Prognostic value of CD8+ tumor-infiltrating T cells in patients with breast cancer: A systematic review and meta-analysis

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Abstract. CD8+ tumor-infiltrating lymphocytes have been regarded as potential biomarkers for cancer prognosis, while the prognostic effect of CD8+ tumor-infiltrating T cells remains controversial in breast cancer. In the present study, a meta-analysis was performed to evaluate the prognostic value of CD8+ T cells in breast cancer and the associations between CD8+ T cells and the pathological characteristics. The PubMed, Embase and the Cochrane Library were systematically searched entries added from the establishment of the database to November 2021 and prospective or retrospective studies of patients with breast cancer were included. The Newcastle-Ottawa Scale was used to assess the quality of evidence for each study. STATA 15.1 was used for the data analysis. A total of 14 studies comprising 22,222 patients were included in the final analysis and the pooled results suggested that a high CD8+ T-cell infiltration level was significantly related to better overall survival [hazard ratio (HR)=0.70, 95% confidence interval (CI): 0.60-0.82, P<0.001] and disease-free survival (HR=0.63, 95% CI: 0.49-0.81, P<0.001) for patients with breast cancer. In addition, a high CD8+ T-cell infiltration level was significantly associated with decreased expression of estrogen receptor [odds ratio (OR)=1.92, 95% CI: 1.30-2.85, P=0.001] and progesterone receptor (OR=1.66, 95% CI: 1.14-2.42, P=0.008), and increased human epidermal growth factor receptor 2 expression (OR=0.79, 95% CI: 0.66-0.94, P=0.010) in patients with breast cancer, while there was no significant association between CD8+ T-cell infiltration and age, tumor size or lymph node status of patients with breast cancer (P>0.05). In conclusion, CD8+ T-cell infiltration is of prognostic value in patients with breast cancer. High levels of CD8+ T-cell infiltration were related to improved prognosis, including OS and DFS, in patients with breast cancer.

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Introduction

Breast cancer, one of the most common malignant tumor types in females, is the leading cause of death in women worldwide (1). According to global cancer statistics, in 2020, 2.26 million new cases of female breast cancer were diagnosed, accounting for about a quarter of female malignancies, and the death toll from breast cancer was 680,000, ranking first among female malignancies (2). Although early detection, early diagnosis and early treatment have contributed to a gradual decline in breast cancer mortality over the past few decades, the prognosis of patients remains poor (3). New prognostic biomarkers are still needed to develop targeted therapies and improve patient survival.

Recently, several studies have demonstrated the importance of the tumor immune microenvironment in cancer progression (4). Among them, the interaction between tumor cells and immune cells has become the focus of current attention (5). Tumor-infiltrating lymphocytes (TILs) are important components of the tumor immune microenvironment and have a key role in the local immune response of cancer (6). The appearance of TILs is a sign of the immune response of the host's immune system to tumor antigens and reflects the local immune response of the tumor. The level of TILs in the primary tumor reflects the body's anti-tumor potential and their quantity also indicates the therapeutic effect against the tumor (7,8). In the adaptive immune system, cytotoxic (CD8+) T cells are the primary immune cells involved in recognizing and killing tumors (9,10). In breast cancer, the relationship between the expression of CD8+ TILs and prognosis has remained to be fully elucidated. Most previous studies on CD8+ T lymphocytes in breast cancer reported that CD8+ T cells were associated with improved prognosis (11-13). However, other studies were in disagreement with this (14). In addition to their association with survival, certain studies have also found a link between the presence of immune cells and the effect of chemotherapy (15,16). Therefore, analyzing the prognostic value of CD8+ T cells in breast cancer may improve prognosis and enhance the application of individualized and customized therapy.

The objective of the present systematic review and meta-analysis was to investigate the prognostic value of CD8+ T cells in breast cancer and to explore the association between CD8+ T cells and the pathological characteristics of

patients with breast cancer in order to provide new prognostic biomarkers for the clinical treatment of breast cancer.

Materials and methods

Protocol and registration. The present systematic review and meta-analysis were performed based on the requirements of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (17). The protocol was registered at PROSPERO with the following ID: CRD42022313171.

Literature inclusion and exclusion criteria. The inclusion criteria were as follows: Prospective or retrospective studies were included in this meta-analysis; patients with breast cancer were the subject of the research; the language was limited to English. The following exclusion criteria were applied: Duplicate publications; research without full text, incomplete information or inability to conduct data extraction; animal experiments; reviews and systematic reviews.

Search strategy. For the present meta-analysis, the Pubmed (https://pubmed.ncbi.nlm.nih.gov/), Embase (https://www.embase.com) and Cochrane Library (https://www.cochranelibrary.com/) databases were searched from the establishment of the database up until to November 2021. The search terms were mainly as follows: 'Breast neoplasm', 'breast tumor', 'breast cancer', 'breast carcinoma' AND 'CD8-positive T-lymphocytes', 'CD8-positive lymphocyte', 'CD8+T' AND 'prognosis', 'prognostic', 'overall survival', 'disease-free survival' and 'progression-free survival'. As the analysis was based on published studies, neither ethical approval nor patient consent was required.

Literature screening and data extraction. The literature search, screening and information extraction were all independently completed by two researchers (YPS and YLK). In case of any doubts or disagreements, the decision was made after discussion or consultation with the third researcher (XL). The data extracted included the author, year of publication, country, study design, sample size, median age, median follow-up time, location of $CD8^+$ TILs and the indicators for evaluating outcome, including hazard ratio (HR) for overall survival (OS) and disease-free survival (DFS).

Literature quality assessment. The quality of evidence for each study was assessed by two independent researchers (YPS and YLK) using the Newcastle-Ottawa Scale (NOS) (18). The NOS includes 4 items (4 points) for 'Research Subject Selection', 1 item (2 points) for 'Comparability between Groups' and 3 items (3 points) for 'Result Measurement', with a full score of 9 points and studies with ≥7 are regarded as high-quality literature, while those with <7 are classified as lower-quality literature. When the ratings were inconsistent, the score was decided through discussion or consultation with the third researcher (XL). Publication bias was assessed by two independent researchers (YPS and YLK) using funnel plots and Egger's test. The sensitivity analysis was performed by two independent researchers (YPS and YLK) if necessary. In case of any doubts or disagreements, the decision was made after discussion or consultation with the third researcher (XL).

Data synthesis and statistical analysis. STATA 15.1 (Stata Corporation) was used to analyze the data, with $P<0.05$ suggesting a statistical significance. The HR (95% CI) was used to evaluate the OS and DFS. Cochran's Q-test and I^2 statistics were used to evaluate the heterogeneity among the included studies. If the heterogeneity test result was $P\geq0.1$ or $I^2\leq50\%$, it indicated that there was no significant heterogeneity among studies. Subsequently, the fixed-effects Mantel-Haenszel model (19) was used for combined OR analysis and the fixed-effects Inverse-Variance model was used for pooled HR evaluation. Otherwise, if $P<0.1$ or $I^2>50\%$, it indicated that there was statistically significant heterogeneity and the random-effects model (REM) according to DerSimonian and Laird (20) was used to analyze the pooled results. Sensitivity analysis and subgroup analysis were used to identify the source of heterogeneity. A funnel plot and Egger's test were jointly used to assess the publication bias.

In a fixed-effects model (FEM) (21), it is assumed that all included studies share a common true effect size. The sampling error is the only one source of variation, which is equal to the within-study error variance. The weight assigned to each study is based on the inverse of the within-study error variance, which is related to the precision of the estimation of each study. In general, studies with a larger sample yield a more precise estimate of the population mean and thus will be assigned more weight compared to those with a smaller sample. Therefore, studies with a small sample will be neglected more easily. By contrast, in an REM (22,23), the true effect size changes according to different included studies. There are two levels of sampling, and therefore two sources of variance. The overall study error variance in a random-effects meta-analysis contains two components: One is the within-study error variance due to sampling error (same as in the FEM) and the other is the inter-study variance resulting from the difference in effect size distribution. Therefore, the weight assigned to each study is based on the inverse of the sum of the within-study error variance and the inter-study variance. Different from the FEM, studies with a small sample may also be assigned more weight if the inter-study variance is small. If there is no significant heterogeneity between studies, the inter-study variance is zero. The result estimated from the REM would then be identical to that of the FEM. The challenge in the REM is how to estimate the inter-study variance. The DerSimonian and Laird estimate is easy to compute and is qualitatively consistent with the heterogeneity test based on the Q statistic.

Results

Results of the literature search. A total of 305 studies were retrieved from the Pubmed, Embase and Cochrane Library databases via a literature search. After the removal of duplicates, 156 studies were preliminarily screened by browsing the titles and abstracts with 49 studies excluded, retaining 107 studies for further full-text screening. After browsing through the full text, 14 studies were retained as eligible for inclusion. Finally, 14 studies were included in the meta-analysis. Fig. 1 illustrates the flowchart of the selection process with reasons for exclusions.Baseline characteristics and quality assessment of the included studies. A total of 14 retrospective studies (11,12,14,24-34) were included in the

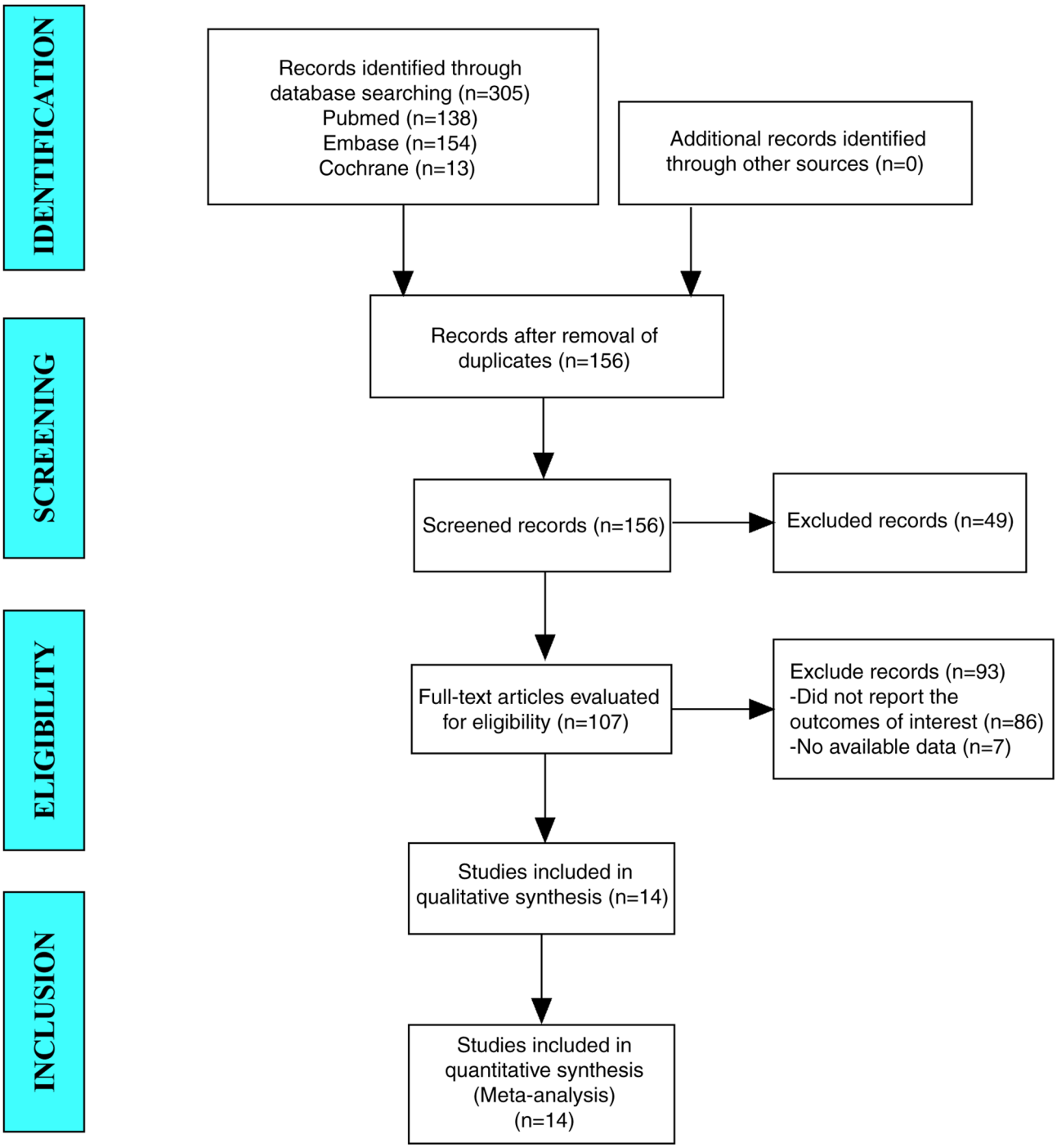

Figure 1. Flow diagram for the selection of included studies.

present meta-analysis. The sample size of patients was 22,222 in total. The patients from 8 articles were from Asia, while the patients from 5 articles were from Europe. Furthermore, the patients from Ali *et al* (29) were from several countries. All of the studies were published between 2011 and 2019. Locations of $CD8^+$ TILs included Intratumoral, Peritumoral and Intratumoral & Peritumoral. The NOS scores used for quality assessment were all >7. The baseline characteristics of all included studies are listed in Table I. The CD8+ T-cell infiltration level was derived from the original studies. The CD8+ T-cell infiltration was assessed via immunohistochemistry staining and evaluated manually by the number of positive cells or the density of positive cells. Since the evaluation methods and cutoff points to separate high and low infiltration levels vary between studies as indicated in Table I, it is hard to use the same criterion to evaluate the CD8+ T-cell infiltration level for all the included studies. Therefore, the cutoff value for defining high-level CD8+ T-cell infiltration or positive CD8+ T-cells was determined according to each included study separately, as indicated in Table I.

Results of the meta-analysis

$CD8^+$ T-cell infiltration and OS. The results of the heterogeneity test indicated significant heterogeneity among the

Table I. Baseline characteristics and quality assessment of the included studies.

First author, year	Country	Sample size	Median age, years	Median follow-up time, months	Location of CD8+ TILs	Evaluation method	Cutoff (positive/ high level)	Outcome	NOS score	(Refs.)
Mahmoud, 2011	UK	1902	55	127	P	Number of positive cells	≥2	OS	7	(12)
Liu, 2011	China	1270	52	66	I/P	Infiltrating density	Median	OS/DFS	7	(14)
Liu, 2012	China	3992	58.9	151.2	I	Count of positive cells	≥1	OS	7	(11)
Ma, 2012	China	81	/	/	I&P	Positive cell number	Median	OS	8	(24)
Mohammed, 2013	UK	338	/	164	I&P	Number of lymphocytes	≥40	OS	7	(25)
Kim, 2013	Korea	72	49	33.7	I&P	Number of CD8+ T cells	>60	DFS	7	(26)
Sun, 2014	China	218	57.6	72	I&P	Infiltrating density	Median	OS/DFS	8	(27)
Chen, 2014	China	332	/	152	I&P	Intensity of infiltrate	Infiltrate score >0	OS/DFS	8	(28)
Ali, 2014	Several countries	12439	/	114.8	I	Absolute count	>0	OS	7	(29)
Chung, 2017	Korea	377	51	69	I&P	Number of TIL subsets	Median	DFS	7	(30)
Liu, 2017	China	647	55	21.5	I/P	CD8+ TIL count	sTIL ≥3, iTIL ≥1	OS	7	(31)
Papaioannou, 2019	Greece	207	60	70	P	Absolute number	Median	OS/DFS	8	(32)
Groot, 2019	Netherlands	196	/	55.2	I	Number of CD8+	Median	DFS	8	(33)
Catacchio, 2019	Italy	151	57	63	I&P/I/P	Number of TILs	Median	DFS	8	(34)

P, peritumoral; I, intratumoral; OS, overall survival; DFS, disease-free survival; NOS, Newcastle-Ottawa Scale; TIL, tumor-infiltrating lymphocyte; sTIL, stromal TILs; iTIL, intratumoral TILs.

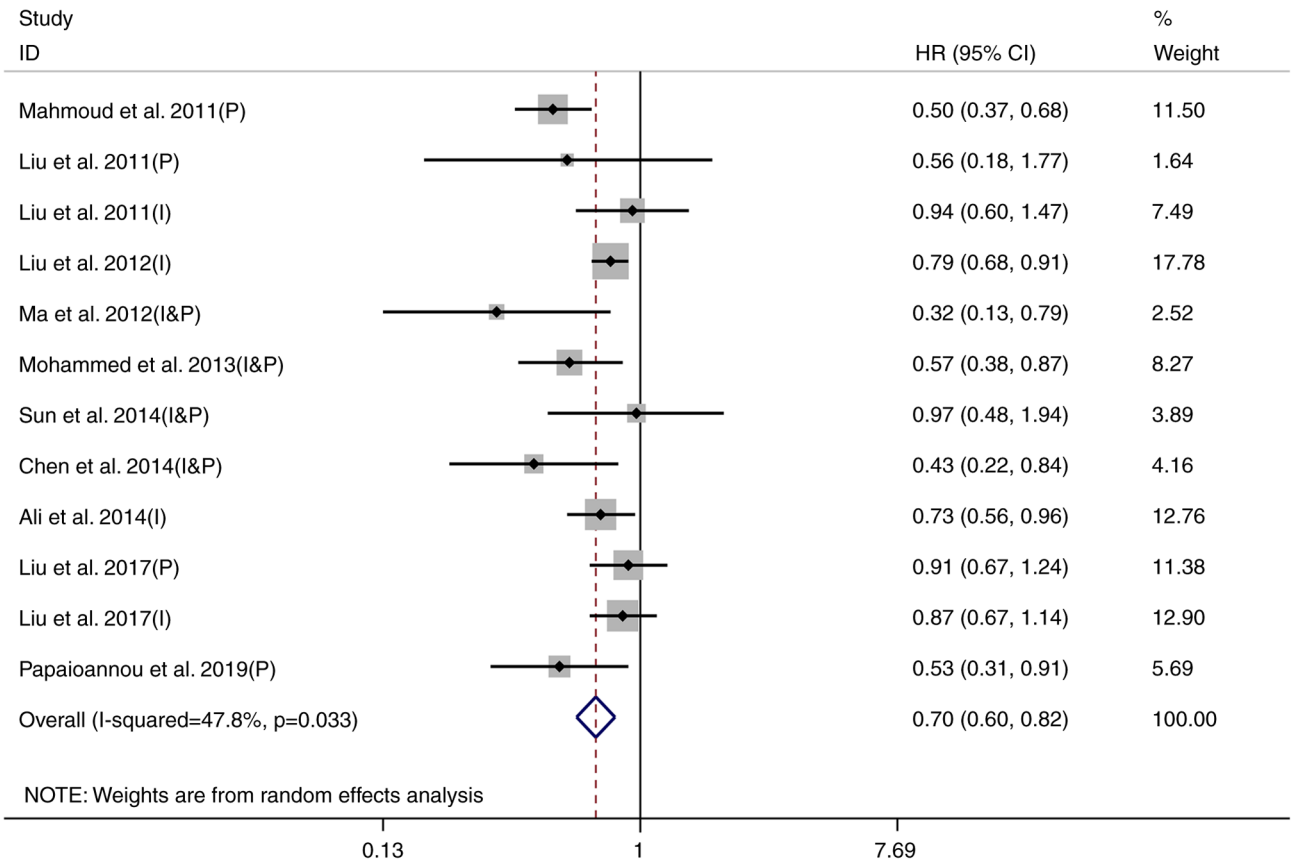

Figure 2. Relationship between CD8+ T-cell infiltration and overall survival. I, intratumoral; P, peritumoral; HR, hazard ratio.

studies (I^2=47.8%, P=0.033). Since the sensitivity analysis did not find any individual study that had a significant impact on the results of the meta-analysis, the REM was used to pool the results. The pooled results indicated that a high CD8+ T-cell infiltration level was significantly related to better OS of patients with breast cancer (HR=0.70, 95% CI: 0.60-0.82, P<0.001), as indicated in Fig. 2.

CD8+ T-cell infiltration and DFS. In addition, the relationship between CD8+ T-cell infiltration and DFS in patients with breast cancer was explored. By excluding each included study one by one and analyzing the pooled results of the remaining studies (HR and 95% CI), the sensitivity analysis indicated that the study on the CD8+ T-cell infiltration within the tumor (intratumoral TILs) and DFS by Catacchio *et al* (34) had an excessive impact on the results, as indicated in Fig. 3 and Figs. S1-S3. The results of the I^2 heterogeneity test were reduced from 33.7 to 0.0% when the study of Catacchio *et al* (34) (intratumoral TILs) was excluded. Therefore, in the subsequent analysis of the relationship between CD8+ T-cell infiltration and DFS, the study by Catacchio *et al* (34) (intratumoral TILs) was excluded. The results of the heterogeneity test revealed no significant heterogeneity among the studies (I^2=0.0%, P=0.498), and thus, an FEM was used. The pooled results suggested that a high CD8+ T-cell infiltration level was significantly related to better DFS of patients with breast cancer (HR=0.63, 95% CI: 0.49-0.81, P<0.001), as presented in Fig. 3.

CD8+ T-cell infiltration and clinicopathological characteristics of breast cancer. Furthermore, the relationship between CD8+ T-cell infiltration and clinicopathological characteristics, including clinical stage, N stage and performance status, was analyzed (Fig. 4). Both random-effects (D+L) and fixed-effects (M-H) models were used to analyze the pooled OR value. It was indicated that the estimation from the REM was different from the FEM when there was significant heterogeneity between studies, and the difference vanished when no heterogeneity existed (i.e. for HER2 status). These results agree with the above discussion regarding the REM and FEM. The result yielded from the REM (D+L) was adopted when there was significant heterogeneity among studies (I^2>50%); otherwise, the estimation from the FEM (M-H) was used.

A high CD8+ T-cell infiltration level was significantly associated with decreased expression of ER (OR=1.92, 95% CI: 1.30-2.85, P=0.001; I^2=75.8%, P<0.001) and PR (OR=1.66, 95% CI: 1.14-2.42, P=0.008; I^2=79.9%, P<0.001) and increased HER2 expression (OR=0.79, 95% CI: 0.66-0.94, P=0.010; I^2=2.4%, P=0.407) in patients with breast cancer, while there was no significant association between CD8+ T-cell infiltration and age, tumor size and lymph node status of patients with breast cancer (P>0.05), as indicated in Table II.

Subgroup analysis. In order to explore whether the location of CD8+ TILs affects the relationship between CD8+ T-cell infiltration and the prognosis of patients with breast cancer, a subgroup analysis was further carried out. The pooled results are listed in Table III. The result yielded from the REM (D+L) was adopted when there was significant heterogeneity among studies (I^2>50%); otherwise, the estimation

Table II. Relationship between CD8+ T-cell infiltration and clinicopathological characteristics of patients with breast cancer.

Characteristic	Number of studies	Test for relationship		
		OR	95% CI	P-value
Age, years (≤50 vs. >50)	3	1.07	0.73-1.58	0.717
Tumor size, cm (≤2 vs. >2)	5	1.14	0.94-1.40	0.211
Lymph node status (negative vs. positive)	4	1.18	0.69-2.00	0.549
ER status (negative vs. positive)	7	1.92	1.30-2.85	0.001
PR status (negative vs. positive)	6	1.66	1.14-2.42	0.008
HER2 status (negative vs. positive)	7	0.79	0.66-0.94	0.010

OR, odds ratio; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

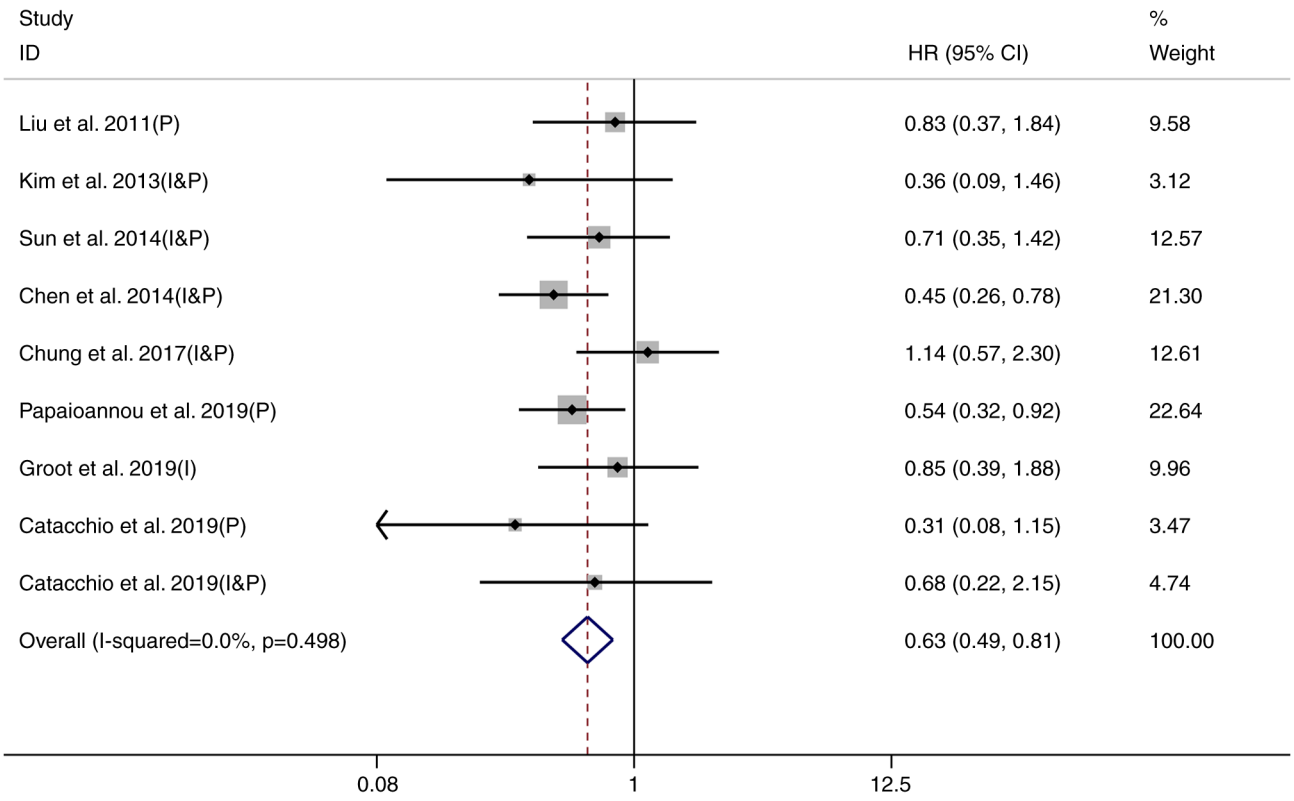

Figure 3. Relationship between CD8+ T-cell infiltration and disease-free survival [Catacchio *et al* (34) 2019 (I) excluded]. HR, hazard ratio.

from the FEM (I-V) was used. It was indicated that a high infiltration level of CD8+ T cells in the peritumoral group (HR=0.63, 95% CI: 0.43-0.91, P=0.015), intratumoral group (HR=0.80, 95% CI: 0.72-0.90, P<0.001) and intratumoral and peritumoral group (HR=0.56, 95% CI: 0.41-0.75, P=0.002) were all significantly related to better OS of patients with breast cancer, as presented in Fig. 5. In addition, the pooled results illustrated that a high infiltration level of CD8+ T cells in the peritumoral (HR=0.58, 95% CI: 0.38-0.87, P=0.010) and intratumoral and peritumoral group (HR=0.64, 95% CI: 0.46-0.89, P=0.009) was significantly related to better DFS of patients with breast cancer, but not in the intratumoral group (HR=0.85, 95% CI: 0.39-1.87, P=0.685), as indicated in Fig. 6.

Sensitivity analysis. By excluding each included study one by one and analyzing the impact on the results of the remaining studies (OS and DFS), a sensitivity analysis was performed. The analysis did not indicate any study that had any excessive impact on the OS (Fig. 7) and DFS (Fig. 8). This means that the results of the present meta-analysis are stable and reliable.

Publication bias. The funnel plots for publication bias are provided in Figs. 9 and 10. It may be observed that the funnel plots are near-symmetrical. The P-values of Egger's test were P=0.141 for the studies regarding OS and P=0.897 for the studies regarding DFS, indicating that there is no obvious publication bias in the present study. No small-study effects were indicated, since all the P-values were >0.05.

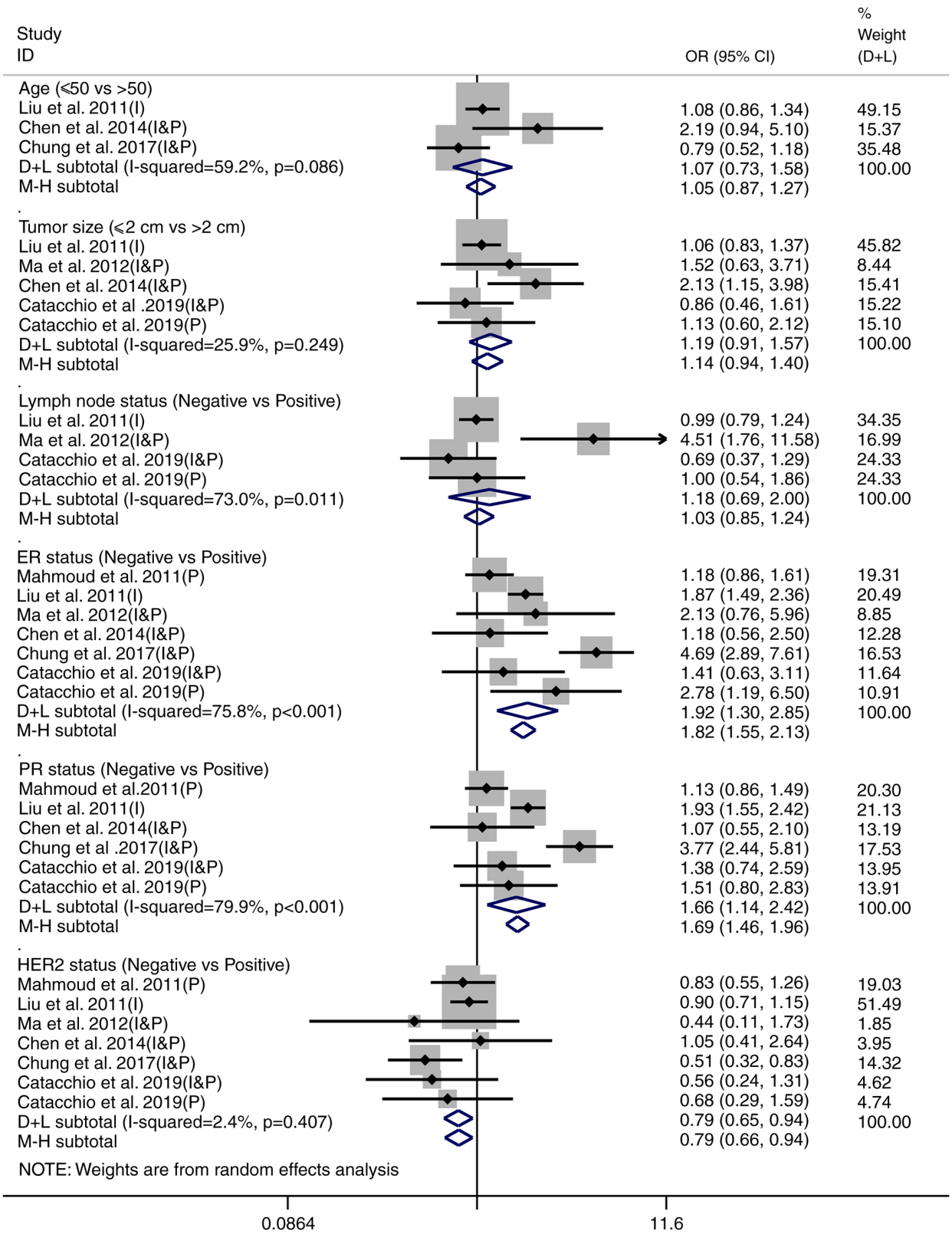

Figure 4. Relationship between CD8+ T-cell infiltration and clinicopathological characteristics of patients with breast cancer. D+L, DerSimonian and Laird model; M-H, Mantel-Haenszel model; I, intratumoral; P, peritumoral; OR, odds ratio; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Discussion

TILs, as an important component of the tumor microenvironment, predict prognosis and therapeutic response to immunotherapy (35,36). High levels of tumor-infiltrating CD8+ T-lymphocytes are characteristic of immunogenic hot tumors, which respond significantly better to immunotherapy (35,36). In the present review and meta-analysis, 14 studies including

Table III. Pooled HRs for OS and DFS according to subgroup analyses.

A, OS

Subgroup	Number of studies	HR (95% CI)	P-value
P	4	0.63 (0.43-0.91)	0.015
I	4	0.80 (0.72-0.90)	<0.001
I&P	4	0.56 (0.41-0.75)	0.002

B, DFS

Subgroup	Number of studies	HR (95% CI)	P-value
P	3	0.58 (0.38-0.87)	0.010
I	1	0.85 (0.39-1.87)	0.685
I&P	5	0.64 (0.46-0.89)	0.009

HR, hazard ratio; P, peritumoral; I, intratumoral; OS, overall survival; DFS, disease-free survival.

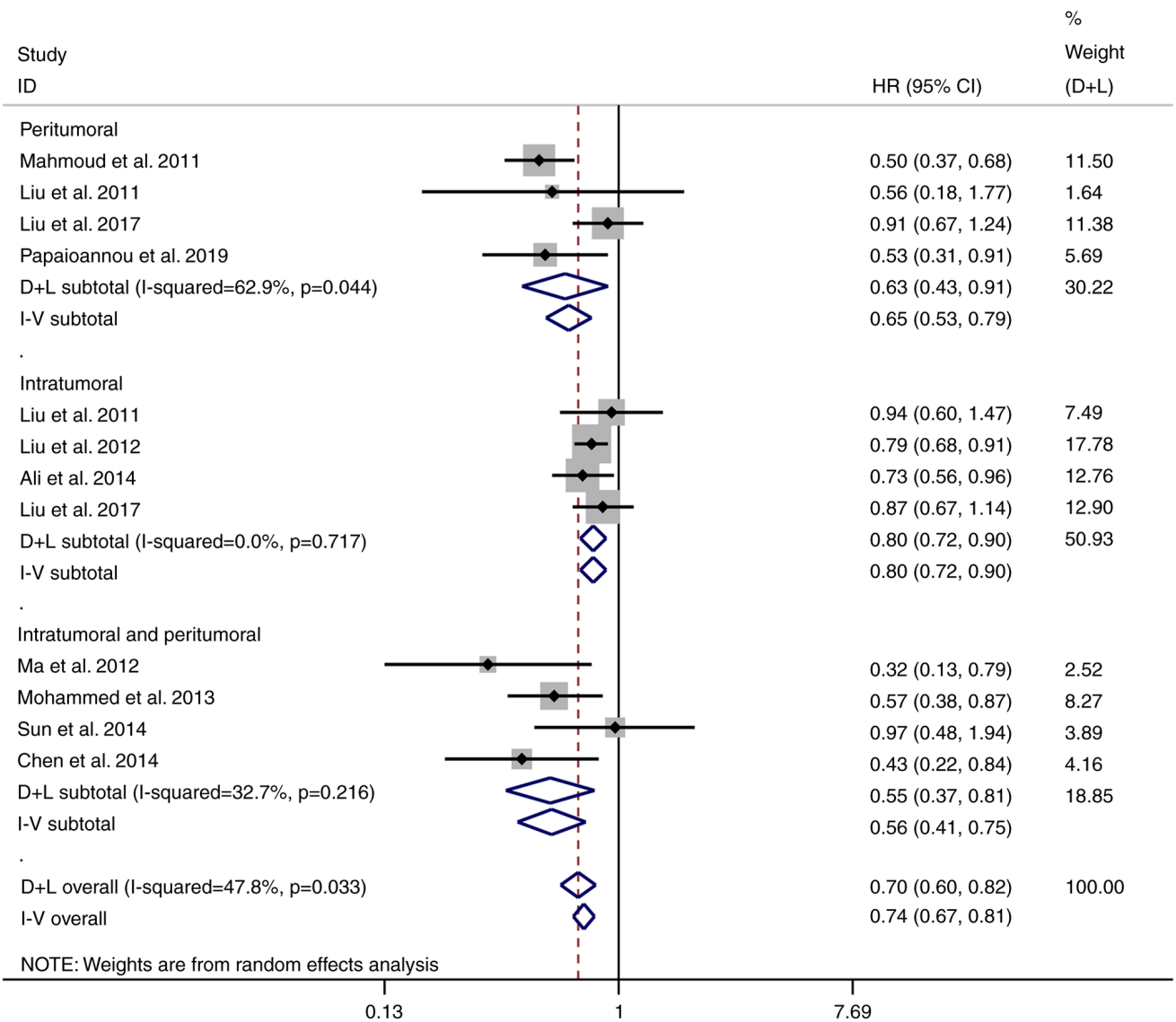

Figure 5. Relationship between the CD8+ T-cell infiltration in different tumor locations and overall survival. D+L, DerSimonian and Laird model; I-V, inverse-variance model; HR, hazard ratio.

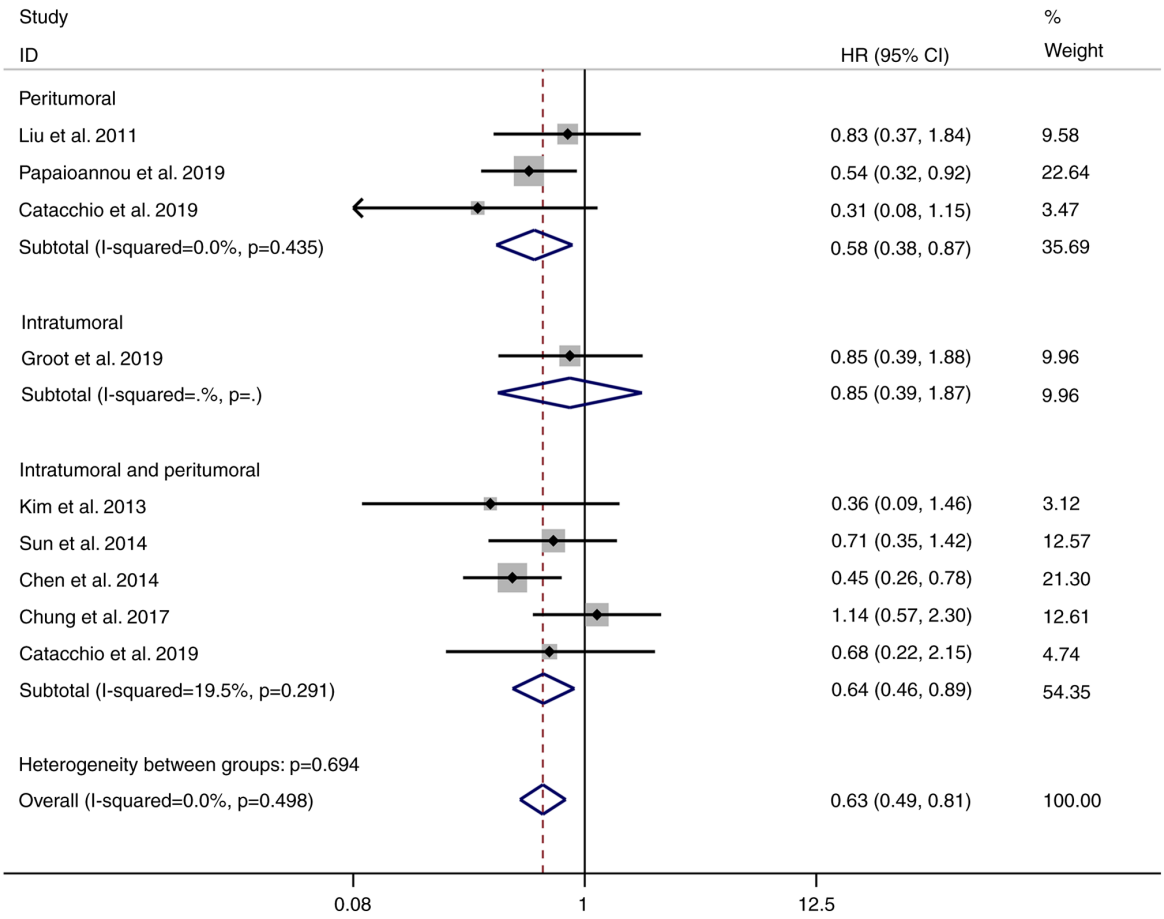

Figure 6. Relationship between the CD8+ T-cell infiltration in different tumor locations and disease-free survival. HR, hazard ratio.

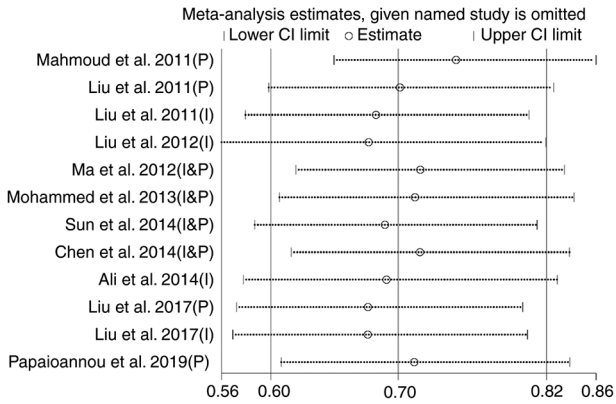

Figure 7. Sensitivity analysis of the relationship between CD8+ T-cell infiltration and overall survival. CI, confidence interval; I, intratumoral; P, peritumoral.

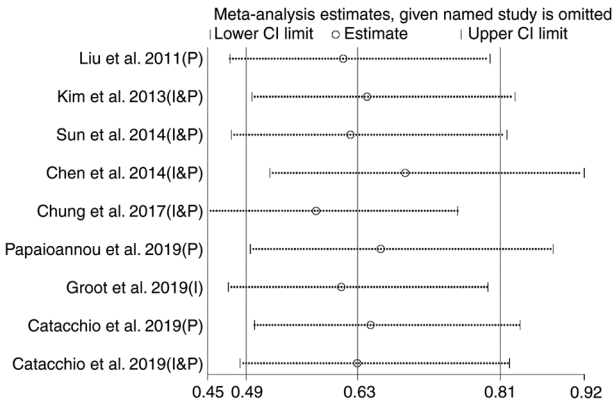

Figure 8. Sensitivity analysis of the relationship between CD8+ T-cell infiltration and disease-free survival. CI, confidence interval; I, intratumoral; P, peritumoral.

22,222 patients were pooled in order to investigate the prognostic value of CD8+ T cells in breast cancer, and to explore the association between CD8+ T cells and the pathological characteristics of patients with breast cancer, with the aim of providing new prognostic biomarkers for the clinical treatment of breast cancer.

The pooled results of the present study suggested that the high CD8+ T-cell infiltration level was significantly associated with better OS and DFS of patients with breast cancer. Cytotoxic T cells are marked by CD8. Cells presenting foreign antigens associated with major histocompatibility complex class I molecules are recognized by CD8+ T cells through specific interactions between the presented antigens and T-cell receptors (37). CD8+ T cells represent a marker of immune response against tumor, directly triggering apoptosis of the target cell via the perforin/granzyme A/B system or through FAS ligand expression (37). Enhancing tumor infiltration by cytotoxic T cells appears to be an important therapeutic strategy, which

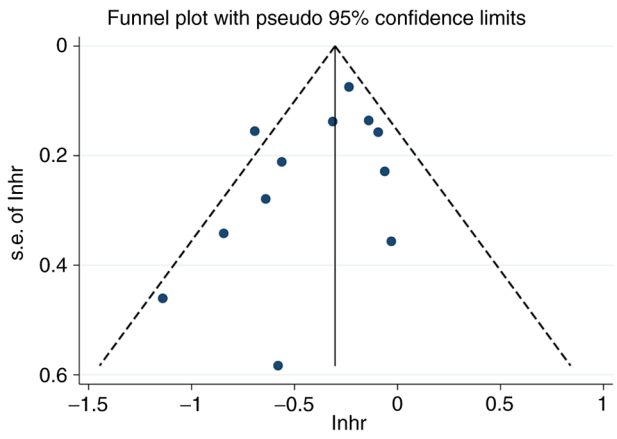

Figure 9. Funnel plot for evaluating the publication bias of the relationship between CD8+ T-cell infiltration and overall survival. S.e., standard error; lnhr, the natural logarithm of HR.

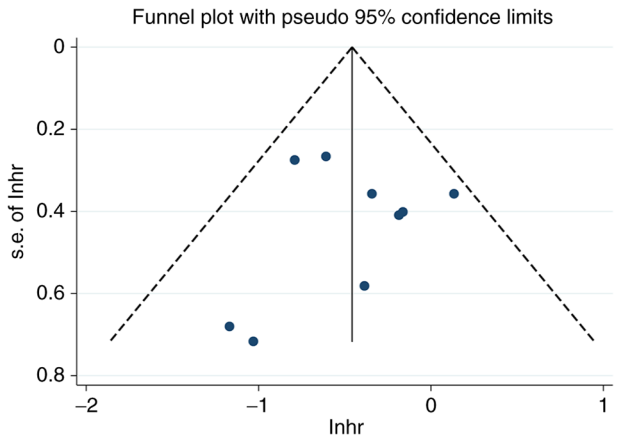

Figure 10. Funnel plot for evaluating the publication bias of the relationship between CD8+ T-cell infiltration and disease-free survival. S.e., standard error; lnhr, the natural logarithm of HR.

may convert immunogenic cold tumors to hot tumors, thereby increasing the response rate to immunotherapy. In order to explore whether the location of CD8+ TILs affects the relationship between CD8+ T-cell infiltration and the prognosis of patients with breast cancer, a subgroup analysis was further performed. The pooled results indicated that a high infiltration level of CD8+ T cells was significantly related to better OS of patients with breast cancer regardless of the location. This suggests that the location of CD8+ T cells in breast cancer does not affect the relationship between CD8+ T cells and OS. However, the pooled results illustrated that a high infiltration level of CD8+ T cells in the peritumoral, and intratumoral and peritumoral, but not in the intratumoral region, was significantly related to better DFS of patients with breast cancer. This suggests that peritumoral CD8+ T cells are more helpful in predicting DFS in patients with breast cancer. Regarding the differences in the prediction results of the two locations, future studies are required in order to reveal the mechanisms in detail.

In addition, the present results suggested that a high CD8+ T-cell infiltration level was significantly associated with decreased expression of ER and PR, as well as increased HER2 expression, in patients with breast cancer. The above results indicate that drugs targeting ER and PR are not suitable for patients with breast cancer with a high CD8+ T-cell infiltration level, while drugs targeting HER2 are more suitable for patients with breast cancer with a high CD8+ T-cell infiltration level. Furthermore, patients with HER2-positive breast cancer may have a better prognosis.

The present review and meta-analysis also has certain limitations. First, since all patients with breast cancer were included in the present study, the heterogeneity of patients with breast cancer itself was not excluded in this study, resulting in mild to moderate heterogeneity of the study results. However, as the included studies did not specify the type of breast cancer, it was not possible to perform any further subgroup analysis. Furthermore, the small number of studies included in the present subgroup analysis of CD8+ T-cell location may challenge the objectivity of the results. Finally, since the evaluation method and cutoff point to separate high and low infiltration levels vary among the studies, it is difficult to use the same criteria to evaluate the CD8+ T-cell infiltration level for all of the studies. These differences may have led to heterogeneity among the included studies.

In conclusion, CD8+ T cells are of value in predicting the prognosis of patients with breast cancer. A high level of CD8+ T-cell infiltration was related to improved prognosis, including OS and DFS, in patients with breast cancer. In addition, a high CD8+ T-cell infiltration level was significantly associated with decreased expression of ER and PR, and increased HER2 expression.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

YPS and XL contributed to the conception, design and modification of the study. YPS and YLK screened articles for inclusion and extracted the data. YPS performed the statistical analysis. YPS and YLK contributed to the interpretation of the results. YPS and XL drafted the manuscript. YPS and XL confirm the authenticity of all the raw data. All authors contributed to manuscript revision, and read and approved the final manuscript. All authors agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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