + ICG were significantly increased compared with MB alone, while the FNR decreased significantly. A systematic review published by Kim *et al.* (65), which included 69 studies investigating SLNB and ALND of early-stage breast cancer, concluded that the dual-tracer mapping method had a higher IR compared with radioisotope or blue dye alone.

The present study evaluated studies that included both MB and MB + Tc99m or MB + ICG. Our NMA showed that the IR, FNR, SEN, and AR using MB + Tc99m were 96%, 7%, 93%, and 96%, respectively, while the IR, FNR, SEN, and AR using MB + ICG were 97%, 7%, 93%, and 97%, respectively. These results are superior to the IR and FNR of the MB single-tracer mapping method reported in previous literature (63).

IR and FNR are important indicators for evaluating the effectiveness of the tracer in SLNB. It is important to note that many factors can influence IR; for example, research has shown that experienced surgeons can achieve a 95.6% IR with blue dye alone (66). However, this meta-analysis suggests that MB combined with Tc99m or ICG can achieve a higher overall IR, which is consistent with the conclusions of studies using other blue dyes combined with radioisotopes or fluorescent tracers (67-69).

With respect to FNRs, the ASBrS previously stated that an FNR below 5% could only be accepted when the AR was greater than 95% (70). However, most recent studies have reported an FNR between 5% and 10%. Our research found that the FNR of both MB + Tc99m and MB + ICG was 7%. Therefore, in terms of clinical practice, the dual-tracer mapping method has significant advantages compared with the use of MB alone, which was shown to have a FNR of 13% in a previous meta-analysis. Wong *et al.* (71) found that when 1 SLN was obtained, the FNR was 14.3%, and that when 2 or more SLNs were obtained, the FNR was 4.3%. Among the 40 studies we included that reported the number of SLNs, only 6 studies reported fewer than 2 SLNs after using MB + Tc99m or MB + ICG. However, it should be noted that not all studies clearly indicated that the identification of SLN involved intraoperative pathological evaluation, and that the studies demonstrated differences in the pathological evaluation of positive lymph nodes. Therefore, we believe that the FNR of MB + Tc99m or MB + ICG is higher than 5% or 4.3%, as the studies in the meta-analysis included some retrospective studies of small samples which did not report the number of SLNs obtained, the pathological evaluation criteria, or whether or not intraoperative pathological evaluation occurred.

When comparing MB + Tc99m and MB + ICG, MB + ICG has some advantages over MB + Tc99m in IR and AR. Compared with the limitations radionuclide

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use in clinical practice, such as the high requirements of personnel qualification and management, the high price of equipment and tracers, the difficulty of storage, and the potential radioactive damage to patients and staff, the use of ICG is easier to promote. The findings of this study provide evidence-based support for the clinical application of MB + ICG.

Although no significant heterogeneity was found in this study, the AR (I^2=61%, P<0.01) using MB + Tc99m demonstrated a degree of heterogeneity. Despite the utility of sensitivity analysis and meta regression, the origin of the heterogeneity could not be thoroughly traced. Many of the studies included in this meta-analysis were retrospective studies of relatively low quality. In terms of publication bias, our application of Begg's tests using the IR of MB + Tc99m (P=0.17) or MB + ICG (P=0.04) show less possibility of publication bias; meanwhile, potential bias may exist due to the tendency for positive results to be published and our strict inclusion criteria. This study also limited the publication language to English or Chinese, so publication bias cannot be totally excluded.

This evidence-based study has demonstrated that the MB single-tracer method can be used safely in clinical practice, especially in areas where access to other tracers is limited (72). Our meta-analysis showed that the MB + Tc99m or MB + ICG mapping methods can be used to obtain higher IR and lower FNR than MB alone. Our NMA showed no statistical significance between MB + Tc99m and MB + ICG with IR and AR.

Acknowledgments

We sincerely thank all the reviewers and editors for their supportive comments.
Funding: This work was funded by The Beijing Medical Award Foundation (Grant number: YXJL-2016-0040-0065).

Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://dx.doi.org/10.21037/tcr-21-1239

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tcr-21-1239). HJL, MSS, ZHY, XXC, QL, YJC, LX, YHL, and JMY report receiving funding from The Beijing Medical Award Foundation (Grant number YXJL-2016-0040-0065). LYL has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Liu HJ, Sun MS, Liu LY, Yu ZH, Chen XX, Liu Q, Cheng YJ, Xu L, Liu YH, Ye JM. The detection rate of methylene blue combined with another tracer in sentinel lymph node biopsy of early-stage breast cancer: a systematic review and network meta-analysis. Transl Cancer Res 2021;10(12):5222-5237. doi: 10.21037/tcr-21-1239

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Supplementary

Table S1 Judgments on Bias and Applicability according to QUADAS 2

Domain		Risk and bias (Signaling question)	Applicability
Patient Selection	Could the Selection of Patients Have Introduced Bias?	Was a consecutive or random sample of patients enrolled?	Are there any differences in TNM stage or age among patients using different mapping methods in SLNB?
		Was a case-control design avoided?	
		Did the study avoid inappropriate exclusions?	
		Did the spectrum of patients enrolled represented the patient population who will actually be tested for the indicator?	
Index Test	Could the Conduct or Interpretation of the Index Test Have Introduced Bias?	Were the index test results interpreted without knowledge of the results of the reference standard?	Are there concerns that the index test, its conduct, or its interpretation differ from the review question? Were patients mapped with patent blue or isosulfan blue excluded?
		If a threshold was used, was it prespecified?	
Reference Standard	Could the Reference Standard, Its Conduct, or Its Interpretation Have Introduced Bias?	Was the reference standard likely to correctly classify the target condition?	Are there concerns that the target condition as defined by the reference standard does not match the question?
		Were the reference standard results interpreted without knowledge of the results of the index test?	
Flow and Timing	Could the Patient Flow Have Introduced Bias?	Did all patients receive ALND?	
		Was there an appropriate interval between the index test and reference standard?	
		Did all patients receive the same reference standard?	
		Were all patients included in the analysis?	
		Was the calculation method or outcome of IR, AR, SEN or FNR in this study consistent with other studies ?	

SLNB, sentinel lymph node biopsy; ALND, axillary lymph node dissection; IR, identification rate; AR, accuracy rate; SEN, sensitivity; FNR, false-negative rate.

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https://dx.doi.org/10.21037/tcr-21-1239

Table S2 Results of quality assessment of the included studies according to QUADAS 2

No.	Study	Risk and bias				Applicability		
		Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
1	Tang *et al.* (9)	☹	☺	☺	☺	☺	☺	☺
2	Zhao *et al.* (10)	☹	☺	☺	☺	☺	☺	☺
3	Lu *et al.* (11)	☹	☺	☺	☺	☺	☺	☺
4	D'Eredita *et al.* (12)	☹	☺	☺	☺	☺	☺	☺
5	Liu *et al.* (13)	☹	☺	☺	☺	☺	☺	☺
6	Lin *et al.* (14)	☹	☺	☺	☺	☺	☺	☺
7	Somashekhar *et al.* (15)	☹	☺	☺	☺	☺	☺	☺
8	Wang *et al.* (16)	☹	☺	☺	☺	☺	☺	☺
9	Chen *et al.* (17)	☹	☺	☺	☺	☺	☺	☺
10	Yang *et al.* (18)	☹	☺	☺	☺	☺	☺	☺
11	Liu *et al.* (19)	☹	☺	☺	☺	☺	☺	☺
12	Chen *et al.* (20)	☹	☺	☺	☺	☺	☺	☺
13	Coskun *et al.* (21)	☹	☺	☺	☺	☺	☺	☺
14	Lu *et al.* (22)	☹	☺	☺	☺	☺	☺	☺
15	Tian *et al.* (23)	☹	☺	☺	☹	☺	☺	☺
16	Cao *et al.* (24)	☹	☺	☺	☺	☺	☺	☺
17	Zhang *et al.* (25)	☹	☺	☺	☹	☺	☺	☺
18	Ji *et al.* (26)	☹	☺	☺	☹	☺	☺	☺
19	Lei *et al.* (27)	☹	☺	☺	☹	☺	☺	☺
20	Yuan *et al.* (28)	☹	☺	☺	☹	☺	☺	☺
21	Zhang *et al.* (29)	☹	☺	☺	☺	☺	☺	☺
22	Liu *et al.* (30)	☹	☺	☺	☺	☺	☺	☺
23	Cui *et al.* (31)	☹	☺	☺	☺	☺	☺	☺
24	Tang *et al.* (32)	☹	☺	☺	?	☺	☺	☺
25	Zhang *et al.* (33)	☹	☺	☺	☹	☺	☺	☺
26	Guo *et al.* (5)	☹	☺	☺	☹	☺	☺	☺
27	Ji *et al.* (34)	☹	☺	☺	☹	☺	☺	☺
28	Heng *et al.* (35)	☹	☺	☺	☺	☺	☺	☺
29	Sun *et al.* (36)	☹	☺	☺	☹	☺	☺	☺
30	Yuan *et al.* (37)	☹	☺	☺	☺	☺	☺	☺
31	Agarwal *et al.* (38)	☹	☺	☺	☺	☺	☺	☺
32	Shen *et al.* (39)	☹	☺	☺	☹	☺	☺	☺
33	Li *et al.* (40)	☹	☺	☺	?	☺	☺	☺
34	Zhang *et al.* (41)	☹	☺	☺	?	☺	☺	☺
35	Lei *et al.* (42)	☹	☺	☺	?	☺	☺	☺
36	Gupta *et al.* (43)	☹	☺	☺	☺	☺	☺	☺
37	Qin *et al.* (44)	☹	☺	☺	☺	☺	☺	☺
38	Zhou *et al.* (45)	☹	☺	☺	☹	☺	☺	☺
39	Zhu *et al.* (46)	☹	☺	☺	☹	☺	☺	☺
40	Zhu *et al.* (47)	☹	☺	☺	?	☺	☺	☺
41	Zhao *et al.* (48)	☹	☺	☺	☺	☺	☺	☺
42	Liu *et al.* (49)	☹	☺	☺	?	☺	☺	☺
43	Zhou *et al.* (50)	☹	☺	☺	?	☺	☺	☺
44	Gong *et al.* (51)	☹	☺	☺	☺	☺	☺	☺
45	Huang *et al.* (52)	☹	☺	☺	☹	☺	☺	☺
46	Bai *et al.* (53)	☹	☺	☺	☺	☺	☺	☺
47	Huang *et al.* (54)	☹	☺	☺	?	☺	☺	☺
48	Zhang *et al.* (55)	☹	☺	☺	☺	☺	☺	☺
49	Fang *et al.* (56)	☹	☺	☺	?	☺	☺	☺

☺ = low risk; ☹ = high risk; ? = unclear risk.

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https://dx.doi.org/10.21037/tcr-21-1239