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# Prognostic significance of CD8 + tumor-infiltrating lymphocytes in operable breast cancer: a meta-analysis

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## Abstract

**Background** As key mediators of antitumor immunity, CD8 + tumor-infiltrating lymphocytes present antigens and initiate robust immune responses against cancer cells. When stratified by location, CD8 + T lymphocytes were counted and classified as intratumoral, stromal, or total CD8 + tumor-infiltrating lymphocytes. Despite their crucial role, the impact, especially the specific type of CD8 + T lymphocytes on breast cancer prognosis remains controversial. This meta-analysis synthesized evidence to delineate the relationship between CD8 + tumor-infiltrating lymphocytes density of different counting methods and breast cancer patient outcomes.

**Methods** PubMed, Embase, and the Cochrane Library were systemically searched from inception through January 2024 for studies evaluating the prognostic significance of CD8 + tumor-infiltrating lymphocytes in breast cancer. The primary endpoint was disease-free survival (DFS), and the second endpoints were overall survival (OS), breast cancer-specific survival (BCSS), and recurrence-free survival (RFS).

**Results** Thirty-four studies encompassing 23,626 breast cancer patients were included. Pooled hazard ratios (HRs) indicated a significant association of high CD8 + TIL presence with improved DFS (HR = 0.63; 95% CI = 0.54–0.73), OS (HR = 0.72; 95% CI = 0.65–0.79), BCSS (HR = 0.67; 95% CI = 0.58–0.78), and RFS (HR = 0.53; 95% CI = 0.38–0.73). Stratification by TIL location (intratumoral [iCD8], stromal [sCD8], or total [tCD8]) did not significantly impact DFS or OS.

**Conclusion** High CD8 + TIL density in breast cancer patients is correlated with a favorable prognosis, irrespective of the location of CD8 + tumor-infiltrating lymphocytes. These findings affirm the prognostic utility of CD8 + TIL assessment and may guide future immunotherapeutic strategies.

**Keywords** Breast cancer, Tumor-infiltrating lymphocytes, CD8, Prognosis, Meta-analysis

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## Introduction

Breast cancer is a formidable challenge in oncology, not only as the most prevalent form of cancer among women but also as a lead contributor to cancer-related mortality worldwide [1]. The global burden of breast cancer is, without a doubt, a reflection of its multifaceted nature, characterized by varied molecular subtypes and divergent clinical outcomes. In 2020 alone, an estimated 2.26 million new cases were identified, and approximately 680,000 mortalities occurred, illustrating the sheer scale of its impact [1]. Although advancements in early detection and novel therapeutic strategies have significantly curbed mortality rates, the persistence of suboptimal outcomes for numerous patients indicates the existence of additional prognostic elements alongside recognized indicators such as age, histological categorizations, tumor size, lymph node involvement, and receptor status, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) status [2].

The tumor immune microenvironment attracts considerable attention within the realm of predictive and prognostic biomarkers. As the nexus of tumor-host interactions, this environment has a considerable influence on tumorigenesis, shaping not only cancer initiation and progression but also the response to therapeutic interventions [3, 4]. Of particular interest in recent years are tumor-infiltrating lymphocytes (TILs), which represent the immune system's front-line defense within the tumor. The diversity and density of TILs have been posited to mirror the immune response, with numerous studies corroborating the impact of these cells on cancer prognosis [5] and affecting the efficacy of immune checkpoint blockade [6].

Cytotoxic CD8 + T lymphocytes, the dominant faction within TILs, can recognize and attack cancer cells, presenting antigens via major histocompatibility complex (MHC) class I molecules [7]. The anticancer mechanisms of these agents are further reinforced by the secretion of interferon-gamma, which promotes the retardation and apoptosis of malignant cells [8, 9]. Additionally, existing studies vary widely regarding design and technical approaches, ranging from tissue sampling techniques to immunohistochemical staining protocols, challenging the composite interpretation of their results [10, 11].

The divergence in findings has sparked debates regarding the stratification of the TILs themselves, with evidence emerging for subclassifications such as intratumoral (iCD8), stromal (sCD8), and total CD8 + TILs (tCD8). The prognostic power of these subsets and their spatial heterogeneity within the tumor structure might hold the key to understanding the nuances in survival rates among breast cancer patients. This line of inquiry underscores the importance of meta-analytic studies that synthesize diverse findings to shed light on the true impact of CD8 + TILs on breast cancer survival.

Breast cancer continues to be a formidable adversary in surgical oncology, not solely due to its prevalence among women but also due to the substantial global health burden it imposes. The heterogeneity of breast cancer, exemplified by its diverse molecular subtypes and variable clinical courses, necessitates refined prognostic indicators that can inform personalized treatment strategies. This meta-analysis, by elucidating the prognostic significance of CD8 + tumor-infiltrating lymphocytes (TILs), directly addresses a pivotal gap in the surgical management of breast cancer. Surgeons, who often serve as the primary coordinators of multidisciplinary care, require robust, evidence-based tools to predict patient outcomes and tailor therapeutic plans accordingly. Our study empowers surgeons with a novel biomarker – CD8 + TIL density – that can be readily assessed in routine histopathological evaluations. This knowledge has immediate practical implications, enabling surgeons to better stratify patients for adjuvant therapies, including the emerging landscape of immunotherapies, and to engage in informed discussions with patients regarding their prognosis.

The present systematic review and meta-analysis were conceived in this context to sift through the cacophony of global research findings and distill a clearer understanding of the prognostic value of CD8 + TILs in breast cancer. By adopting rigorous inclusion criteria, analyzing high-quality studies, and applying meta-analytic techniques, we aimed to reconcile the disparate evidence and present a cohesive narrative on the influence of CD8 + TILs on the outcomes of breast cancer patients. Such insights are vital for personalized medicine strategy development, allowing for treatment pathway optimization and potentially revolutionizing the prognostication and therapeutic landscape of breast cancer.

## Materials and methods

### Protocol and registration

The systematic review and meta-analysis were formulated in adherence to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 statement [12] and AMSTAR(Assessing the methodological quality of systematic reviews) guidelines [13].The protocol for the study was registered in the International Prospective Register of Systematic Reviews (PROSPERO).

### Search strategy

We conducted an exhaustive literature search leveraging the PubMed, Embase, and Cochrane Library databases, encompassing all records from their inception until the predefined cutoff date of January 30, 2024. The search methodology was meticulously crafted following

the Patient-Intervention-Comparison-Outcome (PICO) framework, incorporating Medical Subject Headings (MeSH), Emtree terms, and pertinent text words and keywords. The search strings were tailored for each database to capture studies assessing CD8 + tumor-infiltrating lymphocytes concerning prognostic outcomes in breast cancer patients who underwent surgical intervention for primary malignancies. This comprehensive search was supplemented by hand-searching the reference lists of identified reviews and primary articles to identify additional research not captured by electronic strategies. The detailed search strategies, including the various terms and combinations used, are exhaustively delineated in Supplementary Appendix Table 1.

#### Study selection and inclusion criteria

Following the systematic search, all retrieved citations were imported into reference management software, and duplicates were removed. Independent screening of the remaining titles and abstracts was performed by two reviewers to assess initial eligibility. Studies that met the inclusion criteria or for which there was uncertainty were identified in full-text format for a more thorough evaluation. At this juncture, two reviewers independently assessed the studies against our inclusion criteria.

To qualify for inclusion, studies were required to focus exclusively on patients who underwent surgical treatment for primary breast cancer and to have CD8 + TILs identified via immunohistochemistry in immune cells. Additionally, studies were required to employ either standardized scoring systems or comparable methodologies for CD8 + TIL evaluation to minimize variability in the assessment process. Furthermore, studies are needed to provide prognostic information related to overall survival (OS), breast cancer-specific survival (BCSS), and/or disease-free survival (DFS). Exclusion criteria included studies involving carcinoma in situ, metastatic breast cancer, or those lacking sufficient data to determine hazard ratios (HRs) and 95% confidence intervals (CIs) for the outcomes of interest. Additionally, ineligible study formats included commentaries, animal research, reviews, editorial letters, conference abstracts, and case reports.

#### Immunohistochemistry (IHC) for CD8 + TILs

The assessment of CD8 + TILs was conducted through IHC on tumor sections, which were initially deparaffinized and rehydrated, followed by antigen retrieval using a citrate buffer (pH 6.0). Given the variability in monoclonal antibodies used for CD8 + TIL detection across studies (e.g., C8/144B, Cell Marque, USA; SP16, Cell Marque; and other commonly utilized clones for CD8 detection), with antibody dilutions typically spanning from 1:100 to 1:200, our inclusion criteria encompassed studies that evaluated CD8 + TIL density by quantifying the number of positive cells within high-power fields (HPF) in both intratumoral and stromal regions.

To maintain consistency and minimize methodological variability, only studies employing standardized scoring systems for CD8 + TIL evaluation were included in the analysis. These scoring systems generally delineated high versus low CD8 + TIL expression based on predefined cut-off thresholds, such as median values or receiver operating characteristic (ROC) curves.

Any eligibility during the screening phase was resolved through collegial discussion and, if needed, through the mediation of a third reviewer. All deliberations and selections were documented to ensure transparency and reproducibility of the study selection process.

#### Data extraction and quality assessment

For all studies that met the inclusion criteria, data collection was independently performed by two researchers utilizing a predesigned form to ensure consistency in data extraction. The collected data included key study characteristics and relevant clinical metrics, including the first author's name, nation of origin, study design, year of publication, intended CD8 + TIL evaluation site, follow-up duration, median age of patient group, tumor stage at diagnosis, tissue/material assessed for CD8 + TILs, set threshold for high versus low CD8 + TIL expression, number of patients evaluated, and hazard ratios (HRs) with corresponding 95% confidence intervals (CIs).

In instances where direct quantification of HRs was not explicated within the study, we considered relative risks (RRs) as an acceptable substitute or employed established inferential methods to derive HRs from survival curves, as delineated in the literature by Parmar et al. [14] and Tierney et al. [15]. Studies were evaluated on a preferential basis for HRs derived through multivariate analysis over univariate analysis when both were reported.

In the event of missing or incomplete data within the identified studies, we contacted the corresponding authors for the requisite data. Nonresponsive authors or those unable to furnish the necessary data resulted in excluding the associated studies from the meta-analysis to maintain the integrity of the analysis.

The methodological rigor of each study was scrutinized utilizing the Newcastle–Ottawa Scale (NOS), which allocates up to nine points across three dimensions: selection, comparability, and outcome assessment [16]. High-quality studies were identified as those with a score greater than seven. In addition, the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guidelines were used to further evaluate the quality and reproducibility of the tumor marker data [17].

### Prognostic outcomes and subgroup analysis

The prognostic metrics focused on in this meta-analysis included overall survival (OS), defined as the duration from breast cancer (BC) diagnosis to death from any cause; breast cancer-specific survival (BCSS), which marked the interval from BC diagnosis to death specifically attributable to BC or until the most recent follow-up; and disease-free survival (DFS), which tracked the period from initial treatment culmination to the first sign of BC recurrence or death.

Comprehensive subgroup analyses were performed to investigate the relationship between CD8 + TIL localization and patient outcomes, providing insights into potential sources of heterogeneity. These subgroups were predetermined and included CD8 + TIL distribution (intratumoral, stromal, and total CD8 + TILs), which provided an opportunity to explore prognostic variances contingent on histological localization.

### Statistical analysis

The statistical evaluation for this meta-analysis was conducted using Stata software (version 12.0; StataCorp LP, College Station, TX, USA). We computed pooled hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between CD8 + TIL presence and prognostic outcomes in breast cancer patients. The Der Simonian and Laird random-effects model was employed to integrate study-specific estimates [18]. This approach was selected due to its capacity to accommodate anticipated variability between studies beyond chance alone.

To assess the heterogeneity among the included studies, we utilized the $I^2$ statistic, which quantifies the proportion of total variation across studies attributable to heterogeneity rather than chance. An $I^2$ value exceeding 50% indicated significant heterogeneity, prompting further exploration through subgroup analysis.

Predefined subgroup analyses were conducted to elucidate the potential sources of heterogeneity and investigate the consistency of effect sizes across strata defined by characteristics such as the anatomical location of CD8 + TILs (intratumoral—iCD8, stromal—sCD8, total—tCD8), study design (population-based versus hospital-based), geographic region (America, Europe, Asia, Australia), center type (single or multicenter), patient age at diagnosis (≤ 50 years versus > 50 years), duration of follow-up (≤ 10 years versus > 10 years), methodological quality (moderate versus high), analytical approach to survival data (univariate versus multivariate), threshold for CD8 + TIL determination (median value, ROC curves, among others), lymph node status (negative versus positive), CD8 + TIL assessment method (tissue microarray—TMA or whole-slide analysis), molecular subtype of the tumors, and the influence of neoadjuvant therapy (NAT, without, or both).

The sensitivity of our findings was appraised through the application of a leave-one-out approach [19]. This method involved sequentially omitting individual studies to discern their effect on the overall meta-analysis results and the consistency of the pooled estimates.

Finally, to assess publication bias, we visually examined funnel plots and performed Egger's linear regression tests [20]. A statistically significant deviation from symmetry ($P < 0.05$) suggested the presence of publication bias. In such cases, the Duval and Tweedie trim-and-fill method was applied to adjust the risk estimates, thus providing a more conservative understanding of the effects measured [21].

## Results

### Search results

Figure 1 shows the PRISMA flow diagram of the article selection process. Initially, 1398 articles were identified using our search strategy. Of these articles, 66 were screened for further full-text assessment after reviewing the titles and abstracts of the articles. Among them, 32 articles were excluded for various reasons, as demonstrated in Fig. 1. Finally, 34 articles [22–55] involving 23,626 breast carcinoma patients were identified for quantitative synthesis. The detailed screening process is shown in Supplementary Table S1.

### Study characteristics

The baseline characteristics of the included studies are presented in Table 1. All included studies were published between 2008 and 2023 and had a median sample size of 226 (ranging from 31 to 12439 patients). Eighteen studies were performed in Asia, 14 in Europe, 1 in America and 1 in Australia. Thirty-three of those 34 studies were retrospective in design, and 56% (19/34) were of high quality with a NOS score of 8 or 9, whereas 44% were of moderate quality (NOS score of 6 or 7). All studies used IHC as a method for detecting CD8 + markers on TILs, with 17 studies [24, 27, 30–33, 36, 37, 41, 44, 47, 49, 50, 52–55] investigating the association between stromal CD8 + TILs and survival outcome, 15 studies [23, 24, 27, 28, 30, 34, 36, 42, 44, 45, 50, 52–55] reporting intratumoral CD8 + TILs, and 19 studies [22, 23, 25, 26, 29, 34, 35, 38–41, 43–44, 46, 48, 50, 51, 53, 54] reporting total CD8 + TILs (both stromal and intratumoral). There were diverse scoring systems and cutoff values among the studies, and the most frequently used cutoff values for the high versus low density of CD8 + TILs were based on the median values ($n = 14$)[24,31–34,36–38,41,44,45,47,51] of the examined population, ROC curves ($n = 3$) [23, 41, 43], tertiles ($n = 3$) [22, 26, 29] or 75th percentiles ($n = 5$) [50, 52–55]. A tissue microarray (TMA) was used in 13 studies [22, 23, 28, 31, 32, 35, 37, 42, 44, 47, 49, 50, 55], and the other studies used whole-slide analysis TIL assessment.

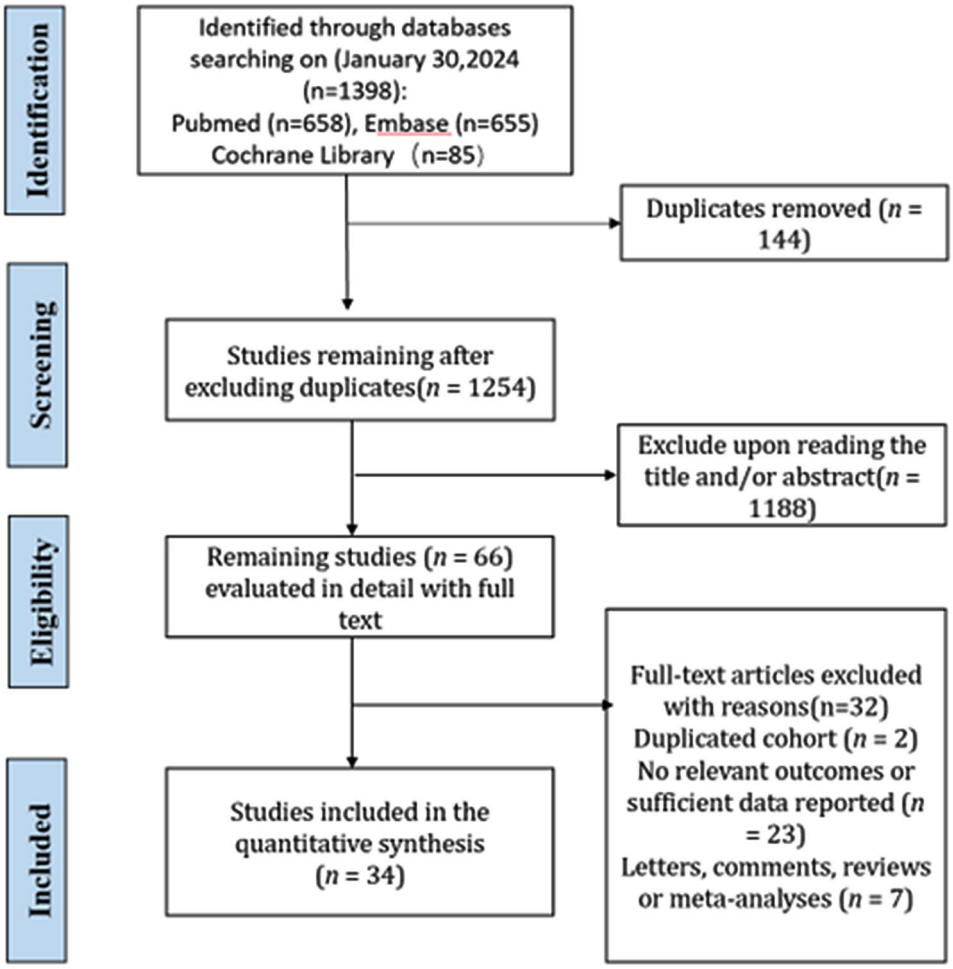


**Fig. 1** Flow chart of study selection based on PRISMA

### Study quality assessment

The results showed that all studies were of excellent quality, with the lowest number of total stars obtained being six. Regarding the selection domain, adequate descriptions of the characteristics and selection criteria for cases and controls were provided for all included studies. Regarding the comparability domain, 14 out of the 30 studies matched for age and at least one additional factor. Moreover, 14 studies lost stars due to inadequate follow-up (Supplementary Table S2).

### Primary outcome: disease-free survival

Twenty studies[25,28,33,34,36,38–45,49–55] involving 5540 patients provided appropriate data concerning the association between CD8 + infiltration and DFS. When stratified by location, CD8 + T lymphocytes were counted and classified as 'intratumoral' CD8+ (iCD8), 'stromal' CD8+ (sCD8) or 'total' CD8+ (tCD8). Subgroup analyses revealed that TIL assessment methods, geographic regions, and patient demographics were key contributors to heterogeneity in the pooled results. As each type of CD8 + T-cell is well documented for analysis, we calculated the relationships between iCD8 + T-cell density, sCD8 + T-cell density, total CD8 + T-cell density and DFS in breast cancer patients.

#### iCD8 + TILs

Ten studies [28, 33, 36, 42, 44, 45, 50, 54, 55] involving 3599 patients investigated iCD8 + T lymphocytes and DFS, and the pooled HR for DFS was 0.63 (95% CI, 0.47–0.84), with significant heterogeneity observed among these studies ($I^2 = 56\%$) (Fig. 2A). To address the significant heterogeneity in the pooled HR for DFS ($I^2 = 56\%$), we performed subgroup analyses (Table 2). Differences in TIL assessment methods, geographic regions, and patient demographics emerged as key contributors. For example, the use of whole-slide methods demonstrated a stronger prognostic association (HR 0.47; 95% CI: 0.29–0.76) and lower heterogeneity ($I^2 = 40.7\%$) compared to tissue microarray (TMA) methods, which exhibited higher variability (HR 0.75; 95% CI: 0.53–1.06; $I^2 = 59.5\%$). This

**Table 1** Design variables of included studies for CD8 + tumor infiltrating lymphocytes analysed

| First author | Year | Country | Sample size | Cut off point | Adjustment variables | Outcome | Follow-up duration |
|---|---|---|---|---|---|---|---|
| Murri et al. | 2008 | British | 168 | Tertiles | Age, type, size, grade, LN status, hormonal receptor status, loco-regional treatment, systemic treatment | BCSS OS | Median: 72 months |
| Liu et al. | 2011 | China | 1270 | Median value | Age, tumor size, lymph node, histological grade, ER/PR/HER2 status, chemotherapy, radiotherapy, endocrine therapy | OS | Median: 66 months |
| Kristi et al. | 2011 | Switzerland | 1953 | ROC curves | Adjuvant therapy, age, tumor diameter, stage | BCSS | Median: 63 months |
| Mohammed et al. | 2013 | UK | 306 | Tertiles | NR | BCSS RFS | Median:64 months |
| Seung et al. | 2013 | Korea | 72 | > 60/HPF | NR | DFS | Median:33.7 months |
| Ali et al. | 2014 | British | 12,439 | > 0 | Tumour size, positive lymph nodes, grade | BCSS | NR |
| Ankita et al. | 2014 | India | 150 | 25th percentile | Age, menstrual status, family history | RFS | Median:43 months |
| Chen et al. | 2014 | China | 332 | > 0 | Age, stage, histological grade, ER, PR, Ki-67, HER-2 | DFS OS | Median:152 months |
| Gujam et al. | 2014 | UK | 361 | Tertiles | Size, involved lymph node, tumor necrosis | BCSS | Median:168 months |
| Minoru et al. | 2015 | Japan | 131 | Median value | Residual tumor size, nodal status | BCSS RFS | Median:43.2 months |
| Hirofumi et al. | 2016 | Singapore | 164 | Median value | Tumor size, LN stage | DFS OS | Not specified |
| Park et al. | 2016 | Korea | 333 | Median value | Tumor size and nodal involvement | DFS OS | Median:117.6 months |
| Giulia et al. | 2016 | Italy | 259 | Median value | Age, histologic grade, nodal status, tumor size, stage | OS RFS | NR |
| Song et al. | 2017 | Korea | 55 | Not defined | NR | DFS | Median:34.9months |
| Mina et al. | 2017 | Japan | 97 | ≥ 50% expression area | Age, tumor size, metastatic lymph nodes, grade | DFS OS | Median:127.3 months |
| Chung et al. | 2017 | Korea | 377 | Median value | Tumor size, LN stage, LVI | DFS | Median:69 months |
| Chen et al. | 2017 | China | 309 | Median value | Residual involved nodes, Ki-67, PD-L1 | RFS OS | Median:70 months |
| Khalid et al. | 2017 | Saudi Arabia | 31 | Median value | Age, histological type, tumor grade, stage, lymphovascular invasion | DFS OS | NR |
| Roni et al. | 2017 | Sweden | 498 | Score based | Age, lymph node metastasis, tumor size, grade, molecular type | BCSS RFS | NR |
| Xu et al. | 2018 | China | 102 | Mean number | Age, tumor size LN status, ER/PR/HER2 status, LVI, endocrine therapy, radiotherapy, Ki67 expression, histological grade | DFS OS | NR |
| Patrick et al. | 2018 | America | 74 | ROC curves/ median value | Age, histologic grade, tumor stage | DFS OS | Median:118 months |
| Vihervuori et al. | 2019 | Finland | 179 | Median value | Tumor size | BCSS | Median:96 months |
| Papaioannou et | 2019 | Greece | 207 | Median value | Age, size, grade, lymph node status, hormone receptor | BCSS DFS | Median:70 months |
| Groot et al. | 2019 | Dutch | 196 | Median value | Hormonal status | DFS | Median:55.2 months |
| Hitomi et al. | 2019 | Japan | 127 | ≥ 30/200 magnification | Tumor size, nodal status, nuclear grade, Ki67, adjuvant treatment | RFS OS | Median:67 months |
| Catacchio et al. | 2019 | Italy | 180 | Median value | NR | DFS OS | Median:63 months |
| Simin et al. | 2019 | Iran | 94 | ROC curves | NR | DFS OS | Median:62.4 months |
| Maria et al. | 2020 | Italy | 244 | Median value | Age, stage at diagnosis, histologic grade and pCR(when applicable) | DFS | Median:81.6 months |
| Ewan et al. | 2020 | Australia | 458 | Median value | Lymph node status, endocrine therapy, tumor size, margin status | DFS OS | Median:192months |
| Triantafyllia et al. | 2020 | Greece | 1011 | 75th percentile | Menopausal status, tumor size, nodal status, grade, radiation, subtypes | DFS OS | Median:130.9 months |
| Zhang et al. | 2021 | China | 596 | 75th percentile | Age | DFS OS | Median:88.41months |
| Nikolaos et al. | 2022 | Greece | 728 | 75th percentile | Menopausal status, tumor size, nodal status, grade radiotherapy | DFS OS | Median:132.5months |

**Table 1** (continued)

| First author | Year | Country | Sample size | Cut off point | Adjustment variables | Outcome | Follow-up duration |
|---|---|---|---|---|---|---|---|
| Shu Yazaki et al. | 2023 | Japan | 125 | 75th percentile | Stage, histologic grade | DFS | Median:77.4months |
| Sun et al. | 2023 | China | 259 | Maximally selected rank statistics | age, grade, stage and lymphovascular invasion | DFS | Median:80 months |

**Abbreviations**: DFS, disease free survival; OS, overall survival; BCSS, breast cancer-specific survival; RFS, relapse free survival; ROC, receiver operator characteristic; NR, not reported

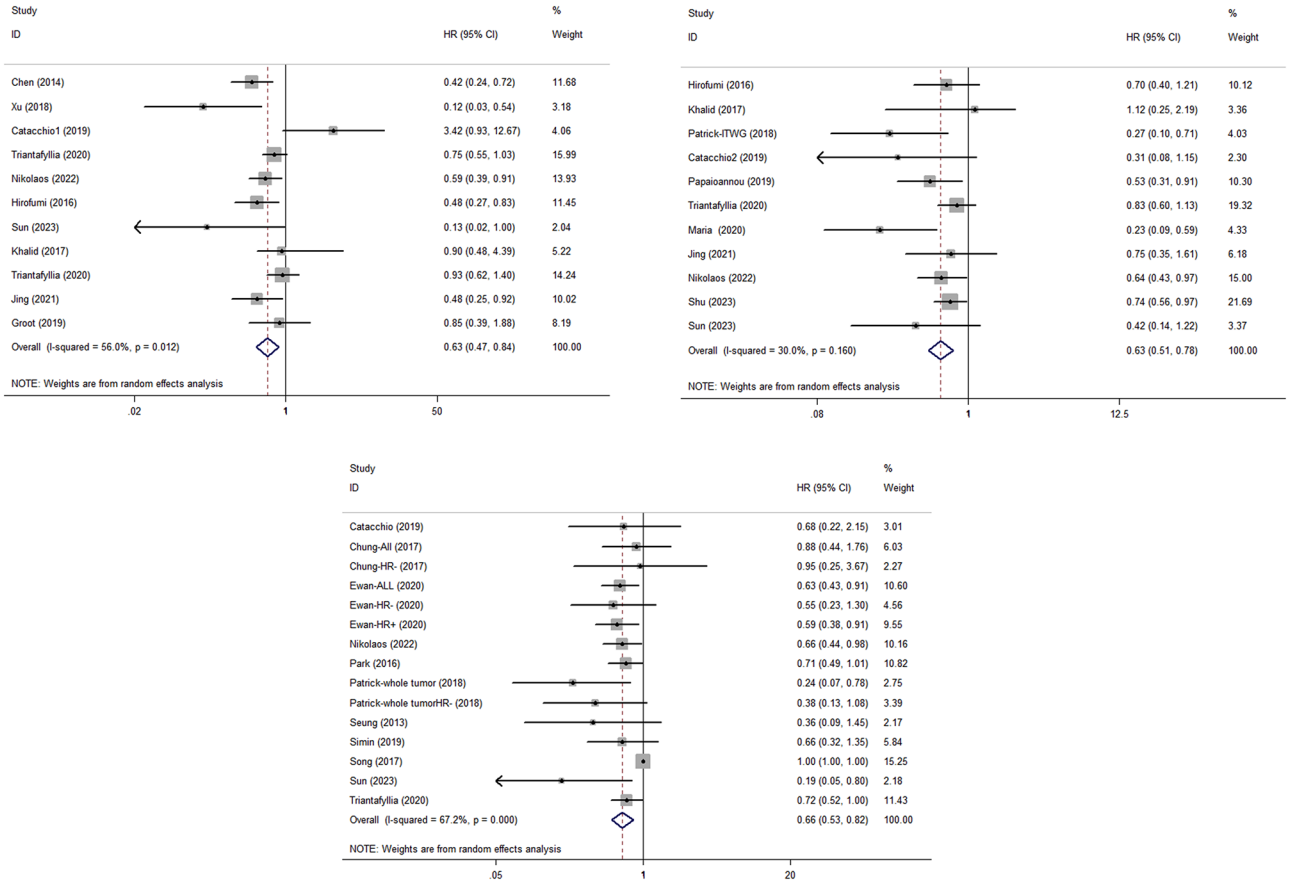


**Fig. 2** Forest plots of hazard ratios for DFS stratified by location (**A**) Forest plots of hazard ratios for iCD8 (**B**) Forest plots of hazard ratios for sCD8 (**C**) Forest plots of hazard ratios for tCD8

disparity may reflect the limitations of TMAs in capturing spatial heterogeneity.

Geographic variations also played a crucial role, with Asian populations showing a robust association with DFS (HR 0.45; 95% CI: 0.32–0.64; $I^2$ = 12.6%) compared to European populations (HR 0.82; 95% CI: 0.60–1.12; $I^2$ = 46%). Such differences could be attributable to genetic, environmental, or healthcare system factors. Study design and setting further influenced the results. Single-center studies displayed a stronger DFS association (HR 0.51; 95% CI: 0.30–0.88) but higher heterogeneity ($I^2$ = 60.2%) compared to multicenter studies, which had no detectable heterogeneity (HR 0.76; 95% CI: 0.61–0.93; $I^2$ = 0). Moreover, high-quality studies (NOS ≥ 8) were associated with significant heterogeneity ($I^2$ = 66.4%), underscoring the methodological challenges inherent in combining diverse study designs. Other demographic factors, such as age and tumor stage, also contributed to variability. For instance, younger patients (≤ 50 years) exhibited a stronger prognostic association (HR 0.61; 95% CI: 0.40–0.94) compared to older patients (> 50 years), who showed a nonsignificant trend (HR 0.62; 95% CI: 0.37–1.05) with higher heterogeneity ($I^2$ = 66%). Similarly, the lack of neoadjuvant therapy was associated with

**Table 2** Subgroup analysis relationships between iCD8 + tumor infiltrating lymphocytes and DFS

| Variables | HR | 95% CI | $I^2$ (%) | No. studies | *P* for heterogeneity between subgroups |
|---|---|---|---|---|---|
| **Overall** | 0.63 | 0.47–0.84 | 56.0 | 10 | NA |
| **Study design** | | | | | 0.023 |
| Population based | 0.81 | 0.63–1.04 | 0.0 | 1 | |
| Hospital based | 0.56 | 0.38–0.82 | 52.6 | 9 | |
| **Geographic regions** | | | | | 0.005 |
| Europe | 0.82 | 0.60–1.12 | 46.0 | 5 | |
| Asia | 0.45 | 0.32–0.64 | 12.6 | 5 | |
| **Methodologic quality (NOS)** | | | | | 0.762 |
| Moderate (6–7) | 0.62 | 0.41–0.95 | 0.0 | 3 | |
| High (≥ 8) | 0.61 | 0.42–0.89 | 66.4 | 8 | |
| **Sample size** | | | | | |
| ≤ 200 | 0.65 | 0.49–0.84 | 44.3 | 4 | 0.656 |
| >200 | 0.60 | 0.28–1.18 | 67.5 | 6 | |
| **Study setting** | | | | | 0.060 |
| Single-center | 0.51 | 0.30–0.88 | 60.2 | 7 | |
| Multi-center | 0.76 | 0.61–0.93 | 0.0 | 3 | |
| **Median age, years** | | | | | 0.300 |
| ≤ 50 | 0.61 | 0.40–0.94 | 56.6 | 5 | |
| >50 | 0.62 | 0.37–1.05 | 66.0 | 4 | |
| **Follow-up period, months** | | | | | 0.608 |
| ≤ 120 | 0.54 | 0.21–1.38 | 72.8 | 5 | |
| >120 | 0.67 | 0.50–0.90 | 50.6 | 4 | |
| **Survival analysis method** | | | | | 0.060 |
| Univariate | 1.54 | 0.40–5.94 | 68.6 | 2 | |
| Multivariate | 0.57 | 0.43–0.76 | 50.0 | 9 | |
| **Cut-off criteria** | | | | | 0.054 |
| Median value | 0.91 | 0.44–1.85 | 61.5 | 4 | |
| 75th percentile | 0.67 | 0.50–0.90 | 41.3 | 5 | |
| **Tumor stage** | | | | | 0.084 |
| I-III | 0.65 | 0.43–0.99 | 66.0 | 7 | |
| II-III | 0.87 | 0.46–1.64 | 0.0 | 2 | |
| **Material** | | | | | 0.035 |
| TMA | 0.75 | 0.53–1.06 | 59.5 | 4 | |
| Whole-slide | 0.47 | 0.29–0.76 | 40.7 | 6 | |
| **Molecular typing** | | | | | 0.288 |
| All types | 0.64 | 0.41–1.01 | 66.5 | 6 | |
| TNBC | 0.75 | 0.48–1.17 | 31.4 | 3 | |
| Luminal type | 0.94 | 0.37–2.39 | 0.0 | 2 | |
| **Neoadjuvant therapy** | | | | | 0.380 |
| NAT | 0.87 | 0.46–1.64 | 0.0 | 2 | |
| Without NAT | 0.61 | 0.42–0.89 | 66.4 | 7 | |

**Abbreviations**: DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; iCD8, intratumoral CD8; sCD8, stromal CD8; tCD8, total CD8; NOS, Newcastle-Ottawa Scale; ROC, receiver operator characteristic; LN, lymph node; TMA, tissue microarray; TNBC, triple-negative breast cancer; NAT, neoadjuvant therapy

significant heterogeneity (HR 0.61; 95% CI: 0.42–0.89; $I^2$ = 66.4%), suggesting that treatment-related immune modulation may mitigate interstudy differences. In addition, triple-negative breast cancer (TNBC) showed a non-significant association with improved DFS (HR: 0.75, 95% CI: 0.48–1.17, $I^2$ = 31.4%), and luminal types also demonstrated weaker and nonsignificant associations (HR: 0.94, 95% CI: 0.37–2.39, $I^2$ = 0%).

To assess the stability of our results, we performed a sensitivity analysis with the leave-one-out method. No individual study significantly altered the summary HR of DFS (lowest HR 0.58, 95% CI 0.42–0.80; highest HR 0.66, 95% CI 0.48–0.90) (Supplementary Figure S1).

The funnel plot showed symmetry upon visual inspection (Supplementary Figure S2), and Egger's test ($P$ = 0.444) did not indicate publication bias. The Duvall and Tweedie trim-and-fill methods did not perform any trimming (Table 5).

### sCD8 + TILs

Eleven studies [33, 36, 41, 44, 47, 49, 50, 55]involving 3619 patients provided appropriate data concerning the association between sCD8 + infiltration and DFS. Pooled analysis revealed significantly increased DFS in the group with high infiltration of $CD8^+$ TILs, with an HR of 0.63 (95% CI: 0.51–0.78) (Fig. 2B). There was no obvious heterogeneity among these 11 studies ($I^2$ = 28.3%; $p$ = 0.107).

The subgroup analysis for sCD8 + TILs and DFS is detailed in Table 3. Significant variability in outcomes was noted across different stratifications, highlighting potential sources of heterogeneity. For example, geographic variations were critical source of heterogeneity. Studies conducted in America showed the strongest association (HR 0.33; 95% CI: 0.16–0.68; $I^2$ = 0%), followed by Europe (HR 0.56; 95% CI: 0.39–0.82; $I^2$ = 54.2%) and Asia (HR 0.75; 95% CI: 0.59–0.94; $I^2$ = 0%). These discrepancies may reflect regional differences in patient characteristics, treatment practices, and genetic factors influencing the tumor microenvironment. Study design and methodological quality also contributed to heterogeneity. Single-center studies demonstrated a stronger association (HR 0.51; 95% CI: 0.37–0.72) with low heterogeneity ($I^2$ = 24.2%), compared to multicenter studies (HR 0.75; 95% CI: 0.62–0.90; $I^2$ = 0%). Similarly, studies with moderate methodological quality (NOS 6–7) showed a greater effect size (HR 0.48; 95% CI: 0.24–0.95) but higher heterogeneity ($I^2$ = 60.7%) than high-quality studies (HR 0.70; 95% CI: 0.60–0.83; $I^2$ = 0%). TIL assessment methods also impacted results. Whole-slide analysis (HR 0.55; 95% CI: 0.38–0.79) yielded stronger associations but higher heterogeneity ($I^2$ = 44.6%) compared to tissue microarrays (HR 0.73; 95% CI: 0.58–0.91; $I^2$ = 0%). Differences in sample size and follow-up duration further influenced heterogeneity, with smaller studies (≤ 200 patients)

**Table 3** Subgroup analysis relationships between sCD8 + tumor infiltrating lymphocytes and DFS

| Variables | HR | 95% CI | $I^2$ (%) | No. studies | *P* for heterogeneity between subgroups |
|---|---|---|---|---|---|
| **Overall** | 0.63 | 0.51–0.78 | 30.0 | 11 | NA |
| **Study design** | | | | | 0.136 |
| Population based | 0.83 | 0.60–1.14 | NA | 1 | |
| Hospital based | 0.59 | 0.47–0.75 | 25.4 | 10 | |
| **Geographic regions** | | | | | 0.100 |
| America | 0.33 | 0.16–0.68 | 0.0 | 2 | |
| Europe | 0.56 | 0.39–0.82 | 54.2 | 5 | |
| Asia | 0.75 | 0.59–0.94 | 0.0 | 4 | |
| **Methodologic quality (NOS)** | | | | | 0.175 |
| Moderate (6–7) | 0.48 | 0.24–0.95 | 60.7 | 4 | |
| High (≥ 8) | 0.70 | 0.60–0.83 | 0.0 | 7 | |
| **Centers involved** | | | | | 0.044 |
| Single-center | 0.51 | 0.37–0.72 | 24.2 | 8 | |
| Multi-center | 0.75 | 0.62–0.90 | 0.0 | 3 | |
| **Sample size** | | | | | 0.308 |
| ≤ 200 | 0.53 | 0.33–0.86 | 25.2 | 5 | |
| >200 | 0.67 | 0.53–0.84 | 36.7 | 6 | |
| **Median age, years** | | | | | 0.384 |
| ≤ 50 | 0.67 | 0.44–1.01 | 35.3 | 5 | |
| >50 | 0.60 | 0.46–0.78 | 31.9 | 6 | |
| **Follow-up period, months** | | | | | 0.358 |
| ≤ 120 | 0.50 | 0.35–0.72 | 43.9 | 7 | |
| >120 | 0.75 | 0.59–0.97 | 0.0 | 2 | |
| **Survival analysis method** | | | | | 0.029 |
| Univariate | 0.28 | 0.13–0.62 | 0.0 | 2 | |
| Multivariate | 0.68 | 0.57–0.82 | 15.6 | 9 | |
| **Cut-off criteria** | | | | | 0.019 |
| Median value | 0.62 | 0.43–0.88 | 0.0 | 4 | |
| 75th percentile | 0.75 | 0.62–0.89 | 0.0 | 4 | |
| **Material** | | | | | 0.365 |
| TMA | 0.73 | 0.58–0.91 | 0.0 | 4 | |
| Whole-slide | 0.55 | 0.38–0.79 | 44.6 | 7 | |
| **Molecular typing** | | | | | 0.215 |
| All types | 0.52 | 0.39–0.71 | 32.9 | 4 | |
| TNBC | 0.73 | 0.60–0.88 | 28.8 | 5 | |
| Luminal type | 0.86 | 0.46–1.60 | 0.0 | 2 | |
| **Neoadjuvant therapy** | | | | | 0.024 |
| Without NAT | 0.70 | 0.60–0.83 | 0.0 | 7 | |
| With or without NAT | 0.25 | 0.13–0.49 | 0.0 | 2 | |

**Abbreviations**: DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; iCD8, intratumoral CD8; sCD8, stromal CD8; tCD8, total CD8; NOS, Newcastle-Ottawa Scale; ROC, receiver operator characteristic; LN, lymph node; TMA, tissue microarray; TNBC, triple-negative breast cancer; NAT, neoadjuvant therapy

showing a more pronounced effect (HR 0.53; 95% CI: 0.33–0.86; $I^2$ = 25.2%) compared to larger studies (> 200 patients; HR 0.67; 95% CI: 0.53–0.84; $I^2$ = 36.7%). Finally, survival analysis methods affected outcomes. Univariate analyses revealed a significantly stronger association (HR 0.28; 95% CI: 0.13–0.62; $I^2$ = 0%) than multivariate analyses (HR 0.68; 95% CI: 0.57–0.82; $I^2$ = 15.6%), likely reflecting unadjusted confounding factors. Moreover, TNBC exhibited a robust prognostic association with DFS (HR: 0.73, 95% CI: 0.60–0.88, $I^2$ = 28.8%). In contrast, luminal subtypes showed nonsignificant results (HR: 0.86, 95% CI: 0.46–1.60, $I^2$ = 0%).

Sensitivity analysis of sCD8 + lymphocytes and DFS revealed an HR of 0.63, consistent with the obtained value. This finding indicated that the result of prolonged disease-free survival was not materially altered by omitting 1 study (lowest HR 0.59, 95% CI 0.47–0.74; highest HR 0.69, 95% CI 0.58–0.81) (Supplementary Figure S1).

A visual inspection of the funnel plot revealed mild asymmetry (Supplementary Figure S2). However, Egger's test ($P$ = 0.034) revealed underlying publication bias. The Duvall and Tweedie trim-and-fill methods did not perform any trimming (Table 5).

#### tCD8 + TILs

For the eleven studies [25, 34, 38, 40, 41, 43, 44, 50, 51, 53, 54] involving 3569 patients whose CD8 + T cells were counted at all locations, the pooled hazard ratio (HR) for disease-free survival (DFS) was 0.66 (95% CI, 0.53–0.82) (Fig. 2C). Significant heterogeneity was observed between studies ($I^2$ = 67.2%).

Subgroup analyses (Table 4) revealed several potential sources of heterogeneity. Geographic region significantly influenced DFS outcomes, with the strongest association observed in the American population (HR 0.28; 95% CI, 0.14–0.55; $I^2$ = 0%), followed by Europe (HR 0.69; 95% CI, 0.54–0.89; $I^2$ = 0%) and Asia (HR 0.87; 95% CI, 0.71–1.07; $I^2$ = 27.5%). These discrepancies may reflect differences in healthcare systems, tumor biology, or immune response due to genetic or environmental factors. The method of TIL assessment also contributed to variability. Whole-slide analysis demonstrated a stronger association (HR 0.58; 95% CI, 0.38–0.88) compared to tissue microarrays (HR 0.67; 95% CI, 0.56–0.80), but with substantially higher heterogeneity ($I^2$ = 71.2% vs. 0%). This variation might arise from differences in sampling techniques or TIL quantification protocols. Study design further impacted the results. Hospital-based studies exhibited higher heterogeneity ($I^2$ = 59.3%) compared to population-based studies ($I^2$ = 0%). Similarly, single-center studies showed higher variability (HR 0.61; 95% CI, 0.41–0.92; $I^2$ = 55.5%) compared to multicenter studies (HR 0.66; 95% CI, 0.56–0.78; $I^2$ = 0%), suggesting potential differences in patient selection criteria or clinical management practices. Patient demographics and clinical factors also played a role. Younger patients (≤ 50

**Table 4** Subgroup analysis relationships between tCD8 + tumor infiltrating lymphocytes and DFS

| Variables | HR | 95% CI | $I^2$ (%) | No. studies | *P* for heterogeneity between subgroups |
|---|---|---|---|---|---|
| **Overall** | 0.66 | 0.53–0.82 | 67.2 | 11 | NA |
| **Study design** | | | | | < 0.001 |
| Population based | 0.66 | 0.55–0.79 | 0.0 | 3 | |
| Hospital based | 0.64 | 0.46–0.89 | 59.3 | 8 | |
| **Geographic regions** | | | | | < 0.001 |
| America | 0.28 | 0.14–0.55 | 0.0 | 2 | |
| Europe | 0.69 | 0.54–0.89 | 0.0 | 3 | |
| Asia | 0.87 | 0.71–1.07 | 27.5 | 5 | |
| **Centers involved** | | | | | < 0.001 |
| Single-center | 0.61 | 0.41–0.92 | 55.5 | 7 | |
| Multi-center | 0.66 | 0.56–0.78 | 0.0 | 4 | |
| **Sample size** | | | | | < 0.001 |
| ≤ 200 | 0.51 | 0.30–0.88 | 66.4 | 6 | |
| > 200 | 0.68 | 0.58–0.79 | 0.0 | 5 | |
| **Survival analysis method** | | | | | < 0.001 |
| Univariate | 0.70 | 0.48–1.03 | 43.2 | 6 | |
| Multivariate | 0.66 | 0.56–0.77 | 0.0 | 7 | |
| **Median age, years** | | | | | < 0.001 |
| ≤ 50 | 0.63 | 0.46–0.88 | 71.6 | 7 | |
| >50 | 0.71 | 0.51–0.98 | 0.0 | 3 | |
| **Follow-up period, months** | | | | | < 0.001 |
| ≤ 120 | 0.66 | 0.48–0.91 | 57.9 | 8 | |
| >120 | 0.65 | 0.54–0.78 | 0.0 | 3 | |
| **Cut-off criteria** | | | | | < 0.001 |
| Median value | 0.65 | 0.53–0.79 | 0.0 | 5 | |
| ROC curves | 0.45 | 0.17–1.17 | 49.8 | 2 | |
| 75th percentile | 0.70 | 0.54–0.90 | 0.0 | 2 | |
| **Molecular typing** | | | | | < 0.001 |
| All types | 0.68 | 0.58–0.81 | 0.0 | 8 | |
| TNBC | 0.46 | 0.22–0.95 | 68.5 | 4 | |
| Luminal type | 0.59 | 0.38–0.91 | NA | 1 | |
| **Material** | | | | | < 0.001 |
| TMA | 0.67 | 0.56–0.80 | 0.0 | 5 | |
| Whole-slide | 0.58 | 0.38–0.88 | 71.2 | 6 | |
| **Methodologic quality (NOS)** | | | | | 0.001 |
| Moderate (6–7) | 0.67 | 0.51–0.87 | 65.3 | 7 | |
| High (≥ 8) | 0.66 | 0.50–0.87 | 10.9 | 4 | |
| **Neoadjuvant therapy** | | | | | < 0.001 |
| Without NAT | 0.65 | 0.56–0.76 | 0.0 | 7 | |
| With or without NAT | 0.32 | 0.16–0.64 | 0.0 | 2 | |

**Abbreviations**: DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; iCD8, intratumoral CD8; sCD8, stromal CD8; tCD8, total CD8; NOS, Newcastle-Ottawa Scale; ROC, receiver operator characteristic; LN, lymph node; TMA, tissue microarray; TNBC, triple-negative breast cancer; NAT, neoadjuvant therapy

**Table 5** Analysis of publication bias with different models for DFS

| Publication bias | Begg's *p* values | Egger's *p* values | T&F(Fill) |
|---|---|---|---|
| DFS-iCD8 | 0.436 | 0.444 | data unchanged(0) |
| DFS-sCD8 | 0.276 | 0.034 | data unchanged(0) |
| DFS-tCD8 | 0.921 | < 0.001 | data unchanged(0) |

**Abbreviations**: T&F, result of trimmed and filled analysis, using assumption of random effects; Fill, number of studies added by trim and fill method; iCD8, intratumoral CD8; sCD8, stromal CD8; tCD8, total CD8

years) exhibited higher heterogeneity ($I^2$ = 71.6%) compared to older patients (> 50 years; $I^2$ = 0%). In addition, heterogeneity was influenced by sample size and follow-up duration. Smaller studies (≤ 200 patients) showed greater heterogeneity ($I^2$ = 66.4%) compared to larger studies (> 200 patients; $I^2$ = 0%), and studies with shorter follow-up periods (≤ 120 months) exhibited higher variability ($I^2$ = 57.9%) compared to those with longer follow-up periods ($I^2$ = 0%). Finally, TNBC had the strongest association with improved DFS (HR: 0.46, 95% CI: 0.22–0.95, $I^2$ = 68.5%). Luminal subtypes showed a weaker but still significant effect (HR: 0.59, 95% CI: 0.38–0.91). These findings may reflect variations in tumor immunogenicity and immune microenvironment across subgroups.

We also performed a sensitivity analysis to assess the robustness of the pooled findings for tCD8 + lymphocytes and DFS (lowest HR 0.64, 95% CI 0.50–0.81; highest HR 0.68, 95% CI 0.54–0.84) (Supplementary Figure S1), which indicated that excluding any study did not explain the heterogeneity, suggesting that the tCD8 + aggregated outcomes were robust.

The funnel plot exhibited an asymmetrical distribution (Supplementary Figure S2), but Begg's test showed no publication bias ($P$ = 0.921). Egger's test revealed statistical significance ($P$ < 0.001). Consequently, the "trim and fill" analysis was further performed, and the recalculated result did not change, indicating the stability of our pooled results (Table 5).

### Secondary outcomes: overall survival, breast cancer-specific survival and recurrence-free survival

#### *Overall survival*

Seventeen studies [22, 24, 28, 32–34, 36, 39, 41–43, 46, 50–54] involving 6205 patients provided appropriate data concerning the association between CD8 + infiltration and OS.

Nine studies [23, 27, 33, 36, 42, 50, 52–54] involving 4493 patients investigated iCD8 + T lymphocytes and OS, and the pooled HR for OS was 0.58 (95% CI, 0.43–0.79), with mild heterogeneity observed among these studies ($I^2$ = 47.2%) (Supplementary Table S3 and Supplementary

Figure S3). Sensitivity analysis revealed that no individual study significantly altered the summary HR of OS (Supplementary Figure S1) (lowest HR 0.52, 95% CI 0.36–0.73; highest HR 0.62, 95% CI 0.46–0.83). For publication bias, the funnel plot exhibited an asymmetrical distribution (Supplementary Figure S2). Additionally, the p-value of Egger's test was 0.001, indicating underlying publication bias. Consequently, the "trim and fill" analysis was further performed, and the recalculated result did not change. In summary, we did not test for publication bias due to the small number of included studies on associations between iCD8 + lymphocytes and OS.

For the relationship between sCD8 + T lymphocytes and OS, nine studies [24, 32, 33, 36, 41, 50, 52–54] with 4392 patients were included. The pooled HR was 0.58 (95% CI, 0.43–0.79) (Supplementary Table S3 and Supplementary Figure S3). There was no among-study heterogeneity ($I^2 = 0\%$). Sensitivity analysis revealed no sources of heterogeneity (Supplementary Figure S1), and there was no between-group heterogeneity ($p = 0.662$). Neither the funnel plot (Supplementary Figure S2) nor Egger's test ($P = 0.726$) showed publication bias. The Duvall and Tweedie trim-and-fill methods did not perform any trimming.

Ten studies [22, 34, 39, 41, 43, 46, 50, 51, 53, 54] involving 3349 patients investigated tCD8 + T lymphocytes and OS, and the pooled HR for OS was 0.73 (95% CI, 0.58–0.91), with mild heterogeneity observed among these studies ($I^2 = 37.2\%$) (Supplementary Table S3 and Supplementary Figure S3). Sensitivity analysis revealed that no individual study significantly altered the summary HR of OS (Supplementary Figure S1) (lowest HR 0.52, 95% CI 0.36–0.73; highest HR 0.62, 95% CI 0.46–0.83). Neither the funnel plot (Supplementary Figure S2) nor Egger's test ($P = 0.388$) showed publication bias. The Duvall and Tweedie trim-and-fill methods did not perform any trimming.

While subgroup analyses stratified by CD8 + lymphocyte location were conducted, we did not perform additional analyses to explore the impact of TIL assessment methods, geographic variations, or patient demographics due to the limited number of studies available for each subgroup. Therefore, while heterogeneity may be partly attributable to these unexamined factors, our results should be interpreted cautiously until validated by future studies with more comprehensive subgroup analyses.

#### Breast cancer-specific survival

Nine studies [22, 23, 26, 27, 29, 31, 35, 47, 48] involving 16,242 patients provided appropriate data concerning the association between CD8 + T-cell infiltration and BCSS. When stratified by the location of CD8 + lymphocytes, the pooled HR was 0.81 (95% CI: 0.66–1.01) in the 'iCD8' subgroup, 0.46 (95% CI: 0.24–0.90) in the 'sCD8' subgroup and 0.65 (95% CI: 0.55–0.78) in the 'tCD8' subgroup (Supplementary Table S3 and Supplementary Figure S3). Heterogeneity was high in the iCD8 ($I^2 = 73.2\%$) and sCD8 ($I^2 = 79.2\%$) subgroups but not in the tCD8 subgroup ($I^2 = 31.9\%$). As a result, the location of CD8 lymphocytes might be a potential source of heterogeneity. Additional subgroup analyses based on baseline study variables were not performed due to the limited number of included studies. Sensitivity analysis revealed that no individual study significantly altered the summary HR of BCSS (Supplementary Figure S1) (lowest HR 0.66, 95% CI 0.58–0.75; highest HR 0.70, 95% CI 0.61–0.80). For publication bias, the funnel plot exhibited an asymmetrical distribution (Supplementary Figure S2). Additionally, the p-value of Egger's test was 0.002, indicating underlying publication bias. Consequently, the "trim and fill" analysis was further performed, and the recalculated result did not change.

#### Recurrence-free survival

Six studies [30–32, 35, 37, 46] involving 1474 patients provided appropriate data concerning the association between CD8 + infiltration and RFS in breast cancer patients stratified by the location of T lymphocytes. The pooled HR was 0.43 (95% CI: 0.29–0.64) in the 'sCD8' subgroup, 0.46 (95% CI: 0.24–0.90) in the 'sCD8' subgroup and 0.73 (95% CI: 0.40–1.35) in the 'tCD8' subgroup. Heterogeneity was high in the 'tCD8' subgroup ($I^2 = 70.7\%$) but not in the 'sCD8' subgroup ($I^2 = 41.3\%$) (Supplementary Table S3 and Supplementary Figure S3). The observed heterogeneity in the tCD8 subgroup highlights the importance of further exploring potential sources of variability, including tumor microenvironment and immune response differences. However, the small number of studies limited the feasibility of such analyses in this study.

## Discussion

Our pooled data from 34 cohort studies including 23,626 patients showed that high $CD8^+$ TILs could be a relatively pronounced predictive marker, with better associated outcomes than low ICs in terms of DFS, OS, BCSS and RFS, irrespective of the location and cutoff value of $CD8^+$ TILs.

Our pooled analyses also revealed significant heterogeneity in the prognostic implications of CD8 + TILs across breast cancer molecular subtypes. Notably, TNBC demonstrated the strongest association with improved DFS, supporting the immune-responsive nature of this aggressive subtype. High CD8 + TIL density may reflect robust antitumor immunity in TNBC, consistent with prior studies emphasizing its immunogenicity and response to immune checkpoint inhibitors. In contrast, luminal A and B subtypes showed weaker associations, likely due

to lower immunogenicity and the dominant role of hormonal pathways in these subtypes.

The results of our study were not the same as those of the relevant meta-analysis. For example, Sun et al. [56] showed that high CD8 + TIL levels were associated with better OS regardless of location and that high CD8 + T cells in the peritumoral region, but not in the intratumoral region, were significantly related to better DFS in patients with breast cancer. However, in our study, high CD8 + T cells could be a relatively pronounced predictive marker for both OS and DFS, irrespective of the location of the CD8 + TILs. In another study exploring the relationship between CD8 + tumor-infiltrating lymphocytes and triple-negative breast cancer, Gao et al. [57] showed that high CD8 + TIL levels were associated with improved DFS (HR 0.55, 95% CI 0.38–0.81) but not OS (HR 0.70, 95% CI 0.46–1.06). In addition, Ezzeldin et al. [58] reported both superior OS and DFS benefits of high CD8 + lymphocytes, with estimated HRs of 0.58 (95% CI, 0.52–0.65) and 0.24 (95% CI, 0.12–0.45), respectively, which presents a lower risk of relapse than our study. The reason for this difference may be that more large-power cohort studies were included in our meta-analysis.

Like other malignant tumors, breast cancer can be highly heterogeneous and complex [59], and many mechanisms are involved in the occurrence and development of malignant tumors. Currently, the immune system, which consists of innate and adaptive immunity, influences tumor formation and progression. Adaptive immunity plays an important role in immune defense against tumors [60]. In other words, the emergence of an active adaptive immune response is often aimed at eradicating the tumor. In adaptive immunity, $CD8^+$ TILs, the main immune cell killer, are responsible for inhibiting tumor proliferation and disrupting metastasis by directly recognizing and killing tumor cells via intracellular antigens [61]. In naïve CD8 + T cells, intracellular antigens that are presented by major histocompatibility complex class I (MHC-I) molecules are expressed by all malignant cells [62]. Once activated, these cells are programmed to proliferate and differentiate into effector cells, known as CD8 + cytotoxic T lymphocytes (CTLs). To destroy their tumor target, CTLs migrate to the tumor site, infiltrate the tumor tissue, and interact with cancer cells to ultimately trigger effector functions via two independent mechanisms, the perforin/granzyme pathway and the FAS/FAS ligand (FASL) pathway [63, 64]. In addition, CD8 + T-cell-mediated type 1 immune responses can enhance the accumulation of distinct endogenous CD8 + and CD4 + T cells and facilitate their antitumor function within the tumor microenvironment [64, 65]. Generally, CD8 + T cells naturally protect normal host tissues and eliminate tumor cells. The extent of CD8 + T cells in the tumor site was positively correlated with patient prognosis.

This meta-analysis has several strengths. One strength of our study was that we conducted a comprehensive literature search regarding $CD8^+$ T cells and the prognosis of patients with breast cancer, which is the largest ($n = 34$) and most comprehensive study to date to quantify. Second, the methodology was consistent within the included studies, designed as cohort studies examining breast cancer through IHC. In addition, in studies with multivariate analysis, the adjusted parameters were similar. Third, to determine the effects of $CD8^+$TILs, we assessed four outcomes, namely, DFS, OS, BCSS and RFS. Thus, we could estimate the most appropriate prognostic markers for future clinical use. Finally, we performed a comprehensive subgroup analysis of the primary outcome, and we separately analyzed tumor-infiltrating cells by different counting methods considering that the location of lymphocytes may impact the results.

Limitations to this study were as follows. First, there was a lack of prospective articles. Second, although we performed subgroup analysis on prognostic associations between tumor location and CD8 + TILS, the immune tumor microenvironment is a complex network of tumors, immune cells, stromal cells and extracellular matrix [66]; moreover, stromal components surrounding tumor cells and other tumor-infiltrating lymphocytes in the tumor immune microenvironment may affect the prognosis of patients with breast cancer, and there may be interactions between different types of immune cells. In addition, differences in tumor size and chemotherapy regimens may also cause differences in the prognosis of patients with breast cancer, and these potential confounding variables vary greatly among individuals. Third, there was heterogeneity in the studies used to analyze the association between CD8 + TILs and breast cancer prognosis. This heterogeneity may be related to the study design, sample size, analysis strategy, participant characteristics, TMAs, and cutoff criteria. Given the heterogeneity, we chose a random-effects model in our meta-analysis, but the results were not materially altered when we used a fixed-effects model. Fourth, despite an extensive search and including the largest number of studies investigating the relationship between CD8 + TILS and breast cancer prognosis, we may still have missed some published or unpublished studies with negative results, which may have affected our pooled estimate. Finally, although we performed separate subgroup analyses of the relationship between CD8 + TILs by site and breast location for the primary outcome variable DFS, we recognize that the use of relatively few studies may have reduced the power of this study to detect publication bias.

The findings of this meta-analysis hold significant implications for both the surgical community and

healthcare policymakers. From a clinical standpoint, our results underscore the importance of considering immunological parameters, particularly CD8 + TILs, in surgical decision-making and postoperative management. By demonstrating the correlation between high CD8 + TIL counts and improved survival outcomes, we advocate for the integration of TIL assessment into routine diagnostic workups, thereby guiding decisions on the extent of surgery, the need for systemic treatments, and the potential utility of immunomodulatory interventions.

For policymakers, these insights highlight the potential value of investing in infrastructure that supports the routine quantification of CD8 + TILs in breast cancer biopsies. This includes the development of standardized protocols for TIL evaluation, training programs for pathologists, and the allocation of resources to facilitate research into the underlying mechanisms of TIL-mediated tumor control. Furthermore, recognizing CD8 + TILs as a prognostic factor may inform reimbursement policies for immunotherapy regimens, ensuring that patients with a favorable immune profile have access to cutting-edge treatments that could prolong survival and improve quality of life. Ultimately, this study contributes to a growing body of evidence supporting the centrality of immune contexture in breast cancer prognosis, thereby informing surgical practices and health policies that strive for precision medicine approaches.

## Conclusions

In conclusion, our meta-analysis provides strong evidence that a high density of CD8 + TILs could serve as an effective marker for evaluating the prognosis of patients with breast cancer. In the future, a larger number of prospectively designed randomized controlled trials with high-quality, multicenter samples are needed to verify the conclusions of this meta-analysis.

## Supplementary Information

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Supplementary Material 1

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There is no one who has contributed to the manuscript but does not qualify as a collaborator.

**Author contributions**
Study concept and design: Z.M.; Acquisition of data: R.N., C.W. and Y.X.; Analysis and interpretation of data: R.N., Y.X., S.L.and Q.Z.; Drafting of the manuscript: R.N. and Z.M.; Critical revision of the manuscript for important intellectual content: all authors; Study supervision: C.W., Y.C., and Z.M.

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**Data availability**
The datasets yielded during the study process are available from the first author (Ruijie Niu) on reasonable request.

## Declarations

**Ethics approval and consent to participate**
No ethical approval and patient consent were required for all analyses were based on literature research.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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