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Tumor-Infiltrating Lymphocytes: A Promising Biomarker in Breast Cancer

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Summary

There is clear evidence that the immune system plays an essential role in tumor defense. By determining tumor-infiltrating lymphocytes (TILs), the individual immunological response becomes more apparent and measurable. In breast cancer, high levels of TILs are associated with a more favorable clinical course. In this review, we describe how TILs are determined with emphasis on daily routine diagnostics. We further discuss their impact as a prognostic and predictive biomarker in the neoadjuvant and adjuvant therapy setting as well as in residual disease. We also discuss their potential future implications on further stratifying prognostic subgroups of breast cancer, thereby possibly influencing future therapy considerations.

Introduction

In healthy individuals, the immune system is able to protect against transformed cells, a process that is called immunosurveillance [1]. Nevertheless, as malignant tumors become clinically apparent, the immunological defense is ineffective. Furthermore, treatment with immunosuppressive drugs increases the risk of developing malignant tumors such as lymphoma or non-melanoma skin cancer [2]. The interaction between immune system and cancer cells is called 'cancer immunoediting' and consists of 3 phases [3]: (1) elimination: cancer cells are eliminated due to immunosurveillance; (2) equilibrium: the transformed cells persist but are kept in check by the immune system; and (3) escape: malignant cells escape the control and the tumor progresses. These processes are mainly regulated by leukocytes and cytokines.

In addition, there is increasing evidence that the immune system essentially contributes to the antitumor effects of cytotoxic regimens and antibody-based therapy. Chemotherapeutic agents such as anthracyclines and taxanes, which are part of the routine management of breast cancer, lead to immune response after cell death by release of factors that activate both innate and adaptive immune responses [4, 5].

Trastuzumab is a monoclonal antibody that blocks the human epidermal growth factor receptor 2 (HER2) in HER2-positive breast cancer by binding to the extracellular domain. Therefore, it prevents homo- and heterodimerization of the receptor and inhibits the downstream signals [6]. Besides that, preclinical models strongly suggest an additional, immune-mediated effect by antibody-dependent cellular cytotoxicity (ADCC). Antibody-tumor cell binding activates immune effector cells to eliminate the antigen-expressing cells [7]. This is further supported by the fact that the trastuzumab effect is significantly reduced in B or T cell-deficient mice, followed by a rapid tumor relapse [8].

This overview is not meant to be exhaustive; it should rather demonstrate the strong interaction between immune system, cancer cells and conventional therapeutic strategies. Consequently, the question arises of whether some therapies need an activated immune system to work or if they work better in patients with an already activated immune system.

Tumor-Infiltrating Lymphocytes

Tumor-infiltrating lymphocytes (TILs) are a specific histological feature of various cancers and are believed to reflect an indi-

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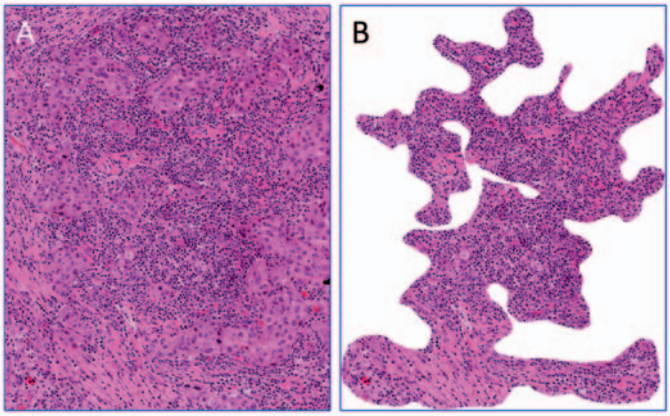

Fig. 1. a Lymphocyte predominant breast cancer (LPBC). **b** The infiltrate is predominantly located in the tumor stroma.

vidual immunological tumor response. In contrast to malignant melanoma, breast cancer has not been traditionally considered as immunogenic [9]. However, some time ago breast carcinomas with a dense lymphocytic infiltrate were described as a subgroup of medullary carcinoma [10]. These carcinomas exhibit additional histological characteristics such as syncytial growth pattern and pushing borders. Although typically poorly differentiated, they tend to have a relatively favorable prognosis compared to grade-matched invasive carcinomas of no special type (NST) [11]. It is proposed that this is related to the lymphocytic infiltrate.

Several strategies to measure the interaction between tumor and immune system have been published. These include analyses by hematoxylin/eosin (H&E) staining [12], evaluation of specific subgroups of immune cells by immunohistochemistry, immunofluorescence or flow cytometry [13, 14] as well as measuring expression of immune system-related genes [15].

The most frequently applied method to detect TILs is the semiquantitative evaluation by light microscopy on H&E-stained slides. This method can easily be integrated in daily routine diagnostics in which most techniques are based on formalin-fixed paraffin-embedded (FFPE) tissue. It is time saving and inexpensive and, therefore, also suitable for evaluation of large study cohorts. On H&E slides, TILs can typically be detected in 2 compartments: the stromal and the intratumoral compartments [16]. Intratumoral TILs – defined as lymphocytes inside tumor cell clusters – are less frequent, and therefore more difficult to detect and less reproducibly assessable. Stromal TILs are detectable in the desmoplastic stroma between the tumor cell clusters. They are more frequent and more numerous [16]. It is obvious that this method does not allow for evaluation of the specific subtypes of immune cells that comprise the TILs, such as T cells, B cells, natural killer cells or macrophages, each of which may contribute differently to the antitumoral activity [17]. However, as evidence was growing that TILs are of prognostic as well as predictive value, a standardized evaluation approach was urgently needed. Therefore, the 'International TILs Working Group 2014' published recommendations for a pragmatic, reproducible and simple assessment of TILs in breast cancer on H&E sections [16]. TILs can be assessed as a continuous parameter, which is recommended as it reflects the continuity of the immune infiltrate. In addition, the classification of 'lymphocyte predominant breast cancer' (LPBC) is frequently applied, especially for statistical analyses. Depending on the study, LPBC is defined as having 50–60% stromal lymphocytes, referring to a tumor that shows more lymphocytes than carcinoma cells (fig. 1) [16].

Interestingly, the frequency of high TIL levels seems to depend on the intrinsic subtype. TILs are more frequent in the aggressive subtypes of breast cancer. In a pooled analysis of 4 prospective adjuvant trials [18], high levels of TILs were significantly more frequent in hormone receptor-negative tumors. In the adjuvant BIG 02-98 trial, the median percentage of TILs was the highest in estrogen receptor (ER)-negative and HER2-negative tumors [19].

TILs in the Neoadjuvant Setting

The concept of neoadjuvant chemotherapy (NACT) has several advantages. It may lead to down-staging of the disease and, therefore, enables a breast-conserving surgery. Furthermore, it allows monitoring of therapy response. In particular, in HER2-positive and triple-negative breast cancer (TNBC) the achievement of a pathological complete response (pCR) is associated with a favorable long-term survival [20]. However, there are no well-established surrogate markers that can help to predict the probability of pCR in an individual case. TILs may be helpful as an additional parameter in this regard. Pre-therapeutic core biopsies of breast cancer patients are an excellent tool to test potential prognostic and predictive markers using FFPE-based methods in the neoadjuvant setting. Over the past few years, several studies have examined TILs on H&E slides and evaluated their association with pCR, therapy response and/or prognosis in the neoadjuvant context [21–23]. The strongest evidence for TILs being a predictive marker for pCR was achieved in retrospective-prospective evaluations of clinical trials [24]. This accessed data from large-scale homogeneously treated case series that were randomized and provided well-documented pathological, clinical and follow-up information [12, 25–27]. These studies mainly comprised HER2-positive or triple-negative tumors. For example, in the neoadjuvant GeparDuo and GeparTrio trials, we demonstrated that high levels of TILs (evaluated as a continuous parameter as well as LPBC tumors) were significantly associated with increased pCR rates [25]. In the neoadjuvant GeparQuattro [28], GeparQuinto [26] and GeparSixto [12] trials, these results were confirmed in triple-negative and HER2-positive cases. Furthermore, cases with high-level TILs seemed to gain an additional benefit from carboplatin treatment [12]. In the recently published secondary analysis of the NeoALLTO trial [29], the association of TILs and pCR rate in early stage HER2-positive breast cancer was again validated. Furthermore, the authors showed an independent positive prognostic impact of TILs since, independent of the anti-HER2 therapy applied, for every 1% increase of TILs the rate of an event decreased by 3%. Interestingly, there are sparse data on the role of TILs in luminal tumors [25].

Table 1. Studies assessing tumor-infiltrating lymphocytes (TILs) in breast cancer

Trial name	Assessment	BC subtype	n	Result [ref]
A) In neoadjuvant setting				
–	retro	TNBC HER2+	180	predictive for pCR [21]
–	retro	HER2+	116	predictive for pCR and improved EFS [22]
GeparDuo/ GeparTrio	retro-pro	all	1058	predictive for pCR [25]
GeparQuattro	retro-pro	HER2+	156	predictive for pCR [28]
GeparQuinto	retro-pro	TNBC	313	predictive for pCR [26]
GeparSixto	retro-pro	TNBC HER2+	580	predictive for pCR [12]
NeoALLTO	retro-pro	HER2+	387	predictive for pCR and improved EFS [29]
B) In adjuvant setting				
BIG 02-98	retro-pro	all	1632	improved prognosis in TNBC subgroup [19]
FinHER	retro-pro	all	934	improved DDFS only in TNBC subgroup
N9831	retro-pro	HER2+	945	improved RFS in subgroup treated with CT alone [34]
ECOG 2197 ECOG 1199	retro-pro	TNBC	481	improved DFS [33]
Phase III randomized adjuvant	retro-pro	all	781	improved OS in TNBC and HER2+, but not in HR+HER2- subtype [37]
Pooled analysis	retro-pro	all	2613	improved DFS [18]
C) In residual disease				
Multicenter	retro	TNBC	278	improved MFS and OS [36]

[a] TILs were all assessed on hematoxylin and eosin (H&E)-stained specimens.
BC = breast cancer, retro = retrospective, pro = prospective, CT = chemotherapy, DDFS = distant disease-free survival, DFS = disease-free survival, EFS = event-free survival, HER2 = human epidermal growth factor receptor 2, HR = hormone receptor, MFS = metastasis-free survival, OS = overall survival, pCR = pathological complete remission, RFS = recurrence-free survival, TNBC = triple-negative breast cancer.

TILs in the Adjuvant Setting

In the adjuvant setting, therapy decisions and prognosis are based on the intrinsic subtype of an individual tumor as defined by the St. Gallen Consensus Conference [30]. Although the molecularly defined intrinsic subtype is not exactly identical to the immunohistochemically determined one, the expression profile of hormone receptors (ER, progesterone receptor (PR)), HER2 and Ki-67 is seminal for further therapy decisions. Tumor size, nodal status and differentiation grade in combination with the receptor status and proliferation rate are well-established prognostic markers. In addition, gene expression-based tests are additional tools to further subdivide the cohorts [31].

The prognostic value of TILs seems to depend on the intrinsic subtype of breast cancer. In ER-positive HER2-negative breast cancer, it appears that TILs have no significant impact on prognosis [19, 32]. For TNBC, there are several studies that show an increase in recurrence-free survival (RFS) with increasing levels of TILs [19, 32, 33]. For example, in the adjuvant FinHER trial, every 10% increase of TILs was significantly associated with decreased distant recurrence, defined as distant disease-free survival (DDFS) [32].

In the subgroup of HER2-positive breast cancer, results are more heterogeneous, which may be influenced by whether an anti-HER2 therapy has been applied, and if so, which one. In the FinHER and BIG 02-98 trials, there was no significant association between TILs and prognosis [19, 32]. However, in the adjuvant N9831 trial, early stage HER2-positive tumors with high levels of TILs, evaluated as LPBC tumors, were associated with higher 10-year Kaplan-Meier estimates for RFS when treated with chemotherapy alone, but not when treated with chemotherapy and trastuzumab [34]. In 2 French adjuvant trials [35], 10-year overall survival rates of patients with HER2-positive tumors were significantly higher for those bearing high-TIL versus low-TIL tumors. However, these tumors were not treated with an anti-HER2 therapy.

Since the effect of trastuzumab is partly immune mediated, the predictive value of TILs on trastuzumab treatment was evaluated. Results from the FinHER trial showed a positive interaction of TILs with trastuzumab [32]. In the N9831 trial, this effect could not be confirmed [35], so that additional investigations are needed.

TILs and Residual Disease

In general, residual tumor after NACT is associated with poor prognosis [20]. There are only a few studies evaluating TILs in residual disease, mainly in TNBC. A retrospective analysis showed that a higher level of TILs in residual tumor is associated with an improved outcome [36]. In the NeoALLTO trial in HER2-positive tumors, TILs were a prognostic marker even if a patient did not achieve pCR. Patients with high TIL levels at the time of diagnosis had a more favorable outcome [29].

TILs in Breast Cancer – Future Perspectives

The amount of data corroborating the promising potential of TILs as a prognostic and predictive biomarker in breast cancer is ever increasing (table 1). To integrate this marker in our daily clinical routine, it became essential to generate standardized and reproducible evaluation criteria as suggested by the 'International TILs Working Group 2014' [16]. TILs seem to be of more relevance in HER2-positive cancer and TNBC than in the luminal subtypes. In the adjuvant setting, TILs are associated with improved survival endpoints. Therefore, they may be integrated as an additional risk factor complementing the well-established ones like receptor status, tumor size or nodal status [38]. They may help to identify more delicate prognostic subgroups and, therefore, patients who are more likely to benefit from adjuvant chemotherapy.

In the neoadjuvant setting, TILs may have even more potential. Being a positive predictor for pCR, they identify tumors that may have a good outcome per se. Some authors suggest the combination of both parameters – TILs and pCR – to identify tumors that may have a negligible risk of recurrence (high TILs/pCR) or a high risk of recurrence, thereby helping to guide further treatment decisions. Tumors showing a high TIL/pCR have an excellent prognosis, which may allow a de-escalation of the therapy regimen. Even high-level TIL tumors that do not achieve pCR have a favorable prognosis [29]. Tumors with low TILs/no pCR are believed to have the poorest outcome. They may profit from different treatment strategies inducing an immune response and promoting TILs [39].

For future clinical trials, one of the most important question is which tumors may benefit from an additional therapy with immune-modulating drugs such as immune-checkpoint inhibitors, e.g. blocking the PD-1/PD-L1 axis [40]. TILs might be a relevant biomarker in this regard, which should be integrated in future clinical trials.

Disclosure Statement

There is no conflict of interest to declare.

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