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Review

Tumor-infiltrating lymphocytes in breast cancer according to tumor subtype: Current state of the art

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ABSTRACT

The recent success of the immune checkpoint blockade in cancer immunotherapy has modified the treatment algorithms in a variety of aggressive neoplastic diseases. Nevertheless, optimal selection of ideal candidates to these drugs remains a challenge. The presence, location and composition of a pre-existing tumor immune infiltrate seem to impact on the benefit from these treatments. The association between the presence of baseline tumor-infiltrating lymphocytes (TIL) and patients' outcomes has been widely investigated in breast cancer, although immunotherapeutic strategies have historically been less successful with respect to other neoplastic diseases such as melanoma and kidney cancer. TIL extent varies and has different associations with outcomes in the various breast cancer subtypes. Furthermore, the presence of baseline high TIL has been associated with an increased benefit from some chemotherapeutic and targeted agents even though some conflicting results have been observed on this regard. This review aims to summarize the state of the art of TIL in breast cancer with a focus on their assessment, prevalence and clinical implications in the different subtypes.

1. Introduction

The recent success of the immune checkpoint blockade in cancer immunotherapy has modified the treatment algorithms in a variety of aggressive neoplastic diseases [1–4]. Nevertheless, since these new agents appear to be active only in a subset of patients, optimal selection of ideal candidates to these drugs remains a challenge. Preliminary observations revealed that the presence, location and composition of a pre-existing tumor immune infiltrate impact on the benefit from these treatments [5,6]. This has led to a rising interest in understanding the influence of the immune response on the clinical outcomes of cancer patients [7–10].

In early breast cancer, the association between the presence of a baseline immune response and patients' outcomes has been widely investigated [11]. Over the past years, a growing amount of data have shown potential clinical implications for tumor-infiltrating lymphocytes (TIL) [12–14] and immune gene signatures (which reflect the presence and the activity of the immune infiltrate) [15–18]. Specifically, the evaluation of TIL in several randomized controlled trials (RCT) revealed that TIL levels vary and have a different association with outcomes in the various breast cancer subtypes [12,19]. The association between the presence of TIL and the benefit from some chemotherapeutic and targeted agents has also been investigated [12,20–24]. However, so far, more conflicting results have been observed on this regard.

This review aims to summarize the state of the art of TIL in breast cancer with a focus on their assessment, prevalence and clinical implications in the different subtypes.

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2. Tumor-infiltrating lymphocytes (TIL)

2.1. Definition and assessment

The method for the assessment of TIL on hematoxylin and eosin (H&E) slides was first introduced by Denkert *et al.* [25]. Lately, international guidelines have been developed as a guide for pathologists to harmonize the scoring of this novel biomarker [12]. Stromal TIL are defined as the percentage of tumor stromal area containing a lymphocytic infiltrate with no direct contact with tumor cells. Intratumoral TIL are defined as intraepithelial mononuclear cells found within tumor cell nests or in direct contact with tumor cells. In breast cancer, TIL are usually more abundant in stromal compared to intratumoral areas, and the assessment of TIL in the stroma emerged as the most reproducible immune parameter scored by pathologists.

Standardization of TIL scoring was the main goal of the International Breast Cancer Immuno-Oncological Biomarkers Working Group (previously named as the TIL Working Group) which includes pathologists, oncologists and immunologists [12]. A recent work from this group demonstrated that the reproducibility of TIL assessment is feasible in breast cancer, even though more efforts are needed for the improvement of the interobserver variability [26]. This study acts as an important step towards a potential use of TIL as a future biomarker in breast cancer.

2.2. Composition and organization

TIL are mostly composed by T cells, followed by macrophages, B, natural killer and other immune cells [12]. Immunohistochemistry (IHC) allows the identification of the various subpopulations of TIL, by using specific leukocyte markers. A recent study demonstrated a more accurate and reproducible scoring of both stromal and intratumoral TIL by IHC with respect to H&E slides [27].

Use of specific markers for T and B cells in double stained IHC slides permits a more accurate detection of tertiary lymphoid structures (TLS) [27]. TLS are organized aggregates of B cells surrounded by T cells often localized in peritumoral areas [17,27,28]. These structures are thought to be the sites where an adaptive memory immune response can be generated [17] and their prognostic role in breast cancer remains controversial [29,30].

3. TIL in luminal breast cancer

3.1. Incidence of TIL in luminal breast cancer

Luminal tumors, defined by the expression of hormone receptors (estrogen receptor [ER] and/or progesterone receptor [PgR]), represent the most common and the least immune infiltrated breast cancer subtype [19]. Median infiltration of stromal TIL ranges between 7 and 10% [20,21,23], usually higher than intratumoral TIL (1.5–5%) [20,21,31].

In early-stage luminal breast cancer the prevalence of the lymphocyte predominant breast cancer (LPBC) phenotype (defined by the presence of ≥50% or ≥60% of either stromal or intratumoral TIL, with this cut-off differing according to each study definition) varies between 2.9 and 15% (≥50% cut-off [20,21]) and 10–12% (≥60% cut-off [25,32]).

A well annotated retrospective study including 614 primary invasive lobular breast tumors (most of whom of luminal subtype), described median stromal TIL levels equaling to 5%, with only 9% of the tumors having ≥20% stromal TIL [33].

Overall, these data suggest that luminal breast tumors are characterized by low levels of stromal and intratumoral TIL with a heterogeneous prevalence of LPBC cases.

3.2. TIL in luminal early-stage breast cancer

The majority of patients with luminal breast cancer included in TIL studies received adjuvant chemotherapy before starting endocrine therapy. No statistically significant correlation between TIL and survival was observed [20,21,23]. A large study including 12,439 patients found no association between cytotoxic ($CD8^+$) and Forkhead box protein 3 positive ($Foxp3^+$) T cells (quantified using IHC) and breast cancer specific survival (BCSS) [34]. A meta-analysis of RCT evaluating TIL in primary breast tumors confirmed that baseline TIL levels in luminal tumors (n = 2132) did not correlate with outcomes in the adjuvant setting [35]. Taken together, these data suggest that TIL extent does not add prognostic information in this setting (Table 1).

Interestingly, in primary invasive lobular breast tumors, higher levels of TIL (≥20%) were associated with worse prognosis at the univariate analysis and were associated with the presence of specific somatic mutations (i.e. *ARID1A*, *BRCA2* and *TP53*) [33]. These results suggest that the immune infiltrate and its clinical significance may differ by breast tumor histotype, even though further confirmation from prospective cohorts would be desirable.

Several studies evaluated the association between a baseline lymphocytic infiltrate scored in pre-treatment core biopsies and the likelihood of responses after neoadjuvant therapies (Table 2). In the GeparDuo and GeparTrio RCT, patients were given neoadjuvant anthracyclines and taxanes, higher baseline TIL levels were associated with higher chance of pathologic complete response (pCR) in the overall population and in specific subgroups including the ER-positive/PR-positive (any HER2) subtype [25]. Similar findings were observed in patients with luminal tumors enrolled in the neoadjuvant GeparQuinto trial (n = 209) receiving neoadjuvant anthracyclines (± bevacizumab) and taxanes (± everolimus in tumors not responding to anthracyclines ± bevacizumab) [32]. In this study, the pCR rate was 28.2% for LPBC and 8.2% for non-LPBC luminal tumors. The meta-analysis by Carbognin *et al.* confirmed a strong association between baseline TIL and pCR in neoadjuvant trials, regardless of the breast cancer subtype [35]. A recent pooled analysis of individual patients data (n = 3771) from six neoadjuvant trials of the German Breast Group (GBG) confirmed that baseline TIL were associated with increased pCR rates in all breast cancer subtypes [36]. However, in luminal tumors (n = 1366), low TIL (<10%) were associated with improved overall survival (OS). Authors speculated that high TIL infiltration in ER-positive tumors might be linked to more aggressive features and/or be associated with endocrine resistance. As of today, mechanistic data or correlative studies to support these findings are lacking.

One explanation for the presence of high TIL and the link with worse prognosis may derive from the specific TIL subsets infiltrating these tumors [19]. Retrospective studies revealed that high infiltrating $Foxp3^+$ regulatory T cells (Treg) correlated with a worse prognosis in patients with luminal early-stage breast cancer treated with adjuvant tamoxifen and/or chemotherapy [19,37,38], particularly in tumors lacking concomitant $CD8^+$ TIL [39]. Therefore, it is possible that the presence of different leukocyte populations infiltrating the luminal breast cancer microenvironment may have different prognostic implications.

A recent study evaluated the association between TIL and response after neoadjuvant therapy in luminal breast cancer [31]. Two prospective trials were included in this analysis, the LETLOB (n = 73; neoadjuvant endocrine-based treatment) and the GIOB (n = 38; neoadjuvant chemotherapy-based treatment) trials. Stromal TIL were centrally evaluated on samples from diagnostic core-biopsies. Tumors with stromal TIL ≥10% (n = 28) were more often of ductal histology (p = 0.02), high grade (p = 0.049), and high Ki67 (p = 0.02) and achieved more frequently a relative ≥50% Ki67