



Tumour-infiltrating lymphocytes in non-invasive breast cancer: A systematic review and meta-analysis



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ABSTRACT

Background: The role of tumour infiltrating lymphocytes (TILs) as a biomarker in non-invasive breast cancer is unclear. This meta-analysis assessed the prognostic impact of TIL levels in patients with non-invasive breast cancer.

Methods: Systematic literature search was performed to identify studies assessing local recurrence in patients with non-invasive breast cancer according to TIL levels (high vs. low). Subgroup analyses per local recurrence (invasive and non-invasive) were performed. Secondary objectives were the association between TIL levels and non-invasive breast cancer subtypes, age, grade and necrosis. Odds ratios (ORs) and 95% confidence intervals (CI) were extracted from each study and a pooled analysis was conducted with random-effect model.

Results: Seven studies (N = 3437) were included in the present meta-analysis. High-TILs were associated with a higher likelihood of local recurrence (invasive or non-invasive, N = 2941; OR 2.05; 95%CI, 1.03–4.08; $p = 0.042$), although with a lower likelihood of invasive local recurrence (N = 1722; OR 0.69; 95%CI, 0.49–0.99; $p = 0.042$). High-TIL levels were associated with triple-negative (OR 3.84; 95%CI, 2.23–6.61; $p < 0.001$) and HER2-positive (OR 6.27; 95%CI, 4.93–7.97; $p < 0.001$) subtypes, high grade (OR 5.15; 95%CI, 3.69–7.19; $p < 0.001$) and necrosis (OR 3.09; 95%CI, 2.33–4.10; $p < 0.001$).

Conclusions: High-TIL levels were associated with more aggressive tumours, a higher likelihood of local recurrence (invasive or non-invasive) but a lower likelihood of invasive local recurrence in patients with non-invasive breast cancer.

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1. Introduction

The large-scale implementation of screening methods has increased the detection of breast cancer at its early stages worldwide, with non-invasive breast cancer currently representing around 20% of all newly diagnosed cases [1,2].

Patients diagnosed with non-invasive breast cancer have approximately a 6% risk of presenting an invasive recurrence and a 3% risk of dying from metastatic breast cancer when receiving standard treatment consisting of surgery with or without adjuvant radiotherapy and the possibility of receiving endocrine therapy in those with hormone receptor-positive tumours [3,4]. Some characteristics such as young age, high histological grade, presence of necrosis and large tumour size also increase the risk of presenting an invasive recurrence [3]. In this regard, it is essential to develop new tools to refine the prognostic classification of patients with

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non-invasive breast cancer, in order to differentiate those with a higher risk of recurrence that may require more aggressive treatment strategies from those with a favourable prognosis that may benefit from treatment de-escalation [5].

The induction of an effective immune response against tumour cells is the rationale that supports immune checkpoint inhibitor treatment, which has significantly improved the outcomes of patients with breast cancer in recent studies [6–8]. The interactions between lymphocytes and tumour cells may regulate tumour progression and ultimately influence the activity of anticancer treatments such as chemotherapy and targeted therapies [9,10]. Supporting this concept, high levels of tumour-infiltrating lymphocytes (TILs) have been demonstrated to be a favourable prognostic factor and a predictor of response to neoadjuvant treatment in patients with early-stage invasive breast cancer [11–13]. Although the prognostic and predictive value of TILs have been demonstrated in patients with invasive breast cancer, their role in non-invasive disease remains unclear [5,14]. In this context, we performed a systematic review and meta-analysis to assess the prognostic impact of TIL levels in patients with non-invasive breast cancer.

2. Methods

The present study is a quantitative synthesis and meta-analysis based on published or publicly available data from studies that assessed local recurrence rates in patients with pure non-invasive breast cancer according to TIL levels.

2.1. Objectives and endpoints

Primary objective was to assess the association between overall local recurrences (invasive or non-invasive) and TIL levels (high vs. low) in patients with non-invasive breast cancer. The primary endpoint was local recurrence, defined as the occurrence of either a non-invasive or an invasive ipsilateral local recurrence of breast cancer. Subgroup analyses were performed for the association between TIL levels and local recurrence type (invasive and non-invasive).

Secondary objectives were the association between non-invasive breast cancer subtypes (triple-negative, HER2-positive and luminal), age (≤ 50 years vs. > 50 years), histological grade (high vs. low-intermediate) and necrosis (yes vs. no) with TIL levels (high vs. low).

2.2. Data sources, search strategy and study selection

A literature search in PubMed, Embase, the Cochrane Library, and conference proceedings from major oncology conferences (American Society of Medical Oncology [ASCO], European Society for Medical Oncology [ESMO], San Antonio Breast Cancer Conference [SABCS] and ESMO breast) was performed with no date restriction up to May 10th, 2021. The search strategy was developed using the Patient, Intervention, Comparator and Outcome (PICO) framework and comprised keywords related to “breast”, “non-invasive” and “tumour-infiltrating lymphocytes”. The detailed search strategy used in one database (PubMed) is provided as **Supplementary material**.

Two reviewers (RC and EA) independently evaluated the titles and the abstracts of the identified studies and reviewed search results to apply eligibility criteria; two additional authors (EdA and RS) were invited to solve any potential discrepancies whenever they occurred. Cross-referencing from relevant studies and review articles on the topic was performed to confirm that all eligible studies were included. This meta-analysis was conducted and

reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines for systematic reviews (**Supplementary material**), and registered before study initiation in the PROSPERO database (registration number CRD42020170431; full protocol available on the website) [15].

2.3. Selection of the articles

Eligible studies had to meet the following criteria: had published, presented, or otherwise publicly available data; included only patients with pure non-invasive ductal breast cancer (or reported local recurrences in this population as a subgroup); reported rates of local recurrence (invasive, non-invasive, or both) according to TIL levels; and be published in English.

The cut-off to categorize patients into different TIL levels was adopted according to each study (high vs. low, or present vs. absent, or dense vs. sparse, depending on the study), and whenever 3 or more categories of TIL levels were present in a same study, the one with the lowest levels was considered as “low”, whereas the remaining categories were pooled as “high”. The definition of local recurrence was adopted according to each study, as long as both local invasive and local non-invasive breast cancer recurrences were part of this endpoint.

No restrictions according to the histotype were applied and both DCIS and LCIS were included in the present analysis.

Studies for which insufficient or no results were available at the time of the literature search, those including only patients with micro-invasive or invasive breast cancer, studies with insufficient methodological details on the assessment of the TILs around non-invasive breast cancer, or those in which TILs were evaluated using non-quantitative manners (for example with symbols like “+” for minor and “+++” for extensive infiltration) were excluded.

2.4. Statistical analysis

For each study, we extracted the number of events (local recurrences, invasive and/or in situ) in patients whose tumours had high-TIL levels versus those whose tumours had low-TIL levels, both in the overall population and according to breast cancer subtype, to age, to histological grade, and to necrosis. For the primary objective and subgroup analyses, odds ratios (ORs) were calculated for each study for the comparison between local recurrences in patients whose tumours had high-TIL levels versus those whose tumours had low-TIL levels. For the secondary objectives, ORs were calculated according to the frequency of each variable (non-invasive breast cancer subtype, age, histological grade and necrosis) in patients whose tumours had high-TIL levels versus those with low-TIL levels. An OR > 1 represents an association of that variable with high-TIL levels, whereas an OR < 1 denotes an association with low-TIL levels. For each OR estimate, 95% confidence intervals (CIs) were computed.

Pooled ORs using the random-effects model were computed with the method of DerSimonian and Laird. The Higgins' I^2 index was computed to obtain a quantitative measure of the degree of inconsistency in the results of the included studies. To assess whether the pooled OR estimates were stable or strongly dependent on one or few studies, sensitivity analyses were conducted by interactively recalculating the pooled OR estimates after exclusion of each single study. Egger's test was applied to assess the occurrence of publication bias. All reported p values were two-sided. All statistical analyses and the generation of forest plots were conducted using Stata Software Version 13.1 (Stata-Corp LP).

The Newcastle-Ottawa Scale (NOS) was employed to assess the quality of the data obtained and the risk of bias in each study (**Supplementary Tables 1 and 2**).

3. Results

From the 291 records initially identified, 290 remained after duplicate removal and were screened, with 246 being excluded for the following reasons: 112 did not report data on TILs, 103 had only patients with invasive breast cancer, 20 had patients with tumours other than breast cancer, and 11 were preclinical studies. The remaining 44 records were fully assessed for eligibility, with 37 being excluded for not meeting eligibility criteria. Overall, 7 studies with a total of 3437 patients were considered eligible and included in the present meta-analysis (Fig. 1). The characteristics of each study are presented in Table 1 [16–22].

The study from “Toss et al.” had 2 separate cohorts of patients eligible for this meta-analysis (“training set” and “validation set”), and thus data from this study were extracted and reported as 2 independent cohorts [18].

In all the studies included in this meta-analysis, TILs were assessed based on the criteria established by the International Immuno-Oncology Biomarker Working Group (or a slightly modified version of these recommendations; Supplementary Table 3).

In the study from “Beguinet et al.” the data was not fully available in the published manuscript. Our group contacted the corresponding author, who kindly agreed to provide access to their

dataset allowing its inclusion in this meta-analysis [19].

3.1. Local recurrence according to TIL levels

Six studies reported ORs for local recurrence rates according to TIL levels (N = 2941). With a total of 416 events observed in the overall population, high-TIL levels were significantly associated with overall local recurrence (invasive or non-invasive; OR 2.05; 95%CI, 1.03–4.08; $p = 0.042$). However, significant heterogeneity was observed in this analysis ($I^2 = 77.3\%$, $p_{\text{heterogeneity}} < 0.001$; Fig. 2). In sensitivity analysis, exclusion of each study individually did not eliminate heterogeneity (Supplementary Table 4). Egger's test did not suggest the occurrence of publication bias ($p = 0.118$).

Three studies (N = 1722) reported local invasive recurrence rates according to TIL levels, with a total of 150 events observed. Low-TIL levels were significantly associated with local invasive recurrence (OR 0.69; 95%CI, 0.49–0.99; $p = 0.042$). No significant heterogeneity was observed in this analysis ($I^2 = 0.5\%$, $p_{\text{heterogeneity}} = 0.366$; Fig. 3A). Sensitivity analysis is provided as Supplementary Table 5A.

Three studies (N = 1722) reported local non-invasive recurrence rates according to TIL levels, with a total of 133 events observed. No significant difference in terms of local non-invasive recurrences

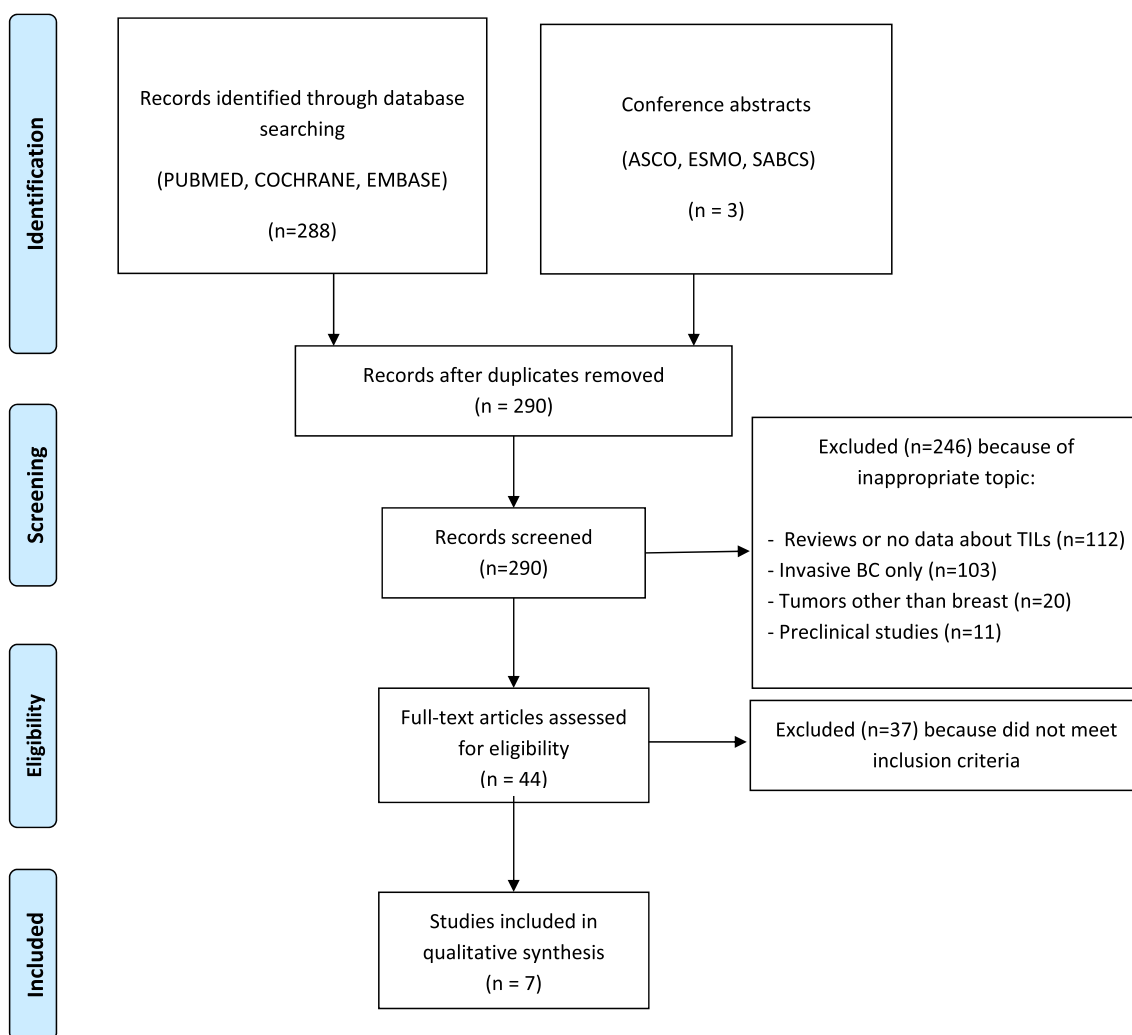


Fig. 1. PRISMA diagram illustrating literature search and study selection for this meta-analysis. **Abbreviations:** ASCO, American Society of Clinical Oncology; BC, breast cancer; ESMO, European Society for Medical Oncology; SABCS, San Antonio Breast Cancer Symposium; TILs, tumor infiltrating lymphocytes.

Table 1
Characteristics of the studies included in the meta-analysis.

First author	Year	Study design	Criteria to classify TILs-high	N (DCIS/LCIS)	Reported outcome	Adjustments factors	Median follow-up
Pruneri G [16]	2017	Retrospective	<1% vs ≥ 1% TILs	1488 (1488/0)	Local recurrences (invasive and non-invasive)	Menopausal status, subtype, necrosis and therapy (surgery, radiotherapy and hormone therapy)	8.2 years
Hendry S [17]	2017	Retrospective	Low (first quartile) vs high (2°, 3° and 4° quartiles)	138 (138/0)	Local recurrences (invasive and non-invasive)	Not adjusted	7.5 years
Toss M [18] (training set)	2018	Retrospective	Mean number of touching TILs/DCIS duct: sparse ≤20 (low) vs. dense >20 (high)	150 (150/0)	Local recurrences (invasive and non-invasive)	Age, size, grade, comedo type necrosis, radiotherapy,	161 months
Toss M [18] (validation set)	2018	Retrospective	Mean number of touching TILs/DCIS duct: sparse ≤20 (low) vs. dense >20 (high)	534 (534/0)	Local recurrences (invasive and non-invasive)	estrogen receptor status, dense TILs (performed for 109 months patients treated with Breast conservative surgery only)	109 months
Beguinet M [19]	2018	Retrospective	Low (0–9%) vs. high (≥10%)	96 (96/0)	Local recurrences (invasive and non-invasive)	Not adjusted	144 months
Darvishian F [20]	2019	Retrospective	Sparse<45% (low) vs dense≥45% (high)	69 (59/10)	Local recurrences (invasive and non-invasive)	Age	6.7 years
Agahozo MC [21]	2019	Retrospective	Low (≤30%) vs high (>30%)	466 (466/0)	Local recurrences (invasive and non-invasive)	Not adjusted	98 months
Farolfi A [22]	2020	Retrospective	Low (≤5%) vs high (>5%)	496 (496/0)	Local recurrences (invasive and non-invasive)	Age, surgery, radiotherapy, grade, necrosis, margins, excision, screen detected, TILs	8.5 years

Abbreviations: N, number of patients; TILs, tumor-infiltrating lymphocytes.

was observed according to TIL levels (OR 1.31; 95%CI, 0.88–1.93; $p = 0.180$). No significant heterogeneity was observed in this analysis ($I^2 = 0\%$, $p_{\text{heterogeneity}} = 0.909$; Fig. 3B). Sensitivity analysis is provided as [Supplementary Table 5B](#).

3.2. TILs and non-invasive breast cancer subtypes

Three studies (N = 2035) reported non-invasive breast cancer subtype distribution according to TIL levels. High-TIL levels were significantly associated with triple-negative (OR 3.84; 95%CI, 2.23–6.61; $p < 0.001$; $I^2 = 0\%$, $p_{\text{heterogeneity}} = 0.439$) and HER2-positive (OR 6.27; 95%CI, 4.93–7.97; $p < 0.001$; $I^2 = 0\%$, $p_{\text{heterogeneity}} = 0.790$) subtypes, whereas low-TIL levels were associated with the luminal subtype (OR 0.14; 95%CI, 0.11–0.17; $p < 0.001$; $I^2 = 0\%$, $p_{\text{heterogeneity}} = 0.723$), with no significant heterogeneity in all these analyses (Fig. 4A–C). Sensitivity analysis is provided as [Supplementary Tables 6A–C](#).

3.3. TILs and clinicopathological characteristics

3.3.1. Age

Two studies (N = 2022) reported patient's age according to TIL levels. No association between TIL levels and age was observed (OR 1.08; 95%CI, 0.60–1.95; $p = 0.803$; Fig. 5A). Significant heterogeneity was observed in this analysis ($I^2 = 86\%$, $p_{\text{heterogeneity}} = 0.007$).

3.4. Histological grade

Seven studies (N = 3276) reported histological grade according to TIL levels. High-TIL levels were significantly associated with high-grade tumours (OR 5.15; 95%CI, 3.69–7.19; $p < 0.001$; Fig. 5B). Significant heterogeneity was observed in this analysis ($I^2 = 62.2\%$, $p_{\text{heterogeneity}} = 0.014$). In sensitivity analysis, heterogeneity was eliminated and the association between high-TIL levels and high-grade remained significant when excluding either the study “Toss M – validation set” ($I^2 = 38.8\%$, $p_{\text{heterogeneity}} = 0.147$; OR 5.78; 95%CI, 4.28–7.80; $p < 0.001$). Sensitivity analysis is provided as [Supplementary Table 7A](#).

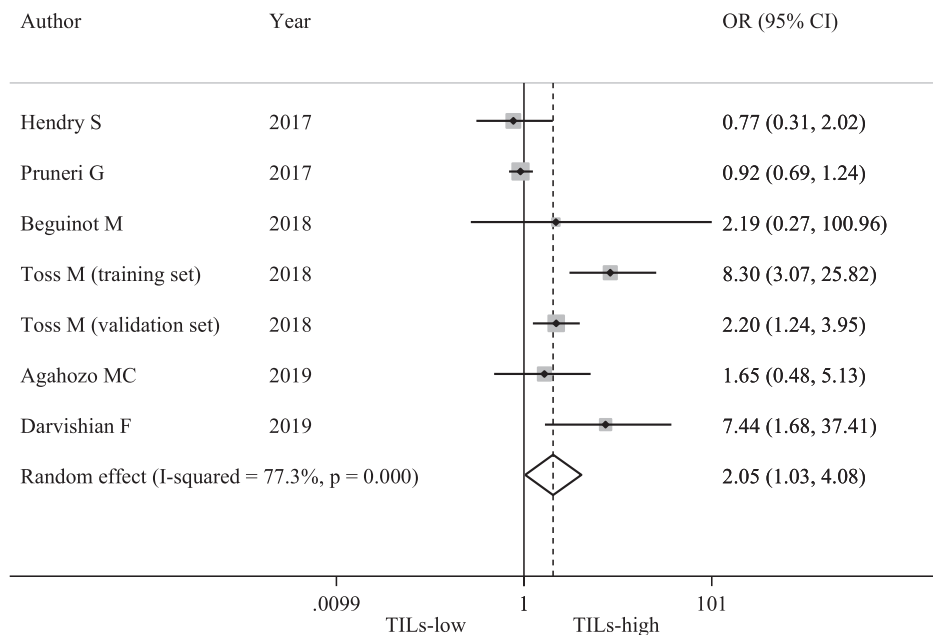
3.5. Necrosis

Seven studies (N = 3257) reported the presence of necrosis according to TIL levels. High-TIL levels were significantly associated with necrosis (OR 3.09; 95%CI, 2.33–4.10; $p < 0.001$; Fig. 5C). Significant heterogeneity was observed in this analysis ($I^2 = 58.6\%$, $p_{\text{heterogeneity}} = 0.025$). In sensitivity analysis ([Supplementary Table 7B](#)), heterogeneity was eliminated and the association between high-TIL levels and necrosis remained significant when excluding either the study “Pruneri G” ($I^2 = 25.8\%$, $p_{\text{heterogeneity}} = 0.241$; OR 2.74; 95%CI 2.10–3.58; $p < 0.001$), or “Toss M – validation set” ($I^2 = 40.7\%$, $p_{\text{heterogeneity}} = 0.134$; OR 3.38; 95%CI, 2.60–4.41; $p < 0.001$).

3.6. Risk of bias assessment

[Supplementary Tables 1 and 2](#) report the quality of data and risk of bias assessment for each study, based on The Newcastle-Ottawa Scale (NOS). Each study was judged on three broad perspectives: the selection of the study groups; the comparability of the groups; and the ascertainment of the outcome of interest. Six and seven studies, respectively, received full score in the evaluation of the study group selection, and of the comparability of the groups. Three out of seven studies did not achieve the full score in the ascertainment of the outcome of interest, due to partially

Local recurrence (invasive and non-invasive)



Random effect: $p=0.042$

Fig. 2. Forest plots and the pooled odds ratios with the respective p values for local recurrence according to TILs levels. **Abbreviations:** OR, odds ratio; CI, confidence intervals; TILs, tumor infiltrating lymphocytes.

missing data.

4. Discussion

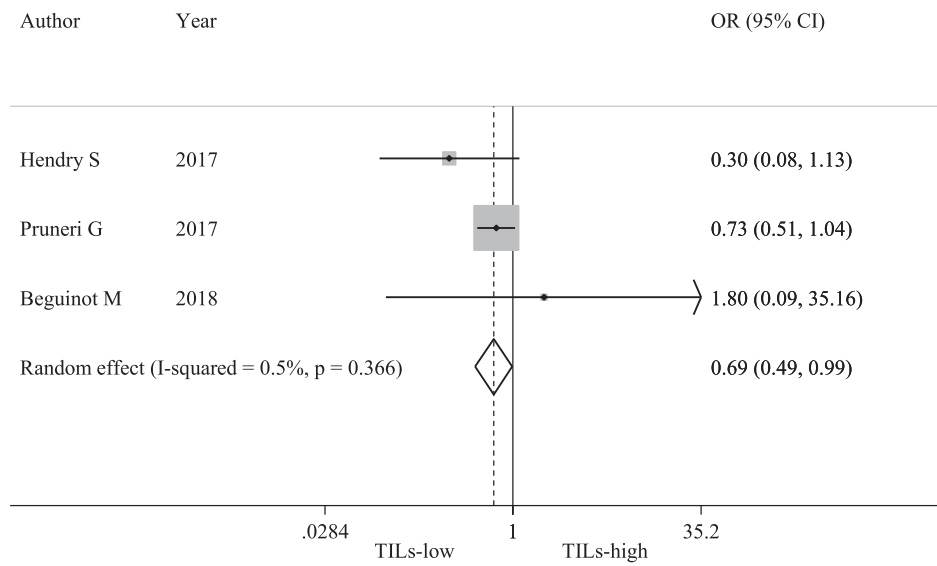
By pooling data from 6 studies (7 cohorts) that included a total of 2941 patients, this meta-analysis demonstrated a significant association between high-TIL levels and local recurrences (invasive and non-invasive) in patients with non-invasive breast cancer. High-TIL levels were also associated with triple-negative and HER2-positive subtypes, with high histological grade and with the presence of necrosis. This association of high-TIL levels with clinicopathological variables of potentially aggressive behaviour confirms findings from previous individual studies and may indeed justify our findings. Nonetheless, the likelihood of presenting invasive recurrences (which may be more clinically relevant) seemed to be higher in patients whose tumours have low-TIL levels, as observed also in patients with invasive breast cancer [11]. In this regard, invasion may be facilitated by the lack of an effective immune surveillance in the stroma of tumours with low-TIL levels, whereas the high-TIL group may correspond to patients who developed an active anti-tumour immune response that may prevent invasiveness [5].

Although high-TIL levels are often interpreted as a sign of anti-tumour immunity, it may not always be the case. In patients with invasive triple-negative breast cancer, the qualitative difference of cells found in the immune infiltrate - rather than TIL levels - seems to have a prognostic impact [23]. Sheu et al. have demonstrated that non-invasive breast lesions have an immune infiltrate enriched with regulatory CD4⁺ T cells, whereas cytotoxic CD8⁺ T cells become more frequent as disease progresses, supporting the

concept that the composition of the immune infiltrate may differ between invasive and non-invasive disease [24]. Different populations of cytotoxic CD8⁺ and regulatory CD4⁺ T cells coexist in the lymphocytic infiltrate, and the balance between immunogenic and immunosuppressive stimuli either activates or down-regulates the immune system in the tumoral stroma [19–21]. Accordingly, the presence of a high proportion of regulatory T cells as part of the lymphocytic infiltrate has been associated with higher recurrence rates in patients with invasive and non-invasive breast cancer [25]. Therefore, not only TIL count, but also the type of cells present in this lymphocytic infiltrate dictate the interactions between the tumour and the immune system.

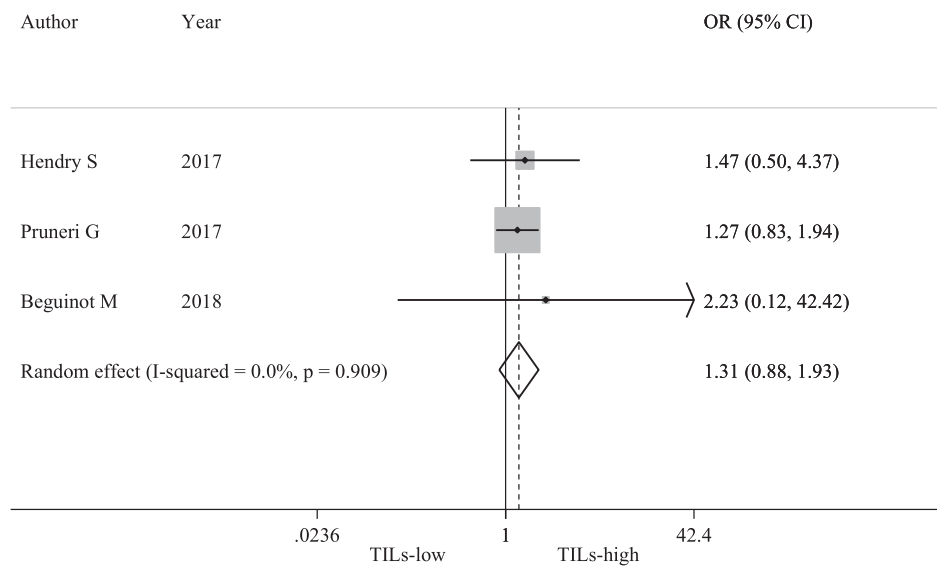
Observational studies suggest that up to 50% of non-invasive breast cancers do not progress to an invasive form without treatment [26–28]. Yet, since it is not possible to anticipate which cases will progress to invasive breast cancer, these patients are often submitted to surgery, followed by radiotherapy and adjuvant endocrine treatment for those with hormone receptor-positive disease [29–31]. Given the potential of these treatments to cause relevant toxicities, efforts to improve the prognosis estimation of this population should be pursued [3]. Characteristics such as high grade, necrosis, tumour size, microinvasion, triple-negative and HER2-positive subtypes have been previously associated with recurrence in patients with non-invasive breast cancer [3,5]. Nonetheless, additional biomarkers are needed to refine the prognosis estimation in this population. Our findings suggest that high-TIL levels may identify patients with a higher overall recurrence risk, although this risk may be mainly driven by non-invasive recurrences, whereas invasive recurrences might be less frequent in patients with high-TIL levels. A better understanding of the

A - Local recurrence (invasive only)



Random effect: $p=0.042$

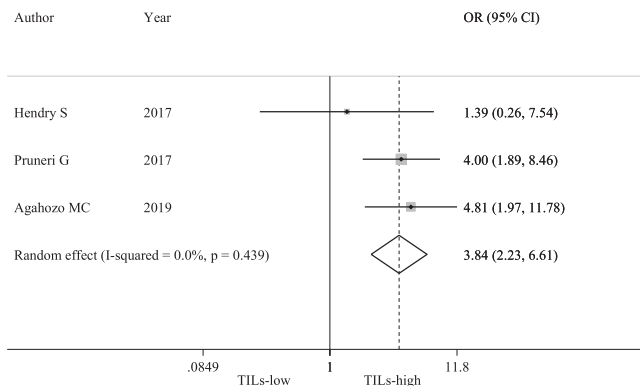
B - Local recurrence (non-invasive only)



Random effect: $p=0.180$

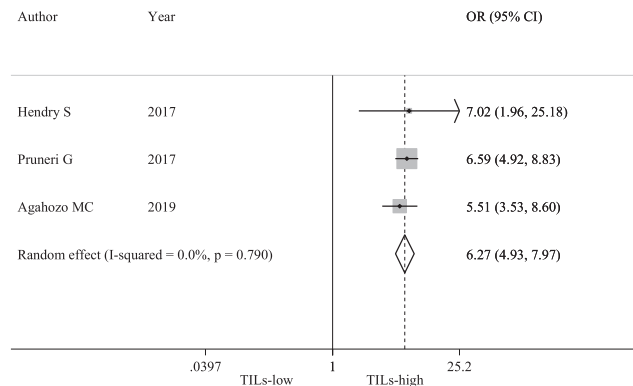
Fig. 3. Forest plots and the pooled odds ratios with the respective p values for invasive local recurrence (A) and non-invasive local recurrence (B) according to TILs levels. **Abbreviations:** OR, odds ratio; CI, confidence intervals; TILs, tumor infiltrating lymphocytes.

A - Triple-negative non-invasive breast cancer and TILs



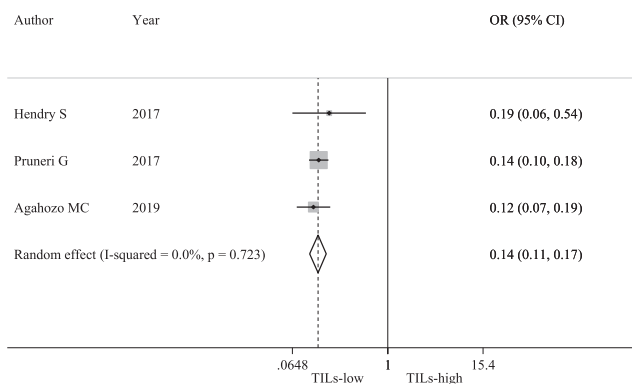
Random effect: $p < 0.001$

B - HER2-positive non-invasive breast cancer and TILs



Random effect: $p < 0.001$

C – Luminal non-invasive breast cancer and TILs



Random effect: $p < 0.001$

Fig. 4. Forest plots and the pooled odds ratios with the respective p values for the association between triple-negative (A), HER2-positive (B) and luminal (C) breast cancer subtypes and TILs levels. **Abbreviations:** OR, odds ratio; CI, confidence intervals; TILs, tumor infiltrating lymphocytes.

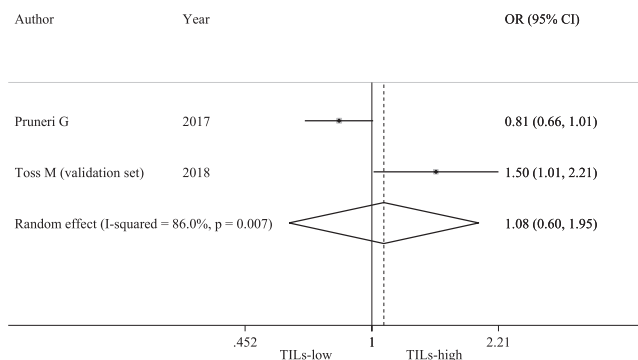
prognostic impact of TILs in non-invasive disease is crucial to identify patients who are candidates for more intensive treatment strategies, such as the administration of anti-HER2 agents for those with HER2-positive or immunotherapy for those with triple-negative disease, and those with a lower recurrence risk who might benefit from treatment de-escalation, which are of particular interest for frail or elderly patients [32–34].

In subgroup analyses, contrary to what was observed in the overall population and in line with previous data from patients with invasive breast cancer, an association between low-TIL levels and local invasive recurrences was observed [11]. Therefore, one cannot exclude that the association between high-TIL levels and local recurrences observed in the overall population of this meta-analysis could have been driven by non-invasive recurrences, meaning that high-TIL levels in non-invasive disease predict mainly the occurrence of non-invasive recurrences. Although non-invasive recurrences may have less potential to result in unfavourable survival outcomes, they often lead patient's to additional procedures that may cause a significant burden and compromise quality of life

[13,4]. Furthermore, these subgroup analyses included only 3 studies, with their results being driven mainly by one study (Pruneri et al.) with a large sample size. Thus, these results need to be interpreted cautiously.

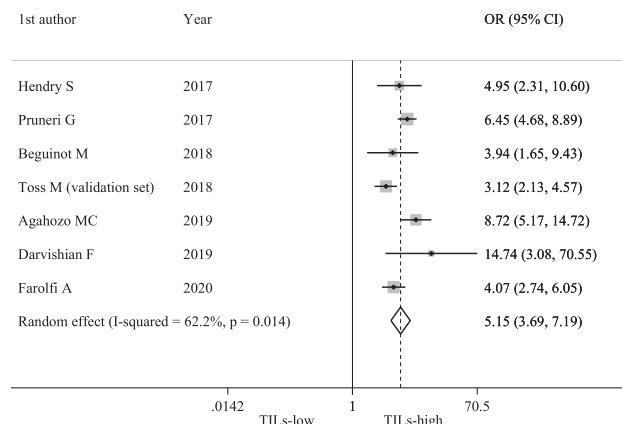
Supporting previous findings, our study has shown a significant association of high-TIL levels with triple-negative and HER2-positive subtypes of non-invasive breast cancer, which usually present high proliferation rates and high risk of invasion [16,21,35–37]. In previous studies, high-TIL levels have been observed in areas of microinvasive breast cancer, suggesting that more aggressive tumours may also be more immunogenic [12,36,38,39]. Additionally, the activation of the HER2 pathway seems to drive the tumoral stroma into a pro-immunogenic state, as HER2-positive tumours have a high frequency of CD8⁺ T cells and a low PD-L1 expression in their lymphocytic infiltrate [21,25,35–37,40]. Conversely, the oestrogen receptor pathway activation observed in luminal tumours creates an immune-suppressive microenvironment that renders these tumours less immunogenic, potentially explaining the observed association of

A – Age and TILs



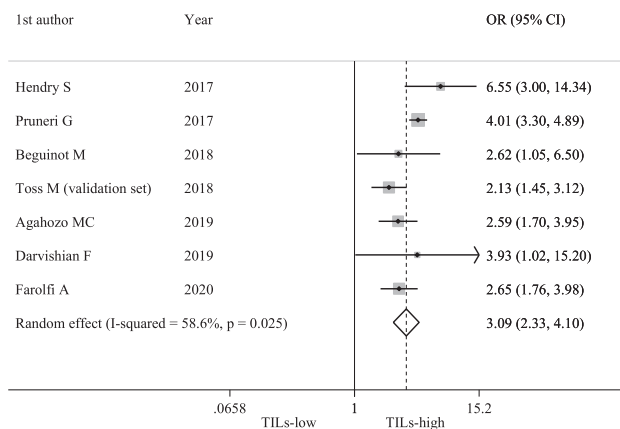
Random effect: $p=0.803$

B - Histological grade and TILs



Random effect: $p<0.001$

C – Necrosis and TILs



Random effect: $p<0.001$

Fig. 5. Forest plots and the pooled odds ratios with the respective p values for the association between age (A), histological grade (B) and necrosis (C) and TILs levels. **Abbreviations:** OR, odds ratio; CI, confidence intervals; TILs, tumor infiltrating lymphocytes.

this subtype with low-TIL levels [41].

The Oncotype DX assay analyses the expression of genes involved in proliferation, invasion, and in the HER2 and oestrogen-receptor pathways to provide prognostic information, but also to estimate the benefit of adjuvant chemotherapy in patients with HER2-negative, hormone receptor-positive, early-stage breast cancer [42,43]. For non-invasive breast cancer, an adapted version of the assay (DCIS Oncotype) relies on the expression of 7 genes related to proliferation and hormone-receptor pathways to estimate the risk of recurrence [44]. In an exploratory study that included patients with non-invasive breast cancer, Knopfmacher et al. observed a positive correlation between mitotic count and TIL levels with DCIS Oncotype scores, suggesting that tumours with high proliferation rates may also be more immunogenic [44]. This hypothesis is in line with the observed association of high-TIL levels with high grade and necrosis in our study.

Potential limitations need to be considered when interpreting our findings. This meta-analysis was not based on individual

patient data (besides for one study [Beguinet et al.]), limiting the number of studies included in subgroup analyses and precluding analyses of additional secondary objectives. All studies were retrospective and had mostly small sample sizes. Although distant recurrences and survival analyses could not be performed, previous studies have shown an increased risk of death in patients with non-invasive breast cancer who present a local recurrence, suggesting that this endpoint may be a surrogate for survival [45,46]. Data on treatments received by patients in each cohort, which may have an impact on local recurrences, was not available. Each study used a different cut-off to categorize patients between high-TIL level and low-TIL level groups. Categorization of ‘high TILs’ was inconsistent in the pooled analysis, with 1%, 5%, 10%, 45% and upper-quartile as thresholds of definitions. This limitation obliges to interpret our study results cautiously and underlines the fact that harmonization of TIL-assessment is a current unmet need that should be addressed in the near future. Although a different methodology to assess and quantify TILs was used in each study, in all of them it was based on

the criteria established by the International Immuno-Oncology Biomarker Working Group (www.tilsinbreastcancer.org), reinforcing the notion that data from these studies can be pooled and highlighting the importance of developing and updating International guidelines to standardize TIL assessment for future studies, preferably using phase 3 clinical trial datasets [47,48].

Despite the aforementioned limitations, to our knowledge this meta-analysis represents the largest and most updated data assessing the prognostic impact of TIL levels in patients with non-invasive breast cancer. Our data can be hypothesis-generating for further studies, as it will be interesting to verify how TILs in DCIS could help to refine prognosis estimation and, consequently, to potentially adapt treatment decision making in this population.

5. Conclusions

High-TIL levels were associated with more aggressive tumours and with a higher likelihood of presenting local recurrences (local and invasive), but with a lower likelihood for invasive recurrence in patients with non-invasive breast cancer. Yet, patients whose tumours have low-TIL levels may present a higher likelihood of invasive local recurrences. Our exploratory data suggest that the assessment of TIL levels might represent a promising prognostic biomarker in patients with non-invasive disease, which could ultimately help clinicians to identify patients who are candidates for more intensive treatment strategies and those who might benefit from treatment de-escalation. Harmonization of TIL-assessment is a critical and currently unmet imperative to be able to perform future studies that can definitively inform on the importance of immunity in DCIS.

Declaration of competing interest

RC received speaker honoraria from Boehringer-Ingelheim, AstraZeneca, and Janssen; and travel grants from Pfizer and AstraZeneca, none related to the present work. EdA has received honoraria from Roche-Genentech, Libbs, Seattle Genetics, Novartis, Pierre Fabre; research grant from Roche-Genentech, Astra Zeneca, GSK/Novartis, Servier (to the institution), and travel grants from Roche-Genentech and GlaxoSmithKline, none related to the present work. All other authors declare no disclosures related to the present work.

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Ethics approval and consent to participate

This study did not perform any intervention and did not require consent from participants nor approval from ethics committee.

Consent for publication

This study does not contain any individual person's data.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Authors' contributions

RC, EA, NRR, MAF, RS and EdA conceived this work. MB and MC performed the statistical analysis. All authors participated in the manuscript elaboration, reviewed and approved its final version before submission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.breast.2021.07.007>.

References

- [1] Lee RJ, Vallow LA, McLaughlin SA, Tzou KS, Hines SL, Peterson JL. Ductal carcinoma in situ of the breast. *Int J Surg Oncol* 2012;2012:123549. <https://doi.org/10.1155/2012/123549>.
- [2] Ernster VL, Ballard-Barbash R, Barlow WE, Zheng Y, Weaver DL, Cutter G, et al. Detection of ductal carcinoma in situ in women undergoing screening mammography. *J Natl Cancer Inst* 2002;94:1546–54. <https://doi.org/10.1093/jnci/94.20.1546>.
- [3] Narod SA, Iqbal J, Giannakeas V, Sopik V, Sun P. Breast cancer mortality after a diagnosis of ductal carcinoma in situ. *JAMA Oncol* 2015;1:888–96. <https://doi.org/10.1001/jamaoncol.2015.2510>.
- [4] Giannakeas V, Sopik V, Narod SA. Association of a diagnosis of ductal carcinoma in situ with death from breast cancer. *JAMA Netw Open* 2020;3:e2017124. <https://doi.org/10.1001/jamanetworkopen.2020.17124>.
- [5] Chen X-Y, Yeong J, Thike AA, Bay BH, Tan PH. Prognostic role of immune infiltrates in breast ductal carcinoma in situ. *Breast Canc Res Treat* 2019;177:17–27. <https://doi.org/10.1007/s10549-019-05272-2>.
- [6] Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, et al. Pembrolizumab for early triple-negative breast cancer. *N Engl J Med* 2020;382:810–21. <https://doi.org/10.1056/NEJMoa1910549>.
- [7] Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med* 2018;379:2108–21. <https://doi.org/10.1056/NEJMoa1809615>.
- [8] Mittendorf EA, Zhang H, Barrios CH, Saji S, Jung KH, Hegg R, et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 tri. *Lancet (Lond Engl)* 2020;396:1090–100. [https://doi.org/10.1016/S0140-6736\(20\)31953-X](https://doi.org/10.1016/S0140-6736(20)31953-X).
- [9] Luen S, Virassamy B, Savas P, Salgado R, Loi S. The genomic landscape of breast cancer and its interaction with host immunity. *Breast* 2016;29:241–50. <https://doi.org/10.1016/j.breast.2016.07.015>.
- [10] Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008;454:436–44. <https://doi.org/10.1038/nature07205>.
- [11] Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol* 2018;19:40–50. [https://doi.org/10.1016/S1470-2045\(17\)30904-X](https://doi.org/10.1016/S1470-2045(17)30904-X).
- [12] Losurdo A, De Sanctis R, Fernandes B, Torrisi R, Masci G, Agostinetti E, et al. Insights for the application of TILs and AR in the treatment of TNBC in routine clinical practice. *Sci Rep* 2020;10:20100. <https://doi.org/10.1038/s41598-020-77043-9>.
- [13] Agostinetti E, Eiger D, Punie K, de Azambuja E. Emerging therapeutics for patients with triple-negative breast cancer. *Curr Oncol Rep* 2021;23:57. <https://doi.org/10.1007/s11912-021-01038-6>.
- [14] Semeraro M, Adam J, Stoll G, Louvet E, Chaba K, Poirier-Colame V, et al. The ratio of CD8(+)/FOXP3 T lymphocytes infiltrating breast tissues predicts the relapse of ductal carcinoma in situ. *Oncol Immunology* 2016;5:e1218106. <https://doi.org/10.1080/2162402X.2016.1218106>.
- [15] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009;339:b2700. <https://doi.org/10.1136/bmj.b2700>.
- [16] Pruneri G, Lazzaroni M, Bagnardi V, Tiburzio GB, Rotmensz N, DeCensi A, et al. The prevalence and clinical relevance of tumor-infiltrating lymphocytes (TILs) in ductal carcinoma in situ of the breast. *Ann Oncol Off J Eur Soc Med Oncol* 2017;28:321–8. <https://doi.org/10.1093/annonc/mdw623>.
- [17] Hendry S, Pang J-MB, Byrne DJ, Lakhani SR, Cummings MC, Campbell IG, et al. Relationship of the breast ductal carcinoma in situ immune microenvironment with clinicopathological and genetic features. *Clin Canc Res* 2017;23:5210–7. <https://doi.org/10.1158/1078-0432.CCR-17-0743>.

- [18] Toss MS, Miligy I, Al-Kawaz A, Alsleem M, Khout H, Rida PC, et al. Prognostic significance of tumor-infiltrating lymphocytes in ductal carcinoma in situ of the breast. *Mod Pathol an Off J United States Can Acad Pathol Inc* 2018;31:1226–36. <https://doi.org/10.1038/s41379-018-0040-8>.
- [19] Beguinot M, Dauplat M-M, Kwiatkowski F, Lebouedec G, Tixier L, Pomel C, et al. Analysis of tumour-infiltrating lymphocytes reveals two new biologically different subgroups of breast ductal carcinoma in situ. *BMC Canc* 2018;18:129. <https://doi.org/10.1186/s12885-018-4013-6>.
- [20] Darvishian F, Ozerdem U, Adams S, Chun J, Pirraglia E, Kaplowitz E, et al. Tumor-infiltrating lymphocytes in a contemporary cohort of women with ductal carcinoma in situ (DCIS). *Ann Surg Oncol* 2019;26:3337–43. <https://doi.org/10.1245/s10434-019-07562-x>.
- [21] Agahozo MC, van Bockstal MR, Groenendijk FH, van den Bosch TPP, Westenend PJ, van Deurzen CHM. Ductal carcinoma in situ of the breast: immune cell composition according to subtype. *Mod Pathol an Off J United States Can Acad Pathol Inc* 2020;33:196–205. <https://doi.org/10.1038/s41379-019-0331-8>.
- [22] Farolfi A, Petracci E, Serra L, Ravaoli A, Bravaccini S, Ravaoli S, et al. Tumor-infiltrating lymphocytes (TILs) and risk of a second breast event after a ductal carcinoma in situ. *Front Oncol* 2020;10:1486. <https://doi.org/10.3389/fonc.2020.01486>.
- [23] Savas P, Salgado R, Denkert C, Sotiriou C, Darcy PK, Smyth MJ, et al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. *Nat Rev Clin Oncol* 2016;13:228–41. <https://doi.org/10.1038/nrclinonc.2015.215>.
- [24] Sheu B-C, Kuo W-H, Chen R-J, Huang S-C, Chang K-J, Chow S-N. Clinical significance of tumor-infiltrating lymphocytes in neoplastic progression and lymph node metastasis of human breast cancer. *Breast* 2008;17:604–10. <https://doi.org/10.1016/j.breast.2008.06.001>.
- [25] Bates GJ, Fox SB, Han C, Leek RD, Garcia JF, Harris AL, et al. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J Clin Oncol Off J Am Soc Clin Oncol* 2006;24:5373–80. <https://doi.org/10.1200/JCO.2006.05.9584>.
- [26] Page DL, Dupont WD, Rogers LW, Jensen RA, Schuyler PA. Continued local recurrence of carcinoma 15–25 years after a diagnosis of low grade ductal carcinoma in situ of the breast treated only by biopsy. *Cancer* 1995;76:1197–200. [https://doi.org/10.1002/1097-0142\(19951001\)76:7<1197::aid-cncr2820760715>3.0.co;2-0](https://doi.org/10.1002/1097-0142(19951001)76:7<1197::aid-cncr2820760715>3.0.co;2-0).
- [27] Page DL, Dupont WD, Rogers LW, Landenberger M. Intraductal carcinoma of the breast: follow-up after biopsy only. *Cancer* 1982;49:751–8. [https://doi.org/10.1002/1097-0142\(19820215\)49:4<751::aid-cncr2820490426>3.0.co;2-y](https://doi.org/10.1002/1097-0142(19820215)49:4<751::aid-cncr2820490426>3.0.co;2-y).
- [28] Sanders ME, Schuyler PA, Dupont WD, Page DL. The natural history of low-grade ductal carcinoma in situ of the breast in women treated by biopsy only revealed over 30 years of long-term follow-up. *Cancer* 2005;103:2481–4. <https://doi.org/10.1002/cncr.21069>.
- [29] Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol Off J Eur Soc Med Oncol* 2019;30:1194–220. <https://doi.org/10.1093/annonc/mdz173>.
- [30] Morrow M, Van Zee KJ, Solin LJ, Houssami N, Chavez-MacGregor M, Harris JR, et al. Society of surgical oncology-American society for radiation oncology-American society of clinical oncology consensus guideline on margins for breast-conserving surgery with whole-breast irradiation in ductal carcinoma in situ. *Pract Radiat Oncol* 2016;6:287–95. <https://doi.org/10.1016/j.prro.2016.06.011>.
- [31] National Comprehensive Cancer Network. *NCCN guidelines for patients ductal carcinoma in situ (breast cancer)*. 2020.
- [32] Cobleigh MA, Anderson SJ, Siziopikou KP, Arthur DW, Julian TB, Rabinovitch R, et al. Primary results of NRG Oncology/NSABP B-43: phase III trial comparing concurrent trastuzumab (T) and radiation therapy (RT) with RT alone for women with HER2-positive ductal carcinoma in situ (DCIS) after lumpectomy. *J Clin Oncol* 2020;38:508. https://doi.org/10.1200/JCO.2020.38.15_suppl.508.
- [33] Lowenfeld L, Mick R, Datta J, Xu S, Fitzpatrick E, Fisher CS, et al. Dendritic cell vaccination enhances immune responses and induces regression of HER2(pos) DCIS independent of route: results of randomized selection design trial. *Clin Cancer Res an Off J Am Assoc Cancer Res* 2017;23:2961–71. <https://doi.org/10.1158/1078-0432.CCR-16-1924>.
- [34] Punglia RS, Bifolck K, Golshan M, Lehman C, Collins L, Polyak K, et al. Epidemiology, biology, treatment, and prevention of ductal carcinoma in situ (DCIS). *JNCI Cancer Spectr* 2018;2. <https://doi.org/10.1093/jncics/pky063>.
- [35] Thompson E, Taube JM, Elwood H, Sharma R, Meeker A, Warzecha HN, et al. The immune microenvironment of breast ductal carcinoma in situ. *Mod Pathol an Off J United States Can Acad Pathol Inc* 2016;29:249–58. <https://doi.org/10.1038/modpathol.2015.158>.
- [36] Lal A, Chan L, Devries S, Chin K, Scott GK, Benz CC, et al. FOXP3-positive regulatory T lymphocytes and epithelial FOXP3 expression in synchronous normal, ductal carcinoma in situ, and invasive cancer of the breast. *Breast Canc Res Treat* 2013;139:381–90. <https://doi.org/10.1007/s10549-013-2556-4>.
- [37] Czerniecki BJ, Koski GK, Koldovsky U, Xu S, Cohen PA, Mick R, et al. Targeting HER-2/neu in early breast cancer development using dendritic cells with staged interleukin-12 burst secretion. *Canc Res* 2007;67:1842–52. <https://doi.org/10.1158/0008-5472.CAN-06-4038>.
- [38] Hussein MR, Hassan HI. Analysis of the mononuclear inflammatory cell infiltrate in the normal breast, benign proliferative breast disease, in situ and infiltrating ductal breast carcinomas: preliminary observations. *J Clin Pathol* 2006;59:972–7. <https://doi.org/10.1136/jcp.2005.031252>.
- [39] Morita M, Yamaguchi R, Tanaka M, Tse GM, Yamaguchi M, Otsuka H, et al. Two progressive pathways of microinvasive carcinoma: low-grade luminal pathway and high-grade HER2 pathway based on high tumour-infiltrating lymphocytes. *J Clin Pathol* 2016;69:890–8. <https://doi.org/10.1136/jclinpath-2015-203506>.
- [40] Chapman C, Murray A, Chakrabarti J, Thorpe A, Woolston C, Sahin U, et al. Autoantibodies in breast cancer: their use as an aid to early diagnosis. *Ann Oncol Off J Eur Soc Med Oncol* 2007;18:868–73. <https://doi.org/10.1093/annonc/mdm007>.
- [41] Svensson S, Abrahamsson A, Rodriguez GV, Olsson A-K, Jensen L, Cao Y, et al. CCL2 and CCL5 are novel therapeutic targets for estrogen-dependent breast cancer. *Clin Canc Res* 2015;21:3794–805. <https://doi.org/10.1158/1078-0432.CCR-15-0204.LP>.
- [42] McVeigh TP, Kerin MJ. Clinical use of the Oncotype DX genomic test to guide treatment decisions for patients with invasive breast cancer. *Breast Cancer (Dove Med Press)* 2017;9:393–400. <https://doi.org/10.2147/BCTT.S109847>.
- [43] Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. *N Engl J Med* 2018;379:111–21. <https://doi.org/10.1056/NEJMoa1804710>.
- [44] Knopfmacher A, Fox J, Lo Y, Shapiro N, Fineberg S. Correlation of histopathologic features of ductal carcinoma in situ of the breast with the oncotype DX DCIS score. *Mod Pathol an Off J United States Can Acad Pathol Inc* 2015;28:1167–73. <https://doi.org/10.1038/modpathol.2015.79>.
- [45] Romero L, Klein L, Ye W, Holmes D, Soni R, Silberman H, et al. Outcome after invasive recurrence in patients with ductal carcinoma in situ of the breast. *Am J Surg* 2004;188:371–6. <https://doi.org/10.1016/j.amjsurg.2004.06.034>.
- [46] Donker M, Litière S, Werutsky G, Julien J-P, Fentiman IS, Agresti R, et al. Breast-conserving treatment with or without radiotherapy in ductal carcinoma in situ: 15-year recurrence rates and outcome after a recurrence, from the EORTC 10853 randomized phase III trial. *J Clin Oncol Off J Am Soc Clin Oncol* 2013;31:4054–9. <https://doi.org/10.1200/JCO.2013.49.5077>.
- [47] Dieci MV, Radosevic-Robin N, Fineberg S, van den Eynden G, Ternes N, Penault-Llorca F, et al. Update on tumor-infiltrating lymphocytes (TILs) in breast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma in situ: a report of the International Immuno-Oncology Biomarker Working Group on. *Br Semin Cancer Biol* 2018;52:16–25. <https://doi.org/10.1016/j.semcancer.2017.10.003>.
- [48] Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol Off J Eur Soc Med Oncol* 2015;26:259–71. <https://doi.org/10.1093/annonc/mdu450>.



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