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CURRENT TOPICS IN BREAST PATHOLOGY

Tumour-infiltrating lymphocytes and the emerging role of immunotherapy in breast cancer

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Summary

Breast cancer has not previously been considered a highly immunogenic cancer. Observations of tumour-infiltrating lymphocytes (TILs) in and around neoplastic cells in tumour samples, and associations with improved pathological complete response and clinical survival end points have changed our perspective on this. Lymphocytic infiltrates have long been observed in breast cancer; however, more recently, retrospective analysis of prospectively collected tissue samples from clinical trials has demonstrated the potential role of host immunosurveillance in influencing the biology of breast cancer. This association appears to be strongest in triple negative and HER2 positive breast cancer subtypes. Contrastingly, the association in luminal tumours is less clear, and is potentially limited by substantial tumoural heterogeneity. Several methodologies have been employed to quantify, and describe the composition of TILs, each with its own advantages and disadvantages. The results of these analyses have been generally consistent, and valuable efforts are currently underway to standardise the evaluation of TILs toward a universal approach. More technical methods of TIL characterisation remain important in the research setting. The evaluation of TILs becomes increasingly relevant with the emerging role of immunotherapy in breast cancer. Early phase trials of checkpoint blockade show promising results; however, it is likely that some patients will require combination treatments to maximise therapeutic benefits. Equally, some patients may not derive any benefit from immunotherapies. This underscores the importance of the development of relevant predictive biomarkers. As a key representative of the immune interaction between host and tumour, lymphocytic infiltrates are ideally placed for continued research into the determinants of immunogenicity, and response to immunotherapeutic approaches. In this review, we will discuss the current methodologies of evaluation, and the clinical relevance of TILs. Additionally, we discuss the emerging role of immunotherapy in breast cancer, and the future of TIL characterisation in this context.

Key words: Tumour-infiltrating lymphocytes; breast cancer; biomarkers; prognosis; immunotherapy; checkpoint blockade.

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INTRODUCTION

Ample evidence confirms host anti-tumour immunity can play a key deterministic role in the outcome of cancerous cells. Evasion of immune influences is considered a hallmark of cancer.[1] An encompassing hypothesis is the immunoediting theory, whereby host immunosurveillance recognises immunogenic tumour antigens and responds in three subsequent phases: elimination, equilibrium, and escape.[2,3] Left unchecked, these processes shape the clonal and genomic structure of tumours towards a state where the tumour can escape immune containment, the most frequent point of diagnosis. More recently, it has become evident that immunoediting alone is insufficient to fully explain the lack or loss of immunogenicity of a tumour. Indeed, as we increasingly explore personalised medicine, we are beginning to unravel genomic alterations that may provide tumour-intrinsic mechanisms of immune escape, demonstrating an intriguing interaction between host and tumour factors.[4–6]

Tumour-infiltrating lymphocytes (TILs), observed as mononuclear immune cells nested in and around neoplastic cells, stand at the very heart of this interaction. Numerous retrospective analyses of tumour samples from prospective trials have confirmed TILs to be a robust and reproducible biomarker of immunogenicity in selected breast cancer subtypes. While providing valuable insights, a more complete understanding of the biological significance of TILs is hampered by heterogeneity of TIL evaluation and trial design. Regardless, as we enter a new era of immunotherapeutics, TILs will become increasingly relevant as a biomarker in our efforts to tailor therapies to maximum effect. This review aims to discuss the evaluation of TILs, its clinical relevance, and its potential role in the future treatment of breast cancer with immunotherapies.

EVALUATING TILs

TILs encompass a mixture of different cell types usually dominated by T cells, with variable proportions of B cells, NK cells, macrophages, and dendritic cells.[7,8] TIL quantification and characterisation therefore have been used as a surrogate for evaluation of tumour immunogenicity. Of note, a chronically sustained immune response is generally considered to be counter-productive in human disease and so mechanisms of immunosupression inevitably establish.[9] Indeed, chronic inflammation can ultimately lead to a protumourogenic environment.[10] Additionally, there is a

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continuous dynamic between active adaptive T-cell responses and exhausted adaptive T-cell responses; qualification of immune cells at a single time point from a biopsy at a single anatomical location may be insufficient to capture the full extent of this dynamic.

Numerous studies have demonstrated the importance of adaptive T-cell mediated cytotoxic responses as a dominant mechanism of host anti-tumour immune responses.[11,12] T-cell responses result from the recognition of tumour-specific peptides that have arisen as the end product of expressed somatic cancer mutations, termed neoantigens.[13–15] These peptides are presented to effector cells of the immune system in association with major histocompatibility complex (MHC) proteins. Additionally, natural killer (NK) cell mediated antibody dependent cell-mediated cytotoxicity[16–18] and B-cell mediated anti-tumour immunity[19,20] may also play important roles in specific disease and treatment contexts.

METHODS OF TIL EVALUATION

TILs have been evaluated by various methodologies described below. Studies evaluating TILs have rapidly expanded in recent years, largely by virtue of retrospective analysis of prospectively collected tumour samples in early breast cancer clinical trials. These have served as ideal platforms to investigate the prognostic and predictive influences of host anti-tumour immunity.

Light microscopy of haematoxylin and eosin (H&E) stained histological slides

Perhaps the most common method of detection and quantification of TILs are via light microscopy of H&E stained histological slides of tumour samples. This involves direct visualisation and measurement of mononuclear cells on representative H&E tumour sections. Given the heterogeneity in methodologies, the International TILs Working Group, recently renamed as the International Immuno-Oncology Biomarker Working Group, has put forward recommendations for standardisation to maximise reproducibility[21] (Table 1). The defined tumour area is divided into a stromal compartment and an intratumoural compartment, and measurements are made per compartment. Most studies measure both stromal and intratumoural compartment TILs, however stromal TILs are considered to be more representative and reproducible. Certainly, stromal TILs are generally more abundant, whereas intratumoural TILs are typically low in number, more heterogeneous across samples, and less visible. Consequentially, significant associations with clinicopathological parameters and disease outcomes are better established with stromal TILs.

The preferred method of quantification of TILs is by percentage as a continuous variable. Some previous studies have utilised the term lymphocyte-predominant breast cancer (LPBC) with discrete cut-offs, typically defined as TILs greater than 50–60% depending on the study. Quantification as a continuous variable is supported by the linear relationships observed between increasing lymphocytic infiltration and survival outcomes in specific breast cancer subpopulations as described in subsequent sections. It also further allows more accurate capture of TIL heterogeneity in a population than an arbitrary discrete cut-off.

Whole tumour sections are preferred to core biopsies where possible, although core biopsy TIL evaluation in neoadjuvant studies has been previously used and displays good concordance with results from tumour section analyses. Tissue microarrays (TMAs) could provide a more rapid solution to evaluation of TILs in large study cohorts; results in several studies utilising TMAs are similar to those observed from full sections,[22–24] however the potential for sampling bias remains unclear.[21] Intratumoural heterogeneity could perceivably cause issues in selected cases. As such, in cases with spatial heterogeneity, it is suggested to evaluate different regions and report the average.

Table 1 Recommendations for assessing tumour-infiltrating lymphocytes (TILs) in breast cancer

Recommendations
1. TILs should be reported for the stromal compartment (= % stromal TILs). The denominator used to determine the % stromal TILs is the area of stromal tissue (i.e., area occupied by mononuclear inflammatory cells over total intratumoural stromal area), not the number of stromal cells (i.e., fraction of total stromal nuclei that represent mononuclear inflammatory cell nuclei).
2. TILs should be evaluated within the borders of the invasive tumour.
3. Exclude TILs outside of the tumour borders, e.g., around DCIS and normal lobules.
4. Exclude TILs in tumour zones with crush artefacts, necrosis, regressive hyalinisation as well as in the previous core biopsy site.
5. All mononuclear cells (including lymphocytes and plasma cells) should be scored, but polymorphonuclear leukocytes are excluded.
6. One section (4–5 µm, magnification 200–400×) per patient is currently considered to be sufficient.
7. Full sections are preferred over biopsies whenever possible. Cores can be used in the pre-therapeutic neoadjuvant setting; currently no validated methodology has been developed to score TILs after neo-adjuvant treatment.
8. A full assessment of average TILs in the tumour area by the pathologist should be used. Do not focus on hotspots.
9. The working group's consensus is that TILs may provide more biological relevant information when scored as a continuous variable, since this will allow more accurate statistical analyses, which can later be categorised around different thresholds. However, in daily practice most pathologists will rarely report for example 13.5% and will round up to the nearest 5-10%, in this example thus 15%. Pathologist should report their scores in as much detail as the pathologist feels comfortable with.
10. TILs should be assessed as a continuous parameter. The percentage of stromal TILs is a semiquantitative parameter for this assessment, for example, 80% stromal TILs means that 80% of the stromal area shows a dense mononuclear infiltrate. For assessment of percentage values, the dissociated growth pattern of lymphocytes needs to be taken into account. Lymphocytes typically do not form solid cellular aggregates, therefore the designation '100% stromal TILs' would still allow some empty tissue space between the individual lymphocytes.
11. No formal recommendation for a clinically relevant TIL threshold(s) can be given at this stage. The consensus was that a valid methodology is currently more important than issues of thresholds for clinical use, which will be determined once a solid methodology is in place. LPBC (lymphocyte predominant breast cancer) can be used as a descriptive term for tumours that contain 'more lymphocytes than tumour cells'. However, the thresholds vary between 50–60% stromal lymphocytes.

Reproduced from Salgado *et al.*,[21] with permission from Oxford University Press on behalf of the European Society for Medical Oncology. TILs, tumour-infiltrating lymphocytes; DCIS, ductal carcinoma *in situ*.