Association between levels of tumor-infiltrating lymphocytes in different subtypes of primary breast tumors and prognostic outcomes: A meta-analysis

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Abstract

Purpose To investigate the impact of the elevation of tumor-infiltrating lymphocytes (TILs) in different molecular subtypes of primary breast cancer, i.e. a 10% increment of TILs in tumor and lymphocyte-predominant breast cancer (LPBC), on long-term survival and pathological complete response (pCR) and to compare the presentation of high-level TILs across these molecular subtypes.

Methods Citation retrieval was performed in the PubMed, Cochrane Library, Embase and Web of Science databases. All statistical calculations were performed by the software of StataSE version 12.0.

Results Twenty-two eligible clinical trials including 15676 unique patients were included for meta-analysis. The 10% increment of TILs in human epidermal growth factor receptor 2 (HER2)-overexpression (pooled Hazard ratio (HR), 0.92; 95% CI, 0.89-0.95) and triple-negative (TN) (pooled HR, 0.90; 95% CI, 0.89-0.92) breast tumors significantly improved overall survival (OS) but in Luminal tumor subtype was inert to improve that (pooled HR, 1.06; 95% CI, 0.99-1.13). It was also associated with an increased pCR rate in breast cancers (pooled Odds ratio (OR), 1.27; 95% CI, 1.19-13.5). LPBC was significantly related with a higher pCR rate (OR, 2.73; 95% CI, 2.40-3.01) than non-LPBC. This significant difference was also shown in different molecular subtypes of LPBC compared with those of non-LPBC. HER2-amplified (OR, 3.14; 95% CI, 1.95-5.06) and TN (OR, 4.09; 95% CI, 2.71-6.19) phenotypes of breast cancers expressed significantly elevated high-level TILs than Luminal tumor subtype, although the presentation of those between the former two subsets was not significantly different (OR, 1.30; 95%CI, 0.83-2.04).

Conclusion The elevation of TILs in breast tumors predicts promising prognostic outcomes, particularly in the HER2-overexpression and TN subtypes. These benefits in Luminal tumor subtype need to be warranted.
Background

Tumor microenvironment is thought to play an important role in the germination, development, invasion and metastasis of tumors and is composed of immune cells, cytokines, adipocytes, and cancer-related fibroblasts, as well as the extracellular stroma. (1, 2) The interaction of immune lymphocytes and tumor cells is cardinal in these procedures. In the immune system, lymphocytes can eradicate tumor cells and prevent neoplasm development through immune surveillance; (3) tumor-infiltrating lymphocytes (TILs) participate in the regulation of the tumor niche and the inhibition of tumor formation and development. (2)

High-level TILs favor a good, long-term prognosis and enhanced chemosensitivity in primary aggressive molecular subtypes of breast cancer, including the human epidermal growth factor receptor 2 (HER2)-positive (HER2/neu oncogene overexpressed, estrogen receptor (ER)-negative) and triple-negative (TN) subtypes. When TN breast cancer patients undergo chemotherapy, each 10% increment of intratumoral TILs (iTILs) and stromal TILs (sTILs) leads to reductions of the recurrence risk of 17% and 15%, respectively, and to reductions of the death risk of 27% and 17%, respectively. (4) The presentation of high-level TILs is also positively associated with the survival benefits of anthracycline-based chemotherapy and anti-HER2 targeted therapy (trastuzumab) in HER2-positive breast tumors. (5) Of note, a pooled analysis of 3371 patients who underwent neoadjuvant therapy had a higher concentration of TILs, which led to a shorter overall survival (OS) than lower concentrations in the luminal phenotype of breast cancer. (6) suggesting a different biological feature of immune infiltration in this tumor subtype.

In this context, the purpose of our study is to settle these issues, including how each 10% increment of TILs and high-level TILs in breast cancer and in three tumor phenotypes (luminal, HER2-overexpression and TN) influence the OS and the pathological complete
response (pCR) rate. We also compare the expression of high-level TILs across these molecular subsets.

Methods

Search strategy

Electronic retrievals were performed from the PubMed, Web of Science, Cochrane Library and Embase databases according to the following search strategy: ((primary breast cancer) OR (primary breast tumor) OR (primary breast tumor)) AND ((tumor-infiltrating lymphocytes) OR (immune cells infiltration) OR (immune cells infiltrating) OR (immune cell infiltration) OR (immune cell infiltrating)) NOT (metastasis OR metastatic OR metastasize). No restrictions were used during the retrieval process. The deadline for retrieval was 25 March 2019.

Inclusion criteria

Clinical trials;
Female patients with primary a breast tumor;
The impact of each 10% increment of TILs or high-level TILs in breast cancer on the OS or on the pCR rate was reported in publications. Studies that documented at least two molecular tumor subtypes with the expression of high-level TILs were also included. TILs were quantified on hematoxylin and eosin–stained sections and evaluated by the usage of the guideline of the International TILs Working Group. (7) OS referred to the duration from the date of diagnosis to the date of death or lost follow-up. pCR was defined as the pathologically absent residual tumor foci in the breast and local regional lymph nodes. The definition of the high-level TILs was the TIL’s concentration in breast tumors greater than 50%.

Exclusion Criteria

Articles not published in English;
Studies referencing forkhead box P3 (FOXP3) + or programmed death 1 (PD-1) + or programmed death ligand-1 (PD-L1) + TILs;
Type of work: reviews, case reports, conference abstracts and conference papers;
Other conditions that did not meet the inclusion criteria.

The retrieved citations were screened by two reviewers (Yaling Wang and Yuhua Song) in terms of duplicated citations, titles, abstract sand full-texts. Only eligible trials that met
the inclusion criteria were included. If there were any inconsistencies, they were addressed by a discussion.

Data abstraction

Two co-authors (Yaling Wang and Yuhua Song) independently used Microsoft Excel version 2016 (Microsoft Corporation, Redmond, Washington, USA) to collect the following information from the eligible papers: the first author, publication year, original nation, median follow-up, median age, total number of analyzed patients, the Hazard Ratio (HR) with its 95% confidence interval (CI) indicating the association of the intervention factor and OS, the event number of pCR in different intervention factor or the Odds ratio (OR) with the 95%CI referencing the association between the intervention factor and pCR, as well as the event number of the presentation of high-level TILs in different subtypes. If some divergences existed, they were resolved by the third co-author (Xuezhen Ma).

Statistical analysis

We protocolled each 10% increment of TILs and high-level TILs in breast tumors as the study groups and non-10% increment of TILs and low-level TILs in tumors as the control groups. If the trials reported the event number of pCR in the study cohort and the control cohort, respectively, the crude OR with its 95% CI was calculated and pooled with that from the other studies. In the analysis of the impact of the intervention factors on OS, the crude HRs with their 95% CIs from the included studies were directly pooled. The comparison of the expression of high-level TILs across the three subtypes was computed in terms of the event and total numbers. If the publication was lacking the event number, it was obtained according to the incidence rate of the event or other information. The heterogeneity among analyzed trials was assessed by the heterogeneity $c^2$ test.
significant level of \( p<0.1 \) with its \( I^2 \) value. The fixed-effect model was used to pool the data if the heterogeneity test of the meta-analysis was not statistically significant; otherwise, the random-effect model was utilized. The publication bias of these analyses was evaluated by the Egger’s test (significant level of \( p<0.05 \)). The ER status, primary endpoint, and the chemotherapy strategy as well as the chemotherapy regimen as well as the TILs subset in the eligible studies were also discussed. All the statistical tests were conducted by StataSE software version 12.0 (StataCorp LP, College Station, TX, USA).

Results

Search Results

After the systematic retrieval from the abovementioned databases, a total of 914 initial citations were obtained by using the search strategy, and 392 potential citations were left for title and abstract screening following the deletions of duplications (n=285), conference papers (n=219), reviews (n=16) and case reports (n=2). Next, 49 articles remained for full-text assessment due to 343 citations being excluded via title and abstract screening; of these, studies that were reviews (n=2), inconsistent to the criteria of the high-level TILs in our study (n=4), devoid of useful data (n=16) and centered on PD-L1+TILs (n=3) or FOXP3+ TILs (n=2) did not meet the inclusion criteria and hence were excluded. Ultimately, 22 qualified studies were included for meta-analysis (Table 1).(5, 6, 8-27) The procedure of qualified article selection is outlined in Fig. 1.

Of those included studies, the publication year ranged from 2010 to 2019, 14 (63.6%) were retrospective studies with a total of 6958 cases, 10 (45.5%) were originally from Asian countries, 9 (40.9%) documented the breast cancer patients with ER-negative status, and the predominately chemotherapy strategy was in the setting of neoadjuvant therapy. Table 2 additionally represented the other details involving the median follow-
up, publication year, the median age, the analyzed cases in each analysis, the primary endpoint, and the detailed chemotherapy regimen, as well as the TILs subsets.

**Association of each 10% increment of TILs and OS**

Four studies recorded each 10% increment of TILs and OS in breast cancers without classification to different molecular subtypes, and the pooled results suggested that each 10% increment of TILs could not significantly improve OS (HR, 0.95; 95% CI, 0.91-1.01). However, there was a significant improvement in OS in terms of the pooled results of multivariate data (HR, 0.92; 95% CI, 0.85-0.98) but not that of univariate data (HR, 1.00; 95% CI, 0.94-1.06) (Fig. 2). In the subgroup analysis of different subtypes, the pooled results showed that, although each 10% increment of TILs in luminal tumor phenotype did not significantly improve OS (HR, 1.06; 95% CI, 0.99-1.13) (eFig. 1, Supplementary page 1), the improvements in OS were attained by it in HER2-overexpression (HR, 0.92; 95% CI, 0.89-0.95) (eFig. 2, Supplementary page 1) and TN (HR, 0.90; 95% CI, 0.89-0.92) subtypes (eFig. 3, Supplementary page 2). The results were both statistically significant in pooling the univariate data and the multivariate data of the latter two molecular phenotypes (these data were shown in eFig. 2 and eFig. 3, respectively).

**Association of each 10% increment of TILs and pCR**

Two studies reported each 10% increment of TILs and pCR in breast tumors, and one (25) of them divided patients into the training cohort and the validation cohort. Thus, three independently relevant data existed. The pooled results indicated that there was a significantly positive correlation between each 10% increment of TILs and the increased pCR rate (OR, 1.27; 95% CI, 1.19-1.35). The results of pooling univariate data (OR, 1.33; 95% CI, 1.19-1.47) and multivariate data (OR, 1.21; 95% CI, 1.14-1.28) were still
Association of high-level TILs and pCR

Eleven studies provided sufficient data to the association of high-level TILs and pCR. There was a significant difference in pCR rate between high-level and low-level TILs (OR, 2.73; 95% CI, 2.40-3.01), and the pooled results of univariate data (OR, 2.84; 95% CI, 2.46-3.21) and multivariate data (OR, 2.35; 95% CI, 1.65-3.05) were also both statistically significant (Fig. 4). In the subgroup analysis, the pooled results all indicated a higher pCR rate in luminal, HER2-overexpression and TN phenotypes with high-level TILs than those with low-level TILs, respectively (these data were outlined in eFig. 4, Supplementary page 3).

Comparison of high-level TILs expression across different breast cancer subsets

Seven studies were collected to perform the comparison of expression of high-level TILs across the different subsets of breast tumors. The pooled data of analysis showed that the presentation of high-level TILs between HER2-overexpression subtype and TN subtype was not significantly different (OR, 1.30; 95%CI, 0.83-2.04), whereas both subtypes experienced a significantly elevated expression of high-level TILs as compared to luminal phenotype (HER2-overexpression vs. luminal, OR, 3.14; 95% CI, 1.95-5.06; and TN vs. luminal, OR, 4.09; 95% CI, 2.71-6.19; respectively) (Fig. 5).

Publication bias

Several meta-analyses manifested moderate-to-considerable heterogeneity, and therefore, the random-effect model was employed to pool the data. With the exception of the impact of each 10% increment of TILs in TN tumor subtype on OS (p=0.001) and that of the high-
level TILs on pCR ($p=0.007$), there was no likelihood of publication bias in others because
the Egger’s tests of them were not statistically significant (eTable 1, Supplementary page 4). The funnel plots for both analyses with significant publication bias were presented in

**eFig. 5 (Supplementary page 5).**

**Discussion**

Previous meta-analyses have shown that the value of total TILs is that they are associated
with an improved outcome in breast cancer following neoadjuvant chemotherapy but not
in ER-negative subtypes.(2) There is, however, already controversy about whether this
benefit is indeed confined to patients with ER-positivity. To investigate this issue, we
evaluate all available evidence regarding ER-positive and ER-negative breast tumors from
a pool of clinical studies and demonstrate that each 10% increment of TILs in breast
tumors improves OS in HER2-amplified and TN molecular subtypes but not in the luminal
phenotype.

Our results also agree with Denkert’s(6) and West’s trials,(10) which both suggest that a
high TILs concentration increases the tumor response to neoadjuvant chemotherapy and
anthracycline-based chemotherapy, and is in association with better long-term survival in
the HER2-overexpression and TN breast tumors. A pooled analysis identified an
appropriate cut-off of sTILs for early-stage, node-negative TN breast cancer patients, in
which those patients with sTILs≥30% who underwent adjuvant treatment benefited the
excellent disease-free survival and OS.(28) Similarly, our meta-analysis confirms a
suitable cut-off of TILs≥50% for all molecular subtypes of primary breast tumors because
the high-level TILs predict a better pCR than the low-level TILs. Further studies need to be
launched to elucidate the best cut-off of TILs associated with the superior long-term
survival in breast tumors.

In the study by Denkert et al.,(6) it is found that the increased TILs may be an adverse
factor to OS in breast cancer patients with the luminal subtype, which differs from our results. This difference may be explained as follows. First, they only evaluated the OS in luminal-HER2-negative tumors, while our study also includes luminal-HER-positive of breast cancer patients. Furthermore, they only center on the assessment of the impact of sTILs on OS, but we additionally assess the iTILs. Last, the treatment strategies are not identical, as only neoadjuvant chemotherapy is included in their study but adjuvant chemotherapy is yet included in ours. Collectively, the elevation of the TILs concentration is not advantageous to predict the prolonged OS in breast cancer patients with the luminal subtype.

A large number of clinical trials are enthusiastic about the association between the presence of TILs and the pCR rate after chemotherapy in ER-negative breast cancers. The 2013 San Antonio Breast Cancer Symposium(5) and 2014 American Society of Clinical Oncology 50th Annual Meeting(29) reported that the presentation of TILs was associated with a higher pCR rate in the HER2-overexpression and TN phenotypes of breast tumors that underwent neoadjuvant chemotherapy (HER2-overexpression breast cancer routinely received trastuzumab treatment). These results map to our findings that each 10% increment of TILs preages a greater pCR rate in breast carcinoma, but it is imperfect as lack of enough data to perform the subgroup analysis of different disease subtypes. Consequently, the understudied association between each 10% increment of TILs and pCR rate in luminal breast cancer needs to be warranted. Of note, the association between different molecular subtypes of the high-level TILs and pCR rate is well delineated in our study, i.e. the increased pCR rate favors all subtypes with the high-level TILs when compared to those with the low-level TILs. Consistently, West and colleagues reaffirm that HER2-amplified and TN subtypes with the high-level TILs have a promising chemosensitivity to anthracycline-based adjuvant or neoadjuvant chemotherapy.(10)
In our study, the HER2-positive and TN subtypes of breast cancers greatly increase the expression of high-level TILs compared with the luminal tumor subtype; however, there is no significant difference in expression between the former two tumor subtypes. These disparate results may be attributed to the evaluation of different subtypes of immune cells. A variety of lymphocyte subtypes form TILs, which have different activities, from cytotoxicity to immune escaping effects. In TILs, the infiltration of cytotoxic CD8+ T cells predicts improved responses to anthracycline- and taxane-based regimens in primary breast cancers and significantly prolongs survival of patients. However, the infiltration of FOXP3+ T cells is a predictor of poor prognosis in that these cells mediate tumor immune escape. A meta-analysis of 17 trials demonstrated that FOXP3+ and PD-1+ TILs were associated with a worse prognosis in breast tumors. (2) Thus, articles with reference to PD-1+, PD-L1+ and FOXP3+ TILs are excluded in our study. Our results at a certain extent explain why luminal breast carcinoma is a predominantly immune cold tumor, the reasons for the good response to anti-HER2 targeted therapy, mediated in part by immune effector mechanisms, of HER2-positive breast cancer and of the strong immunogenic characteristic of breast tumors with the TN subtype.

Admittedly, this meta-analysis has limitations. First, selection bias might exist because of the inclusion criterion that limited the condition to English publications and the exclusion criteria that omitted the immune cell subsets of PD-1+TILs, PD-L1+TILs and FOXP3+TILs. Second, to obtain more evidence and larger scale of cases, analysis of pCR rate between the high-level and the low-level TILs also included trials centering on CD8+LPBC, giving rise to considerable heterogeneity. Finally, there may be clinical heterogeneity among the included studies, such as application of different chemotherapy regimens and treatment strategies, as well as investigation of different TILs subtypes.

Despite these limitations, this was the first meta-analysis that systematically evaluated
the influence of each 10% increment of TILs and the high-level TILs in breast cancer on OS and pCR, and compared the presentation of high-level TILs across different molecular subtypes. Future studies will need to supplement the underrecognized and understudied landscapes whether a higher pCR rate is related to each 10% increment of TILs in the luminal subtype of breast cancer and the high-level TILs in clinical high-risk luminal breast cancer patients can translate into a promising OS.

Conclusions

Each 10% increment of TILs in breast tumors predicts improved OS and pCR rate of patients, specifically in the HER2-overexpression and TN molecular subtypes. Moreover, all subsets with the high-level TILs benefit greater pCR rate than those with the low-level TILs. Although there is no difference between the expression of high-level TILs among HER2-overexpression and TN phenotypes of breast cancer, they both have greater expression than that relative to the luminal tumor subtype.

Abbreviations

TILs, tumor-infiltrating lymphocytes; LPBC, Lymphocyte-predominant breast cancer; HER2, human epidermal growth factor receptor 2; TN, triple-negative DFS, disease-free survival; OS, overall survival; pCR, pathologic complete response; iTILs, intratumoral TILs; sTILs, stromal TILs; PD-1, programmed death 1; PD-L1, programmed death ligand-1; FOXP3, forkhead box P3; HR, Hazard Ratio; CI, confidence interval; OR, Odds ratio; ER, estrogen receptor.

Declarations

-Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.
- Consent to publish

Not applicable

- Availability of data and materials

Not applicable

- Competing interests

The authors declare that they have no competing interests.

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- Authors’ Contributions

Lin He: Writing manuscript; Charting all figures; Drawing the tables.

Yaling Wang: Writing manuscript; Article selection; Data abstract.

Qian Wu: Article selection; Data abstract.

Yuhua Song: Article selection; Data abstract.

Biyuan Zhang: Article selection; Data abstract.

Haiji Wang: Conception/Design; Final approval of manuscript.

All authors reviewed and approved the manuscript prior to submission.

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29. OJotASoC O. Abstracts American Society of Clinical Oncology 50th Annual Meeting: J
| Study (Trial) | Publication Year | Study duration | Original nation | Median follow-up | No. of patient(n) | ER status |
|---------------|------------------|----------------|-----------------|------------------|-------------------|-----------|
| West(1)       | 2011             | Unknow         | Canada          | Unknow           | 111               | Negative  |
| Seo(2)        | 2013             | 2004-2011      | Korea           | Unknow           | 153               | Both      |
| Lee(3)        | 2013             | 2000-2009      | Korea           | Unknow           | 175               | Both      |
| Denkert(4)*   | 2010             | 1999-2001      | Germany         | Unknow           | 218               | Both      |
| Denkert(4)*   | 2010             | 2002-2005      | Germany         | Unknow           | 840               | Both      |
| Denkert(5)    | 2015             | Unknow         | Germany         | Unknow           | 580               | Negative  |
| Watanabe(6)   | 2018             | 2008-2016      | Japan           | 26.1m            | 197               | Both      |
| Galvez(7)     | 2018             | 2003-2014      | Peru            | Unknow           | 435               | Both      |
| Hida(8)       | 2016             | 2007-2014      | Japan           | Unknow           | 159               | Negative  |
| Denkert(9)    | 2018             | Unknow         | Germany         | Unknow           | 3771              | Both      |
| Hwang(10)     | 2019             | 2004-2013      | Korea           | 60.1m            | 248               | Both      |
| Kim(11)       | 2016             | 2004-2011      | Korea           | 6.4y             | 688               | Negative  |
| Sønderstrup(12)| 2019             | 1997-2011      | Denmark         | 5.8y             | 399               | Both      |
| Pruneri(13)   | 2016             | 1995-2010      | Switzerland     | 8.2y             | 897               | Negative  |
| Pruneri(14)   | 2016             | Unknow         | Italy           | 6.9y             | 647               | Negative  |
| Tian(15)      | 2016             | 2008-2012      | China           | 4y               | 372               | Negative  |
| Adams(16)     | 2014             | Unknow         | USA             | 10.6y            | 481               | Negative  |
| Loi(17)       | 2014             | Unknow         | Belgium         | 62m              | 934               | Both      |
| Kochi(18)     | 2018             | Unknow         | Japan           | Unknow           | 40                | Both      |
|               | 2015             | Unknow         | France          | 12.7y            | 781               | Both      |
Table 1: Details of the included trials.

| Study | Year | Location | Duration | Age | Size | Outcome |
|-------|------|----------|----------|-----|------|---------|
| Dieci(19) | 2019 | Unknow | Australia | 6y | 375 | Negative |
| Luen(20) | 2019 | Unknow | Australia | 50m | 678 | Negative |
| Luen(21) | 2019 | Unknow | Australia | 50m | 678 | Negative |
| Burugu(22) | 2017 | 1989-2002 | Canada | 13y | 2497 | Both |

*This article is divided into two researches due to different regimen.

**Abbreviations:** pCR, pathological complete response; OS, overall survival; NAC, neoadjuvant chemotherapy; AC, adjuvant chemotherapy; TNBC, triple-negative breast cancer; HER2+BC, human epidermal growth factor receptor 2 positive breast cancer.

**Regimen explanation:** FEC: fluorouracil, epirubicin, and cyclophosphamide; TET: docetaxel followed by epirubicin plus docetaxel; AC: doxorubicin and cyclophosphamide; ACT: AC followed by docetaxel; AD: doxorubicin and docetaxel; TAC: docetaxel, doxorubicin, and cyclophosphamide; PM: Paclitaxel and non-pegylated liposomal doxorubicin; PMCb: Paclitaxel and non-pegylated liposomal doxorubicin followed by carboplatin; TP: paclitaxel plus platinum; ACP: doxorubicin and cyclophosphamide followed by paclitaxel; CAF: cyclophosphamide, adriamycin and fluorouracil; CMF: cyclophosphamide, methotrexate and fluorouracil; CM: cyclophosphamide plus methotrexate.

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| Characteristic | Studies, No. (%) (N=22) | Primary Breast Cancer Patients, No. (%) (N=15676) |
|----------------|------------------------|-----------------------------------------------|
| **Study type** |                        |                                               |
| Randomized trial | 5 (22.7) | 3578 (22.8) |
| Retrospective | 14 (63.6) | 6958 (44.4) |
| Pooled | 1 (4.5) | 3771 (24.1) |
| Prospective-retrospective | 1 (4.5) | 934 (6.0) |
| Prospective | 1 (4.5) | 435 (2.8) |
| **Publication date, median (range), y** | 2016 (2010-2019) |
| **Follow-up, median (range), mo** | 90.6 (48.0-190.8) |
| **Median age, median (range), y** | 50.0 (46.5-54.0) |
| **10% increment of TILs and OS, total (range), n** | |
| All subtypes | 4460 (399-2346) |
| Luminal | 1886 (463-832) |
| HER2-enriched | 1985 (112-986) |
| TNBC | 3847 (92-897) |
| **10% increment of TILs and pCR, total (range), n** | 1638 (218-840) |
| **LPBC and pCR, total (range), n** | |
| All subtypes | 6697 (40-3771) |
| Luminal | 1717 (91-1366) |
| HER2-enriched | 1801 (40-1379) |
| TNBC | 1425 (48-906) |
| **High TILs across different subtypes, total (range), n** | |
| TNBC vs Luminal | 6524 (138-2297) |
| HER2-enriched vs Luminal | 6696 (149-2745) |
| TNBC vs HER2-enriched | 3722 (105-2285) |
| **Original area** | |
| Asia | 10 (45.5) | 3085 (19.7) |
| America | 4 (18.2) | 3524 (22.5) |
| Europe | 8 (36.4) | 9067 (57.8) |
|                      |                |                |
|----------------------|----------------|----------------|
| ER status            |                |                |
| ER-positive          | 0 (0.0)        | 0 (0.0)        |
| ER-negative          | 9 (40.9)       | 4300 (27.4)    |
| ER-both              | 13 (59.1)      | 11376 (72.6)   |
| Primary endpoint     |                |                |
| pCR                  | 10 (45.5)      | 6834 (43.6)    |
| OS                   | 11 (50.0)      | 6345 (40.5)    |
| Others               | 1 (4.5)        | 2497 (15.9)    |
| Chemotherapy strategy|                |                |
| Neoadjuvant          | 15 (68.2)      | 9669 (61.7)    |
| Adjuvant             | 5 (22.7)       | 5028 (32.1)    |
| Unknown              | 2 (9.1)        | 979 (6.2)      |
| Chemotherapy regimen |                |                |
| Anthracycline-based  | 3 (13.6)       | 2113 (13.5)    |
| Taxanes-based        | 1 (4.5)        | 3771 (24.1)    |
| Anthracycline- and taxanes-based | 10 (45.5) | 3822 (24.4) |
| Methotrexate-based   | 3 (13.6)       | 1915 (12.2)    |
| Unknown              | 5 (22.7)       | 4055 (25.9)    |
| TILs subsets         |                |                |
| TILs                 | 11 (50.0)      | 8014 (51.1)    |
| iTILs                | 3 (13.6)       | 4135 (26.4)    |
| sTILs                | 6 (27.3)       | 3199 (20.4)    |
| CD8+TILs             | 1 (4.5)        | 175 (1.1)      |
| CD4+TILs             | 1 (4.5)        | 153 (1.0)      |

Table 1. Summary of the characteristics of the 21 included Studies.

Abbreviations: TILs, tumor-infiltrating lymphocytes; OS, overall survival; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer; pCR, pathological complete response; LPBC, lymphocyte-predominant breast cancer; ER, estrogen receptor; iTILs, intratumoral tumor-infiltrating lymphocytes; sTILs stromal tumor-infiltrating lymphocytes.

*Median value is calculated in terms of available data.

Figures
Figure 1

Flow diagram of eligible article selection.
### Figure 2

Impacts of each 10\% increment of tumor-infiltrating lymphocytes in breast tumor on overall survival.
Figure 3

Impacts of each 10% increment of tumor-infiltrating lymphocytes in breast tumor on the pathological complete response.
### Table 1: Odds Ratios for High-Level TILs on Pathological Completed Response

| Study ID     | OR [95% CI]   | Weight (%) | Odds ratio I-V, Random, 95%CI |
|--------------|---------------|------------|-------------------------------|
| **Uni-variable analysis** |               |            |                               |
| Denkert (2018) | 2.62 [2.21-3.10] | 54.51      |                               |
| Lee (2013)    | 2.46 [0.93-6.46] | 1.42       |                               |
| Denkert (2010) | 4.12 [2.63-6.47] | 2.93       |                               |
| Denkert (2010) | 6.98 [2.71-17.98] | 0.19       |                               |
| Galvez (2018) | 2.60 [1.40-4.84] | 3.64       |                               |
| Watanabe (2018) | 5.73 [2.73-12.03] | 0.50       |                               |
| Denkert (2015) | 2.92 [1.98-4.31] | 7.97       |                               |
| West (2011)   | 6.33 [2.49-16.08] | 0.23       |                               |
| Seo (2013)    | 7.33 [2.02-26.21] | 0.07       |                               |
| Kochi (2018)  | 3.50 [2.43-5.13] | 5.94       |                               |
| Watanabe (2018) | 5.73 [2.73-12.00] | 0.50       |                               |
| Subtotal (I²=0%; p=0.50) | 2.84 [2.46-3.21] | 77.90       |                               |
| **Multi-variable analysis** |               |            |                               |
| Denkert (2015) | 2.66 [1.76-4.02] | 8.47       |                               |
| West (2011)   | 6.42 [2.08-19.80] | 0.14       |                               |
| Seo (2013)    | 2.38 [0.48-11.69] | 0.34       |                               |
| Kochi (2018)  | 2.02 [1.30-3.14] | 12.78      |                               |
| Watanabe (2018) | 4.97 [1.94-12.80] | 0.37       |                               |
| Subtotal (I²=0%; p=0.65) | 2.35 [1.65-3.05] | 22.10      |                               |
| **Total (I²=0%; p=0.58)** | **2.73 [2.40-3.01]** | **100.00** |                               |

![Figure 4](image)

**Impacts of the high-level TILs on the pathological completed response.**

28
Comparison of the expression of high-level TILs across different subtypes of breast tumors.
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