Research Square

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Predictive and Prognostic Role of Tumor Infiltrating Lymphocytes in Breast Cancer Patients With Different Molecular Subtypes: a Meta-Analysis

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Abstract

Background

Whether TILs plays different roles in different molecular subtypes breast cancer remains unknown. The prognostic and predictive value of TILs in different molecular subtypes breast cancer is still controversy. The aim of our meta-analysis is to assess the prognostic and predictive value of TILs in different molecular subtypes breast cancer by summarizing all relevant studies including multivariate analysis.

Methods

PubMed, Embase, EBSCO, ScienceDirect, Cochrane Database and the Web of Science were comprehensively retrieved (until March 2020). Hazard ratio (HR), odds ratio (OR) and their 95% confidence intervals (CI) were used as effect measures to perform our meta-analysis. Random effect model was used. Stata software, version 15 (2017) (Stata Corp, College Station, TX, USA) was used to carried out statistical analysis.

Results

Thirty-three studies including 18170 eligible breast cancer patients were analyzed. The meta-analysis showed that patients with high TILs expression were significantly correlated with increased pCR after neoadjuvant chemotherapy in HER2 enriched molecular subtype (OR = 1.137, 95% CI [1.061~1.218], p <0.001) and TNBC molecular subtype breast cancer (OR = 1.120, 95% CI [1.061~1.182], p <0.001). But, patients with high TILs expression were not significantly related to high pCR in luminal molecular subtype breast cancer after neoadjuvant chemotherapy (OR =1.154, 95% CI [0.789~1.690], p = 0.460). We carried out this meta-analysis on HR for OS and DFS to assess the prognostic value of TILs in breast cancer with different molecular subtypes more deeply. Our meta-analysis confirmed that high TILs had relationship with a significantly improved DFS in HER2 enriched molecular subtype [HR=0.940, 95%CI (0.903~0.979), *p*=0.003] and TNBC molecular subtype breast cancer patients [HR=0.907, 95%CI (0.862~0.954), *p*<0.001]. However, high TILs was not correlated with a significantly better DFS in luminal molecular subtype breast cancer patients [HR=0.998, 95%CI (0.977~1.019), *p*=0.840]. Furthermore, the results confirmed that high TILs had significant relationship with a better OS in HER2 enriched molecular subtype [HR=0.910, 95%CI (0.866~0.957), *p* <0.001] and TNBC molecular subtype breast cancer patients [HR=0.869, 95%CI (0.836~0.904), *p* <0.001]. Conversely, the summarized results indicated that high TILs was significantly correlated with a poor OS in luminal molecular subtype breast cancer patients [HR=1.077, 95%CI (1.016~1.141), *p*=0.012].

Conclusions

Our meta-analysis confirms that high TILs is correlated with favourable survival and predicts pathological complete response in breast cancer patients with TNBC molecular subtypes and HER2-enriched molecular subtypes.

Background

Breast cancer is one of the most common malignant tumors in women [1], which is still the second reason of cancer-related death in women around the world [2]. At present, the forecast of the prognosis is remain not ideal, and specific predictor is required to enhance the individualized therapy effect. The complex interaction between the immune system and cancer cells plays a vital role in controlling and eradicating cancer, and is regulated by a delicate balance between activation and suppression signals[3]. Research on the microenvironment of tumor can disclose the complex correlation between the immune system and the biological behavior of cancer cells. For restricting the development of breast cancer, it is very important to understand the tumor microenvironment.

More and more evidence indicates that the tumor microenvironment plays an important role in tumor formation, growth, invasion and metastasis. Tumor- infiltrating lymphocytes (TIL) play a vital role in regulating the tumor microenvironment, promoting or inhibiting canceration and cancer progression. Tumor-infiltrating lymphocytes (TIL) have emerged as potentially important prognostic and/or predictive biomarkers for breast cancer [4, 5]. While supplying valuable information, the heterogeneity of experimental design and TILs assessment hindered a more comprehensive understanding of the biological value of TILs. However, the prognostic value of TIL remains complex and controversial. Breast cancer is a clinical and molecular heterogeneous disease, and various factors determine the prognosis and response to treatment.

Thus, we carried out this meta-analysis, aiming to estimate the prognostic and predictive value of tumor infiltrating lymphocytes in patients with different molecular subtypes breast cancer.

Material And Methods

Retrieval strategy

Embase, PubMed, EBSCO, Cochrane Database,ScienceDirect and the Web of Science were comprehensively retrieved for researches exploring the prognostic and predictive relationship between tumor-infiltrating lymphocytes and breast cancer with different subtypes (without time, place of publication or language restrictions until March 2020). No retrieval restrictions were used. In addition, the reference lists of searched reviews and researches were examined to further identify potentially related articles. The main retrieval terms applied were “breast cancer”, “breast carcinoma”, “neoadjuvant chemotherapy”, “TILs”, “Tumor-infiltrating lymphocytes”, “prognosis”, “change”.

Selection standards

In order to ensure the accuracy and reliability of our analysis, we selected qualified studies according to the following standards: (i) The prognostic or predictive value of TILs testing in different subtypes breast cancer with at least one relevant outcome indicator has been reported in the research or can be computed based on published data. (ii) Choose high-quality studies that perform multivariate analysis on pCR or

survival data such as DFS, OS. (iii) Hazard ratio (HR),odds ratio (OR) and their 95% confidence interval (CI) were reported; or, they can be calculated according to the data of outcomes DFS, OS and pCR. (iiii) The samples were taken from the core-needle biopsy specimens or the surgical specimens after operation.

Two authors (Zhao-hua Gao and Ming Liu) independently performed literature retrieval and determined qualified researches according to the inclusion standards. Differences between the authors were settled by discussion and consensus. If no agreement can be reached, the final outcome is determined by a third-party researcher (Cun-xin Li). If there are more than one publications on the basis of the same patient groups, the most informational research was used.

Research quality appraisal and data collection

Our data was collected according to Cochrane guidelines [6]. Two authors (Zhao-hua Gao, Ming Liu) examined eligible researches independently, and any differences between the authors were settled by discussion and consensus. The following data for our meta-analysis were collected: Publication time, first author, country, study design, baseline patient characteristics, age range, treatment type, molecular subtypes, ethnicity, follow-up duration, TILs cutoff value, TILs position, outcomes (pCR, DFS, OS), hazard ratio (HR), odds ratio (OR) and 95% confidence interval (CI). The Newcastle Ottawa scale (NOS) criteria was used to evaluate the quality of the selected eligible researches[7]. Funnel plot was applied to estimate the publication bias. The researches selected in our meta-analysis had obtained written informed consent from all patients and were carried out according to the clinical practice principles, all local regulations and Helsinki Declaration.

Statistic analysis

In this meta-analysis, we chose pathological complete response (pCR) as a predictor of neoadjuvant chemotherapy (NAC) for breast cancer. We assessed the overall odds ratio (OR) and its 95% confidence interval (CI) of qualified studies for the predictive value of TILs in NAC of breast cancer. Overall survival (OS) and disease-free survival (DFS) were used as prognostic outcomes in our meta-analysis. In the meta-analysis, hazard ratio (HR) and its 95% confidence interval (CI) were used as the effect scales of prognosis. The association between TILs and clinicopathological parameters were evaluated using odds ratio (OR) and its 95% confidence interval (CI). If the HR or OR and its 95% CI can not be obtained directly from the original article, we can use the available data to calculate with the software designed by Tierney et al[8]. The Q test was used to calculate the heterogeneity between studies and the I^2 value represents the size of the heterogeneity [9]. If I^2 value > 40% showed high heterogeneity [6]. Supposing that the heterogeneity was high, a random effects model was utilized. If not, a fixed-effect model was applied. P value was set as < 0.05, which was statistically significant. The clinicopathological parameters, prediction and prognostic indicators of all relevant studies were pooled analyzed. At the same time, subgroup analysis was completed based on different ethnicity and different study design. Quality and homogeneity of results were estimated by sensitivity analysis. Utilize a funnel chart to test publication bias.

Stata software, version 15 (2017) (Stata Corp, College Station, TX, USA) was used to carried out statistical analysis. This meta-analysis was guided by the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [10].

Results

Baseline characteristics of qualified researches

In the systematic literature retrieval, we found 617 researches. By reviewing the title and abstract, 74 possible related researches were identified. In the remaining 74 studies, 41 of these studies were later excluded because they did not meet the selection standards. Eventually, we determined that 33 researches met the inclusion standards.[4, 5, 11–41] Fig. 1, summarized the search and screening process. The 33 researches comprised 18170 qualified patients with breast cancer (sample capacity, median: 331 [50–3771], mean: 550). These studies were published between 2010 and 2020 from the Europe, America, Australia and Asia (Spain, France, Italy, United Kingdom, Belgium, Finnish, Germany, Ireland, USA, Canada, Australia, Japan, Korea, China). Eligible researches evaluated TILs by hematoxylin and eosin stained sections. Twelve studies provided OR on pCR to complete the meta-analysis[13, 23, 25, 26, 28, 31, 33–35, 37, 38, 41]. Fifteen of these researches provided HRs data of DFS or OS and we performed the pooled analysis. Twelve researches furnished HRs data of DFS [5, 13, 15–17, 24, 26, 29–31, 37, 40], and ten researches furnished HRs data of OS [5, 13, 15, 17, 18, 20, 21, 24, 26, 29]. Table 1, summarized the main baseline characteristics. We estimated the quality of selected researches based on the NOS and As shown in Table 2.

Relationship of the lymphocyte-predominant breast cancer (LPBC) with clinicopathological parameters

T stage

The incidence of LPBC in the T3,T4 group was lower than T1,T2 group, the difference achieve statistical significance [OR = 0.646, 95%CI(0.542, 0.771), I^2= 0.0%, z = 4.85, p < 0.001]. After that, subgroup analysis were conducted on different ethnicity [Europe: OR = 0.661, 95%CI(0.546, 0.800), I^2=0.0%, z = 4.25, p < 0.001; Asia: OR = 0.516, 95% CI(0.297, 0.898), I^2=0.0%, z = 2.34, p = 0.019; America: OR = 0.695, 95% CI (0.294, 1.643), z = 0.83, p = 0.407] and different study design [RCT: OR = 0.663, 95% CI (0.550, 0.798), I^2= 0.0%, z = 4.33, p < 0.001; retrospective: OR = 0.516, 95% CI (0.297, 0.898), I^2=0.0%, z = 2.34, p = 0.019]. In the Asian group and the European group, the difference were statistically significant.

Lymph node status

The pooled analysis indicated that the incidence of the lymphocyte-predominant breast cancer (LPBC) detection between the lymph node metastasis group and the non lymph node metastasis group has no significant difference (overall: OR = 0.941, 95% CI 0.681, 1.298], I^2= 76.4%, z = 0.37, p = 0.709). After that, subgroup analysis were carried out on different ethnicity [Europe: OR = 0.991, 95% CI (0.633, 1.551), I^2=

80.8%, z = 0.04, p = 0.968; Asia: OR = 1.013, 95% CI (0.595, 1.726), I²=60.8%, z = 0.05, p = 0.962; America: OR = 0.549, 95% CI (0.322, 0.936), z = 2.20, p = 0.028]. The difference was statistically significant in the America group.

Histological type

The incidence of the lymphocyte-predominant breast cancer (LPBC) was significantly different between the invasive ductal carcinoma and invasive lobular carcinoma groups (overall: OR = 2.654, 95% CI [1.132, 6.223], I²= 68.0%, z = 2.24, p = 0.025). Then subgroup analysis were performed on different study design (RCT: OR = 4.735, 95% CI [2.850, 7.867], I²= 0.0%, z = 6.00, p < 0.001; retrospective: OR = 1.101, 95% CI [0.622, 1.951], I²=0.0%, z = 0.33, p = 0.740). The difference was statistically significant in the RCT group.

Histological grade

The detection of the lymphocyte-predominant breast cancer (LPBC) in pathological specimen can show histological grading [III versus □ and I, overall: OR = 2.889, 95%CI (2.218, 3.762), I²=49.5%, z = 7.87, p < 0.001]. After that, subgroup analysis were conducted on different ethnicity [Europe: OR = 2.871, 95% CI (2.290, 3.600), I²= 25.5%, z = 9.14, p < 0.001; Asia: OR = 5.636, 95% CI (3.050, 10.415), I²=0.0%, z = 5.52, p < 0.001; America: OR = 1.659, 95% CI (0.982, 2.804), z = 1.89, p = 0.059] and different study design [RCT: OR = 2.763, 95% CI (2.188, 3.489), I²= 39.7%, z = 8.53, p < 0.001; retrospective: OR = 3.284, 95% CI (1.359, 7.934), I²= 64.0%, z = 2.64, p = 0.008]. In the Asian group and the European group, the difference were statistically significant.

Gene expression of ER, PR and HER2

The lymphocyte-predominant breast cancer (LPBC) incidence rate in the ER□group was significantly lower than that in the ER- group [total: OR = 0.291, 95%CI (0.185, 0.458), I²= 70.0%, z = 5.35, p < 0.001];After that, subgroup analysis were conducted on different ethnicity [Europe: OR = 0.348, 95%CI (0.197, 0.614), I²= 61.1%, z = 3.65, p < 0.001; Asia: OR = 0.154, 95%CI (0.090, 0.264), z = 6.80, p < 0.001; America: OR = 0.342, 95%CI (0.216, 0.540), z = 4.60, p < 0.001]and different study design [RCT: OR = 0.360, 95%CI (0.230, 0.563), I²= 60.1%, z = 4.49, p < 0.001; retrospective: OR = 0.191, 95%CI (0.105, 0.346), I²= 30.4%, z = 5.44, p < 0.001]. In addition, PR□and PR□ groups [total:OR = 0.396, 95%CI (0.173, 0.906), I²=0.0%, z = 2.19, p = 0.028]. Furthermore, the detection rate of lymphocyte predominant breast cancer (LPBC) between HER2□ group and HER2□ group has not significant difference. [total: OR = 1.359, 95%CI (0.646, 2.858), z = 0.81, p = 0.419]. different ethnicity [Europe: OR = 1.443, 95% CI (0.529, 3.933), I²= 92.0%, z = 0.72, p = 0.474; Asia: OR = 1.097, 95% CI (0.539, 2.230), z = 0.25, p = 0.799].

Ki-67 status

The incidence of the lymphocyte-predominant breast cancer (LPBC) was significantly different between the high Ki-67 groups and the low Ki-67 groups (overall: OR = 6.378, 95% CI [3.674, 11.073], I²= 30.1%, z = 6.58, p < 0.001).

Menopausal status

The lymphocyte-predominant breast cancer (LPBC) detection rate between the premenopausal group and the postmenopausal group has not significant difference [total: OR = 0.963, 95%CI (0.716, 1.296), I^2=0.0%, z = 0.25, p = 0.804]. After that, subgroup analysis were conducted on different ethnicity [Asia: OR = 1.036, 95%CI (0.629, 1.708), I^2= 29.3%, z = 0.14, p = 0.888; America: OR = 0.874, 95% CI (0.571, 1.339), z = 0.62, p = 0.537].

TNM stage

The lymphocyte-predominant breast cancer (LPBC) detection rate between the III, Ⅳ group and I, II group has not significant difference [total: OR = 0.825, 95%CI (0.220, 3.095), I^2= 81.4%, z = 0.29, p = 0.775]. After that, subgroup analysis were conducted on ethnicity [Europe: OR = 0.431, 95% CI (0.211, 0.881), I^2=0.0%, z = 2.31, p = 0.021; Asia: OR = 1.268, 95% CI (0.684, 4.050), I^2=0.0%, z = 1.12, p = 0.261]. The difference was statistically significant in the Europe group. The results of pooled analysis were summarized in Table 3.

Impact of TILs on pathological complete response (pCR)

In order to further assess the predictive effect of TILs detection in breast cancer patients with different molecular subtypes, the OR value of PCR was analyzed by meta-analysis. In this meta-analysis, we chose those studies that focused on TILs as a continuous parameter (per 10% increments). OR value of pCR were available in three studies including luminal molecular subtype breast cancer. There was no significant increase of PCR in high TILs group [OR = 1.154, 95%CI (0.789–1.690), p = 0.460]. OR value of pCR were available in seven studies including HER2 enriched molecular subtype breast cancer. The assessed pooled OR value confirmed that high TILs was correlated with a significantly increased pCR [OR = 1.137, 95%CI (1.061–1.218), p < 0.001]. OR value of pCR were available in seven studies including TNBC molecular subtype breast cancer. The estimated pooled OR value showed that high TILs was related to significantly increased pCR [OR = 1.120, 95%CI (1.061–1.182), p < 0.001]. OR value of pCR were available in nine studies including all breast cancer patients. The assessed pooled OR value confirmed that high TILs was related to significantly increased pCR [OR = 1.214, 95% CI (1.108–1.329), p < 0.001]. High quality researches (NOS score > 6) were used to perform the sensitivity analysis and the results were consistent (HER2 enriched molecular subtype breast cancer: OR = 1.133, 95%CI 1.057–1.215, P < 0.001; TNBC molecular subtype breast cancer: OR = 1.237, 95%CI 1.094–1.399, P = 0.001). But in breast cancer patients with luminal molecular subtype, estimated pooled OR value showed that high TILs was correlated with significantly increased pCR [OR = 1.298, 95% CI (1.157–1.456), p < 0.001]. Figure 2 summarized the results of the PCR assessment. The publication bias was detected by Begg's test (Fig. 3).

Effect of TILs on prognosis (OS and DFS)

In order to further estimate the survival impact of TILs detection in breast cancer patients with different molecular subtypes, the HR value of DFS or OS was analyzed by meta-analysis. In this meta-analysis, we chose those studies that focused on TILs as a continuous parameter (per 10% increments). Four studies about luminal molecular subtype breast cancer provide HRs values of DFS. There was no significant improvement of DFS in high TILs group [HR = 0.998, 95%CI (0.977–1.019), p = 0.840]. Four studies about HER2 enriched molecular subtype breast cancer provide HRs values of DFS. The assessed pooled HRs

value confirmed that high TILs was correlated with a significantly increased DFS [HR = 0.940, 95%CI (0.903–0.979), p = 0.003]. Six studies about TNBC molecular subtype breast cancer provide HRs values of DFS. The estimated pooled HRs value showed that high TILs was related to significantly increased DFS [HR = 0.907, 95%CI (0.862–0.954), p < 0.001]. Four studies about all breast cancer patients provide HRs values of DFS. The assessed pooled HRs value confirmed that high TILs was correlated with significantly increased DFS [HR = 0.988, 95%CI (0.979–0.997), p = 0.012]. High quality researches (NOS score > 6) were used to carry out the sensitivity analysis and the results were consistent (breast cancer with HER2 enriched molecular subtype: HR = 0.946, 95% CI 0.913 ~ 0.980, P = 0.002; breast cancer with TNBC molecular subtype: HR = 0.893, 95% CI 0.867 ~ 0.921, P < 0.001; breast cancer with luminal molecular subtype: HR = 0.998, 95% CI 0.977 ~ 1.019, P = 0.840). Figure 4 summarized the results of the DFS assessment. The publication bias was detected by Begg's test. No significant publication bias was found (Fig. 5).

In addition, the HRs values of OS were obtained in four researches. The pooled analysis confirmed that the high TILs group of luminal molecular subtype breast cancer was significantly correlated with unfavorable OS [HR = 1.077, 95%CI (1.016 ~ 1.141), p = 0.012]. By contrary, the HRs values of OS were obtained in three researches about HER2 enriched molecular subtype patients. The assessed pooled HRs value showed that high TILs was related to significantly favourable OS [HR = 0.910, 95%CI (0.866–0.957), p < 0.001]. The HRs values of OS were obtained in eight researches about breast cancer with TNBC molecular subtype. The evaluated pooled HRs value indicated that high TILs was correlated with significantly favourable OS [HR = 0.869, 95%CI (0.836 ~ 0.904), p < 0.001]. The HRs values of OS were obtained in four researches about all breast cancer patients. The estimated pooled HRs value confirmed that high TILs was correlated with significantly favourable OS [HR = 1.017, 95%CI (0.983–1.052), p = 0.324]. High quality researches (NOS score > 6) were applied to conduct the sensitivity analysis and the results were consistent. Figure 6 summarized the results of the OS assessment. The publication bias was tested by Begg's test. No significant publication bias was found (Fig. 7).

Discussion

Breast cancer is a highly heterogeneous disease of clinical process and molecular type. At present, standardized systemic therapy has significantly increased the survival of breast cancer, but metastasis and recurrence remain the determinants of death. Therefore, how to further reduce recurrence and metastasis is still the key issues in clinical practice. The complex interaction between the immune system and cancer cells plays a vital role in controlling and eradicating cancer[3]. A few decades ago, people noticed that the tumor microenvironment contained a variable number of lymphocytes, later called tumor infiltrating lymphocytes (TILs)[42]. Tumor-infiltrating lymphocytes (TILs) have become a potential biomarker for survival prediction of breast cancer patients [4, 5]. In patients with different molecular subtypes, a wide evaluation of the clinical impact of TILs will help to discover the important mechanism of the interaction between tumor and host immunity. Nevertheless,the clinical significance of TILs in different molecular subtypes patients is still unclear. By summarizing and analyzing relevant high-quality

researches, our meta-analysis aims to provide evidences for determining the clinical significance of TILs in different molecular subtypes breast cancer.

Pooled analysis confirmed that lymphocyte-predominant breast cancer (LPBC) was significantly correlated with higher histopathological grade. Moreover, our meta-analysis indicated that LPBC was related to Ki-67, ER and PR status. Afterwards, sensitivity analysis excluding low-quality researches showed consistent results. Whether TILs played different roles in patients with different molecular subtypes remains unknown. We further analyzed the prognostic value and predictive roles of TILs in different molecular subtypes patients. In order to further estimate the survival impact of TILs detection in patients with different molecular subtypes, the HR value of DFS or OS was analyzed by meta-analysis. The assessed pooled OR value confirmed that high TILs was correlated with significantly increased pCR in HER2 enriched molecular subtype breast cancer in multivariate analysis researches. The assessed pooled HRs value confirmed that high TILs was correlated with significantly increased DFS. The assessed pooled HRs value showed that high TILs was related to significantly favourable OS. The sensitivity analysis showed the robustness of the HR estimates.

In TNBC molecular subtype breast cancer, estimated pooled OR value showed that high TILs was related to significantly improved pCR in multivariate analysis researches. Furthermore, the assessed pooled HRs value confirmed that high TILs was correlated with significantly improved DFS and favourable OS in multivariate analysis researches.

In luminal molecular subtype breast cancer, there was no significant increase of PCR in high TILs group. In addition, there was no significant improvement of DFS in high TILs group. Conversely, the pooled analysis confirmed that high TILs group of luminal molecular subtype breast cancer was significantly correlated with unfavorable OS. Due to the small number of studies, the results of this analysis should be interpreted with caution.

Our meta-analysis confirmed that TILs is an ideal biomarker for TNBC molecular subtype and HER2 enriched molecular subtype breast cancer to predict pathological complete response and favourable prognosis. On the contrary, TILs is a biomarker for predicting poor overall survival of breast cancer with luminal molecular subtypes. Therefore, TILs should be monitored in breast cancer patients for rational stratification and adjusting the treatment strategy, and further detailed and in-depth researches on TILs and breast cancers of different molecular subtypes are needed. Further study on the different roles of different TILs subclasses in different molecular subtypes breast cancer will help us to further understand the precise mechanism of TILs and provide more evidences for immunotherapy of breast cancer with different molecular subtypes.

The limitations of this meta-analysis include the following aspects. Firstly, heterogeneity cannot be avoided completely, so we choose random effect model. Secondly, fewer high-quality stratified studies on different molecular subtypes of breast cancer can affect the statistical efficacy of our results. Therefore, it is necessary to conduct more prospective clinical studies to clarify the true usefulness of TILs. Thirdly, our study is according to data provided by different studies, not individual patient data, so reliable

correlation estimates cannot be made. Although our research has some limitations, we systematically evaluated a large number of high-quality researches with multivariate analysis, and the research results may be a reliable reference for guiding clinical practice.

Conclusion

In conclusion, we performed a meta-analysis included thirty-three high quality studies including multivariate analysis and 18170 patients with different molecular subtypes breast cancer were analyzed. our meta-analysis confirms that high TILs is correlated with favourable survival and predicts pathological complete response in breast cancer patients with TNBC molecular subtypes and HER2-enriched molecular subtypes. Conversely, the pooled analysis confirmed that high TILs group of luminal molecular subtype breast cancer was significantly correlated with unfavorable OS. Large scale, multicenter and well-designed high-quality studies are needed to study the role of different TILs subclasses in different molecular subtypes of breast cancer. Moreover, it can afford guidance for clinical practice of breast cancer with different molecular subtypes.

Abbreviations

TILs: tumor-infiltrating lymphocytes; sTILs: stromal tumor-infiltrating lymphocytes; iTILs: intratumoral tumor-infiltrating lymphocytes; OR: odds ratio; HR: hazard ratio; CI: confidence intervals; pCR: pathologic complete response; DFS: disease-free survival; OS: overall survival; RFS: Relapse-free survival; BCSS: breast cancer-specific survival; DRFI: distant recurrence–free interval; MFS: metastases-free survival; DDFS: distant disease-free survival; BCFI: breast cancer-free interval; EFS: Event-free survival; HE: hematoxilin and eosin; IHC: Immunohistochemistry; CNB: core needle biopsy; NAC: Neoadjuvant chemotherapy; CRT: chemoradiotherapy; TMA: tissue microarrays; B-NC: before Neoadjuvant chemotherapy; post: postoperative; NR: Not reported; LN: lymph node; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor-2; TNBC: triple-negative breast cancer; LPBC: lymphocyte-predominant breast cancer; RCT: randomized controlled trial; NOS: Newcastle-Ottawa Scale.

Declarations

Ethics approval and consent to participate

The research was carried out according to the local regulations and was ratified by the Ethics Committee of the Liaoning Province Cancer Hospital and Research Institute.

Consent for publication

Consent for publication was obtained from the participants.

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests.

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None

Authors' contributions

Zhao-hua Gao, Ming Liu, and Cun-xin Li participated in the conception and design of the study. Zhao-hua Gao and Cun-xin Li participated in article selection and data extraction and provided statistical expertise. Zhao-hua Gao and Jia-yuan Jiang did the studies selection, data extraction, statistical analyses and the writing of report. Zhao-hua Gao and Ming Liu contributed to the literature search and figures. Zhao-hua Gao, Cun-xin Li, and Ming Liu participated in the critical revision of the manuscript and interpretation of data. All authors drafted and critically revised the manuscript and approved the final version.

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Tables

Table 1. Baseline characteristics of included studies

first author	year of publication	Country	Study design	Number (n)	Treatment type	sample time	TILs site	TIL evaluation method	curative resection	Endpoint measured	Follow up Median (range)(M)
Hwang, Hye Won[11]	2019	Korea	retrospective	308	NAC	pre-NAC/post-NAC	sTILs	HE	YES	pCR/DFS/BCSS	60.1
Ahn, S. G.[12]	2018	Korea	retrospective	198	not NAC	resection tissue	sTILs	HE	NR	NO	NR
Yang, Xia[13]	2018	China	retrospective	143	NAC+H	pre-NAC/post-NAC	sTILs	HE	YES	pCR/DFS/OS	53(12–102)
Herrero-Vicent, C.[14]	2017	Spain	retrospective	164	NAC	pre-NAC/post-NAC	sTILs	HE	NR	pCR/DFS	78
Luen, S. J.[15]	2019	Australia	retrospective	375	NAC	pre-NAC/post-NAC	sTILs	HE	NR	RFS/OS	72
Fujimoto, Yukie[16]	2019	Japan	retrospective	717	NAC/adjuvant	pre-NAC/post-NAC	iTILs+sTILs	HE	YES	DFS/OS	35.1(1–100.6)
Adams, S.[17]	2014	USA	RCT	481	adjuvant	resection tissue	iTILs+sTILs	HE	NR	DFS/OS/DRFI	127.2
Dieci, M. V.[18]	2014	France/Italy	retrospective	278	NAC/adjuvant	post-NAC	iTILs+sTILs	HE	NR	MFS/OS	75.6
Perez, E. A.[19]	2016	USA	RCT	945	adjuvant	resection tissue	sTILs	HE	NR	RFS	52.8
Dieci, M. V.[20]	2015	France	RCT	781	adjuvant	resection tissue	iTILs+sTILs	HE	NR	OS/DFS	152.4
Loi, S.[5]	2013	Belgium	RCT	2009	adjuvant	resection tissue	iTILs+sTILs	HE	NR	DFS/OS	96
Loi, S.[21]	2014	Finnish	retrospective	934	adjuvant	resection tissue	sTILs	HE	NR	DDFS/OS	62
Yasmin Issa-Nummer[22]	2013	Germany	RCT	313	NAC	pre-NAC	iTILs+sTILs	HE	NR	pCR	NR
Denkert, C.[4]	2010	Germany	RCT	1058	NAC	pre-NAC	iTILs+sTILs	HE	NR	pCR	NR
Denkert, C.[23]	2015	Germany	RCT	580	NAC	pre-NAC	iTILs+sTILs	HE	NR	pCR	NR
Pruneri, G.[24]	2016	Italy	RCT	647	adjuvant	resection tissue	sTILs	HE	NR	BCFI / DFS / DRFI / OS	82.8
Ingold Heppner, B.[25]	2016	Germany	RCT	498	NAC	pre-NAC	sTILs	HE	NR	pCR / DFS	60.4(59.5–61.3)
Denkert, C.[26]	2018	Germany	RCT	3771	NAC	pre-NAC	sTILs	HE	NR	pCR / DFS / OS	62.8
Wang, Qiong[27]	2020	China	retrospective	75	NAC	pre-NAC/post-NAC	sTILs	HE/IHC	YES	pCR / DFS	23.2(6.1–64.5)
Brodsky, Alexander S.[28]	2016	USA	retrospective	50	NAC+H	pre-NAC	sTILs	HE	NR	pCR	127.2
Leon-Ferre, Roberto A.[29]	2018	USA	retrospective	605	adjuvant	resection tissue	iTILs+sTILs	HE	YES	IDFS / OS	127.2
Salgado, Roberto[30]	2015	Australia	RCT	387	NAC+H/L	pre-NAC	sTILs	HE	NR	EFS/ pCR	45.2(42-50.6)
Ignatiadis, Michail[31]	2019	Belgium	RCT	213	NAC	pre-NAC/post-NAC	sTILs	HE	NR	pCR / EFS	56.4
Mori, H.[32]	2017	Japan	retrospective	248	adjuvant	resection tissue	sTILs	HE	YES	RFS / OS	68(2–150)
Dieci, M. V.[33]	2016	Italy	retrospective	105	NAC	pre-NAC/post-NAC	iTILs+sTILs	HE	YES	pCR / EFS	NR
Ruan, Miao[34]	2018	China	retrospective	166	NAC	pre-NAC	iTILs+sTILs	HE	YES	pCR	NR
O'Loughlin, Mark[35]	2018	Ireland	retrospective	75	NAC	pre-NAC	sTILs	HE	NR	pCR	NR
Ali, H. Raza[36]	2016	UK	RCT	614	NAC	pre-NAC/post-NAC	sTILs	HE	NR	pCR	NR
Song, I. H.[37]	2017	Korea	retrospective	108	NAC	pre-NAC/post-NAC	sTILs	HE/IHC	YES	pCR/DFS	31.4(21.1-53.0)
Li, X.[38]	2016	USA	retrospective	129	NAC+H	pre-NAC	iTILs+sTILs	HE	YES	pCR	NR
Würfel, F.[39]	2018	Germany	retrospective	146	NAC	pre-NAC	sTILs	HE	NR	pCR	NR
Hamy, A. S.[40]	2019	France	retrospective	718	NAC±H	pre-NAC/post-NAC	sTILs	HE	YES	pCR/DFS/OS	NR
Khoury, T.[41]	2018	USA	retrospective	331	NAC	pre-NAC	iTILs+sTILs	HE	NR	pCR	NR

Table 2. The evaluation of the risk of bias in research using the Newcastle–Ottawa scale

Study	Selection (0–4)				Comparability (0–2)		Outcome (0–3)			Total
	REC	SNEC	AE	DO	SC	AF	AO	FU	AFU	
Hwang, Hye Won et al.[11]	1	0	1	1	1	1	1	1	1	8
Ahn, S. G et al.[12]	1	0	1	1	1	0	1	0	0	5
Yang, Xia et al.[13]	1	0	1	1	1	0	1	1	1	7
Herrero-Vicent, C et al.[14]	0	0	1	1	0	0	1	1	0	4
Luen, S. J et al.[15]	1	0	1	1	1	0	1	1	1	7
Fujimoto, Yukie et al.[16]	1	0	1	1	1	1	0	1	1	7
Adams, S et al.[17]	1	1	1	1	1	1	1	1	1	9
Dieci, M. V et al.2014[18]	1	0	1	1	1	1	0	1	1	7
Perez, E. A et al.[19]	1	0	1	1	1	1	1	1	1	8
Dieci, M. V et al.2015[20]	1	0	1	1	1	1	1	1	1	8
Loi, S et al.2013[5]	1	1	1	1	1	1	1	1	1	9
Loi, S et al.2014[21]	1	1	1	1	1	1	1	1	1	9
Yasmin Issa-Nummer et al.[22]	1	0	1	1	1	1	1	1	1	8
Denkert, C et al.2010[4]	1	1	1	1	1	1	1	1	1	9
Denkert, C et al.2015[23]	1	1	1	1	1	1	1	1	1	9
Pruneri, G et al.[24]	1	0	1	1	1	1	1	1	1	8
Ingold Heppner, B et al.[25]	1	0	1	1	1	1	1	1	1	8
Denkert, C et al.2018[26]	1	1	1	1	1	1	1	1	1	9
Wang, Qiong et al.[27]	0	0	1	1	1	0	0	1	1	5
Brodsky, Alexander S et al.[28]	0	0	1	1	1	0	0	1	1	5
Leon-Ferre, Roberto A et al.[29]	1	1	1	1	1	1	1	1	1	9
Salgado, Roberto et al.[30]	1	1	1	1	1	1	1	1	1	9
Ignatiadis, Michail et al.[31]	1	0	1	1	1	0	1	0	1	6
Mori, H et al.[32]	1	0	1	1	1	0	1	1	1	7
Dieci, M. V et al.2016[33]	1	1	1	1	1	1	1	0	0	7
Ruan, Miao et al.[34]	1	0	1	1	1	0	1	0	0	5
O'Loughlin, Mark et al.[35]	1	0	1	1	1	0	1	0	0	5
Ali, H. Raza et al.[36]	1	1	1	1	1	1	1	0	0	7
Song, I. H et al.[37]	1	0	1	1	1	1	1	0	0	6
Li, X et al.[38]	1	0	1	1	1	1	1	0	0	6
Würfel, F et al.[39]	1	0	1	1	1	1	1	0	0	6
Hamy, A. S et al.[40]	1	1	1	1	1	1	1	0	0	7
Khoury, T et al.[41]	1	1	1	1	1	1	1	0	0	7

NOTE: REC: Representativeness of the exposed cohort; SNEC: Selection of the non exposed cohort; AE: Ascertainment of exposure; DO: Demonstration that outcome of interest was not present at start of study; SC: study controls for age, sex; AF: study controls for any additional factor; AO: Assessment of outcome; FU: follow-up long enough for outcomes to occur (36 Months); AFU: Adequacy of follow up of cohorts (≥90%). "1" means that the study is meeted the item and "0" means the opposite situation.

Table 3. Detailed subgroup analysis of clinicopathological parameters

clinicopathological parameters		Different ethnicity			Different study design	
	Any	Europe	Asia	America	RCT	Retrospective
Age >50 vs.≤50 (OR)	0.873[0.761,1.002]; I2=0.0% ; z=1.93; p = 0.054	0.868[0.754,1.000]; I2=0.0%; z=1.96; p = 0.049	0.990[0.513,1.912]; z=0.03; p = 0.976	–	0.869[0.753,1.002]; I2= 0.0% ; z= 1.93 ; p = 0.054	0.935 [0.563, 1.554]; I2= 0.0%; z=0.26 ; p = 0.795
pT:T3/T4 vs.T1/T2 (OR)	0.646[0.542,0.771]; I2=0.0%%; z=4.85 ; p <0.001	0.661[0.546,0.800]; I2= 0.0%; z=4.25; p <0.001	0.516[0.297,0.898]; I2= 0.0%; z=2.34; p = 0.019	0.695[0.294,1.643]; z= 0.83; p = 0.407	0.663[0.550,0.798]; I2=0.0%; z=4.33 ; p <0.001	0.516[0.297 , 0.898]; I2= 0.0%; z= 2.34; p =0.019
LN(+) vs. LN(-)(OR)	0.941[0.681 ,1.298]; I2=76.4%; z= 0.37; p =0.709	0.991[0.633 ,1.551]; I2=80.8%; z=0.04; p = 0.968	1.013[0.595 ,1.726]; I2=60.8%; z=0.05; p = 0.962	0.549[0.322,0.936]; z=2.20; p = 0.028	1.003[0.651,1.546]; I2=82.4% ; z= 0.01; p =0.989	0.858[0.501 ,1.468]; I2=66.5%; z=0.56; p =0.576
IDC vs. ILC(OR)	2.654[1.132 ,6.223]; I2=68.0% ; z=2.24; p =0.025	2.642[0.700 ,9.967]; I2=84.0% ; z=1.43; p = 0.152	2.883[0.766 ,10.85]; I2=0.0%; z=1.57; p = 0.118	2.571[0.614,10.77]; z=1.29; p = 0.196	4.735[2.850 ,7.867]; I2=0.0% ; z=6.00; p <0.001	1.101[0.622 ,1.951]; I2=0.0% ; z=0.33; p = 0.740
Histological grade:III vs.I–II(OR)	2.889 [2.218,3.762]; I2= 49.5%; z=7.87; p <0.001	2.871[2.290 ,3.600]; I2=25.5%; z=9.14; p <0.001	5.636[3.050 ,10.42]; I2=0.0%; z=5.52; p <0.001	1.659[0.982 ,2.804]; z=1.89; p =0.059	2.763[2.188,3.489]; I2= 39.7%; z=8.53; p <0.001	3.284[1.359,7.934]; I2= 64.0%; z=2.64; p =0.008
ER (+) vs.(-) (OR)	0.291[0.185,0.458]; I2=70.0%; z=5.35; <0.001	0.348[0.197,0.614]; I2=61.1%; z=3.65; p<0.001	0.154[0.090,0.264]; z= 6.80; p<0.001	0.342[0.216,0.540]; z=4.60; p<0.001	0.360[0.230,0.563]; I2=60.1%; z=4.49; p<0.001	0.191[0.105,0.346]; I2=30.4% ; z=5.44; p<0.001
PR (+) vs.(-) (OR)	0.396[0.173,0.906]; I2=0.0%; z=2.19; p=0.028	–	–	–	–	–
HER2 (+) vs.(-) (OR)	1.359[0.646,2.858]; I2=88.0%; z=0.81; p=0.419	1.443[0.529,3.933]; I2=92.0%; z=0.72; p=0.474	1.097[0.539,2.230]; z=0.25; p=0.799	–	1.871 [0.486,7.205]; I2=95.9%; z= 0.91; p=0.362	0.961[0.544, 1.699]; I2=0.0%; z=0.14; p=0.891
Ki-67: high vs. low	6.378[3.674,11.073]; I2=30.1%; z=6.58; p<0.001	–	–	–	–	–
premenopausal vs. postmenopausal	0.963[0.716,1.296]; I2=0.0%; z=0.25; p=0.804	–	1.036[0.629,1.708]; I2=29.3%; z=0.14; p =0.888	0.874[0.571,1.339]; z=0.62; p =0.537	–	–
TNM stage: III, IV vs. I, II	0.825[0.220,3.095]; I2=81.4%; z=0.29; p =0.775	0.431[0.211,0.881]; I2= 0.0%; z=2.31; p=0.021	1.268[0.684,4.050]; I2=0.0%; z=1.12; p=0.261	–	–	–

Figures

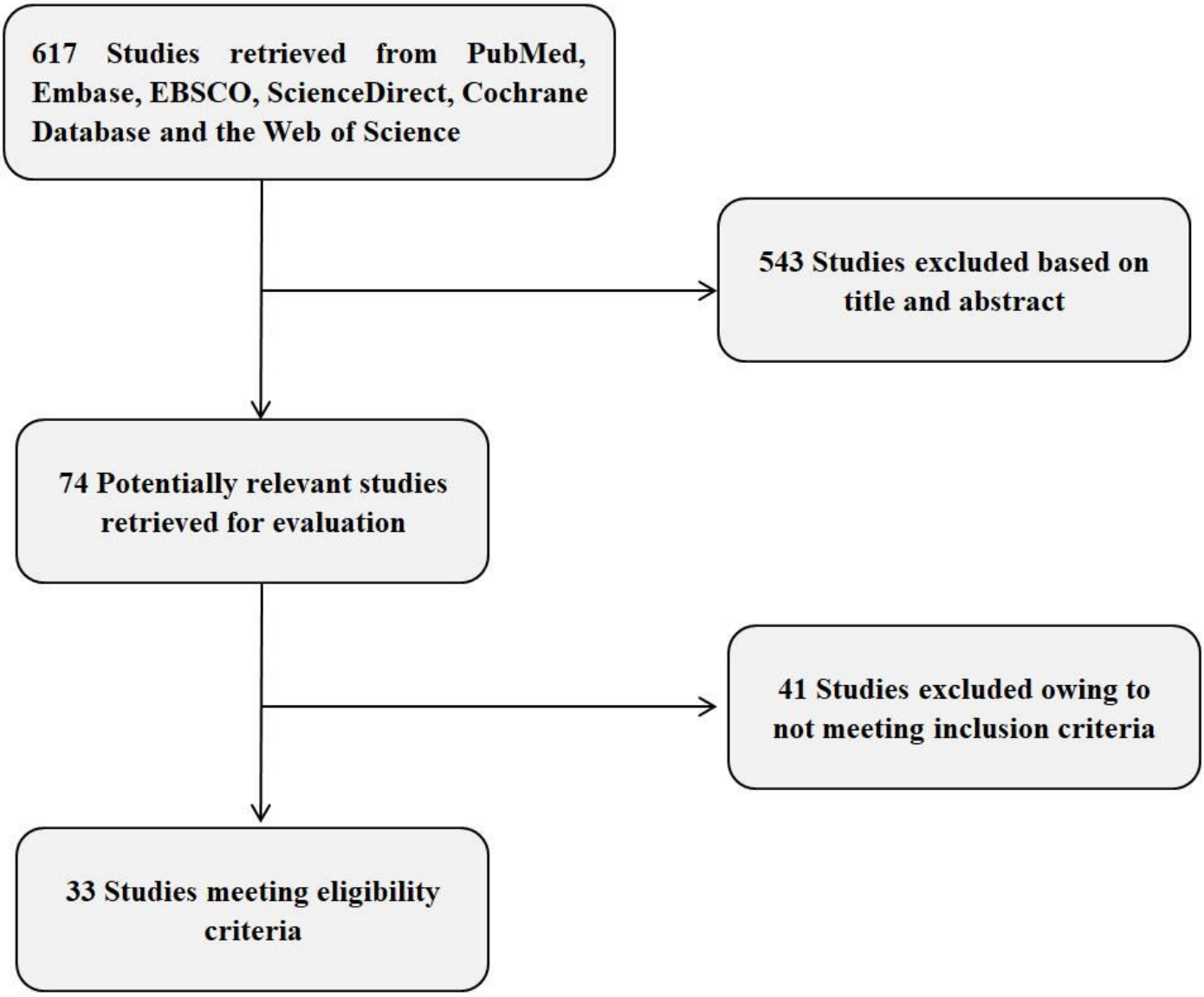

Figure 1

Selection process of included studies.

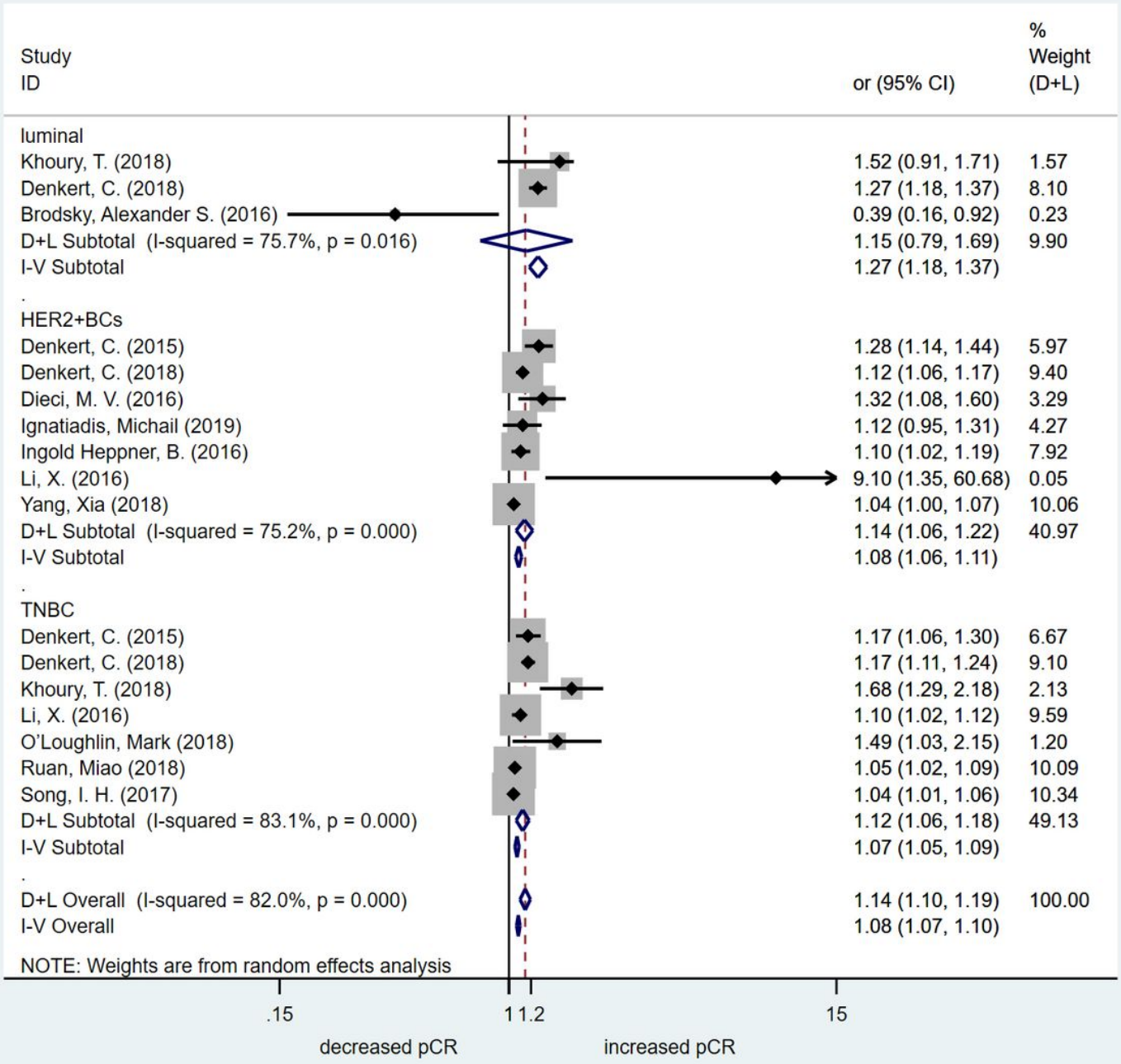

Figure 2

Forest plot of OR for pCR. Pooled assessing OR for pCR.

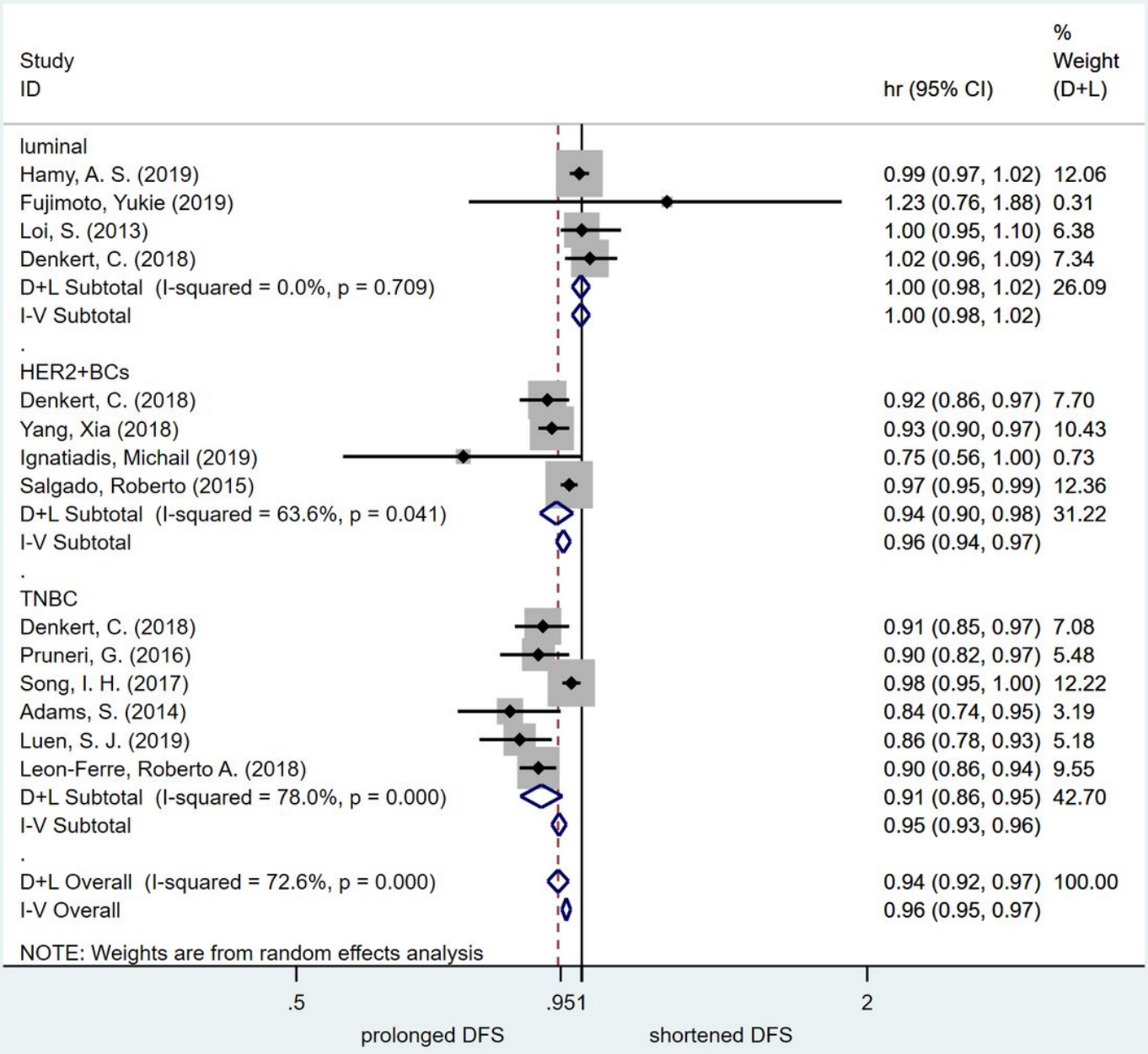

Figure 3

Forest plot of HR for DFS. Pooled assessing HR for DFS.

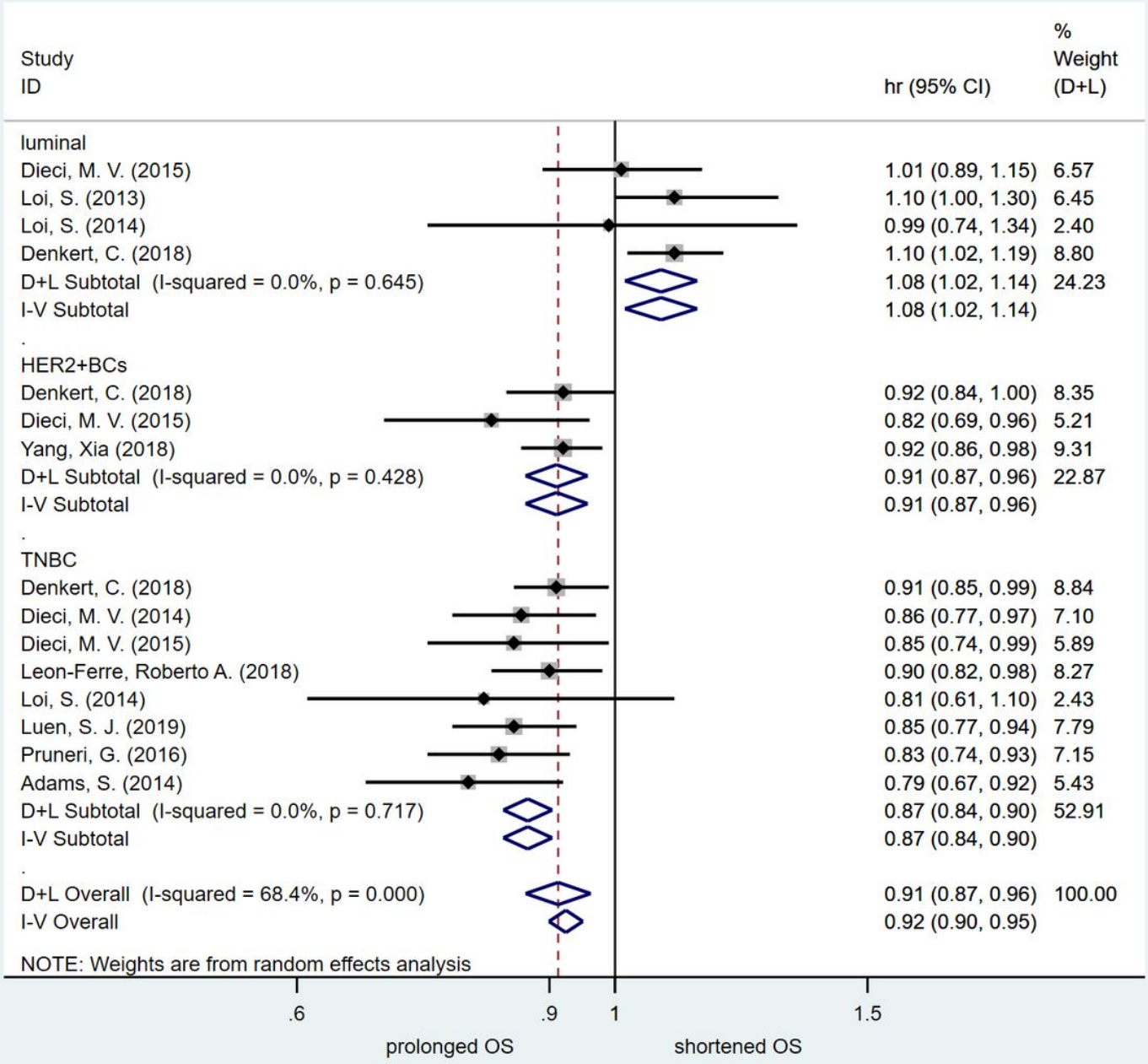

Figure 4

Forest plot of HR for OS. Pooled assessing HR for OS.

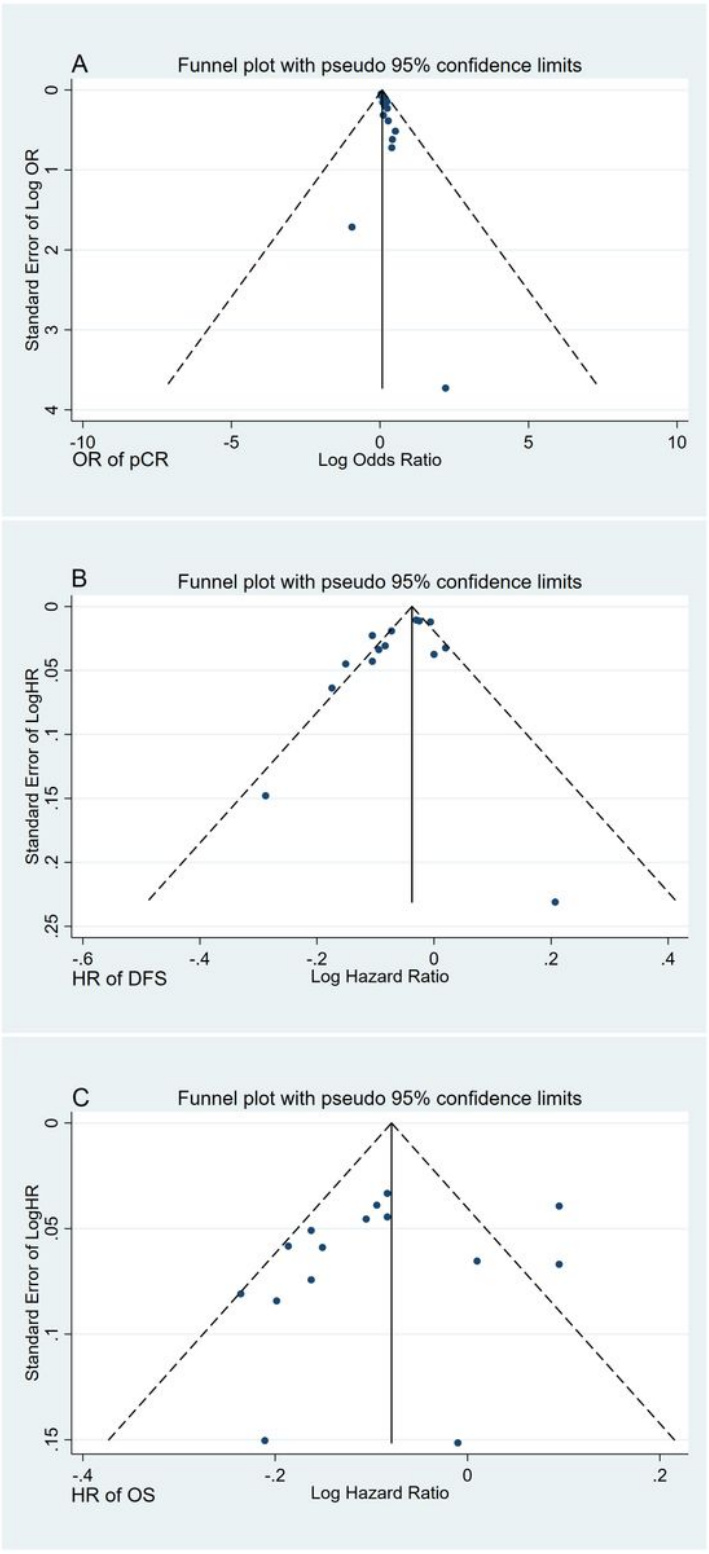

Figure 5

Funnel plot for potential publication bias. A: Funnel plot analysis of studies on pCR. B: Funnel plot analysis of studies on DFS. C: Funnel plot analysis of studies on OS.The funnel plot indicates that there was no significant publication bias.