Predictive Value of Tumor-Infiltrating Lymphocytes for Neoadjuvant Chemotherapy Among Triple-Negative Breast Cancer

Yuan Xu *

School of Food Science and Pharmaceutical Engineering, Nanjing Normal University, Nanjing, 210023, China

* Corresponding Author Email: 28230413@njnu.edu.com

Abstract. Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with high recurrence risk. Currently, neoadjuvant chemotherapy (NAC) serves as the core therapeutic option for TNBC, which requires effective biomarkers to predict patient prognosis. As the key component of the tumor immune microenvironment (TIME) in the tumor microenvironment (TME), Tumor-infiltrating lymphocytes (TILs) have been confirmed to be correlated with NAC prognosis of TNBC by existing studies, yet most of these studies focus only on patients with early-stage TNBC. In addition, no unified interpretation standard has been established. This review reveals the cellular composition of TILs and the anti-tumor immune mechanisms of TILs subsets, summarizes the internationally quantitative evaluation criteria and published clinical research findings and confirms that stromal tumor-infiltrating lymphocytes (sTILs) can facilitate the prediction of the patients prognosis receiving NAC. This review provides a theoretical support for the application of TILs in the precise stratified treatment of TNBC and the efficacy evaluation of new drugs. At present, in-depth research is still needed to clarify the immune pathway mechanisms of each TIL subset, improve stage-specific research, and standardize the interpretation and assessment of TILs. In future research, artificial intelligence can be applied to optimize detection standards, and large-sample clinical trials should be carried out to further explore the clinical potential of TILs.

Keywords: Tumor-infiltrating lymphocytes; triple-negative breast cancer; neoadjuvant chemotherapy.

1. Introduction

With the increasing incidence of breast cancer worldwide, it has become one of the significant factors causing death among women due to cancer [1]. Among them, TNBC is defined by the loss of estrogen receptors (ER), progesterone receptors (PR), as well as human epidermal growth factor receptor-2 (HER2), which accounts for 15% to 20% of all breast cancer cases [2]. The recurrence and distant metastasis risk after traditional chemotherapy treatment of TNBC are relatively high and the prognosis is poor, rendering it one of the most aggressive breast cancer subtypes [3].

NAC is a routine treatment method for TNBC. NAC refers to the systemic treatment using drug prior to surgery. By comparing the tumor samples before and after chemotherapy, we can observe changes at the pathological, biological, and genomic levels, establishing an ideal in vivo research model for exploring the chemosensitivity of tumors [4]. Moreover, NAC can reduce the clinical stage of tumors, eliminate micrometastases, and enhance the success rate of breast-conserving surgery and the completeness of surgical resection. Clinical evidence shows patients achieving pathological complete response(pCR) have a better prognosis [5]. The achievement of pCR is regulated by the number of TILs and is closely related to the initiation and state of tumor immune response [6].

TILs, as a key component of the TME, have been proven to reflect the immunogenicity level of TNBC tumors, and their high infiltration is positively correlated with the improvement of patient prognosis and the enhancement of NAC efficacy [7]. In conclusion, TILs may become an important biomarker for NAC in TNBC. Exploring its clinical value in prognosis prediction and therapeutic efficacy of TNBC is of great significance. This article aims to review relevant literature, elaborate on TILs composition and its immune mechanisms, explore the corrlation between TILs and TNBC

prognosis, summarize the clinical evaluation systems and applications of TILs in neoadjuvant therapy, and discuss future research prospects of TILs.

2. TILs in the TME

2.1. The Composition of TME and TILs

TME is a highly complex system with the composition of tumor cells, tumor-infiltrating immune cells (including macrophages, dendritic cells (DC) and lymphocytes), stromal cells linked with tumors, endothelial cells, adipocytes, extracellular matrix as well as various signaling molecules [8]. As the main components of the TIME, TILs consist of 60% T cells (divided into 20% cytotoxic $CD8^+$T cells, 40% helper $CD4^+$T cells, cytotoxic T lymphocytes (CTLs) and Th cells), 5% NK cells together with 20% B cells, macrophages occupy 5% and DCs account for below 1% of all TILs subsets [9]. TILs in breast cancer lesions scatter in both the intratumoral region and the stromal region, thus they are divided into stromal tumor-infiltrating lymphocytes (sTILs) and intratumoral tumor-infiltrating lymphocytes (iTILs). Compared to sTILs, iTILs have more difficult interpretation and worse detection repeatability [7]. In most TME, TILs are mainly sTILs, which are mostly distributed near tumor cells [10]. The strong immunogenicity of TNBC leads to a higher detection rate of sTILs adjacent to the fibrous stroma of cancer cells, making them key biomarkers [7].

2.2. The Role of TILs in the TME Relating to TNBC Prognosis

TILs play crucial roles in the TME, which are composed of various lymphocyte subsets to execute an anti-tumor response. Focusing on these subsets, CD8+TCRαβCD4+CD8+T cells make up the largest T cell population inside tumor tissue [11]. CD8+TCRαβT cells are abbreviated as CD8+ T cells, commonly known as CTLs, which can produce abundant anti-tumor factors and cytotoxic molecules, such as interferon-γ (IFN-γ), tumor necrosis factor-α(TNF-α). They have strong anti-viral and anti-tumor functions [12]. Therefore, the extensive infiltration of cytotoxic $CD8^+$T cells into tumors indicate strong direct killing of tumors, which is significantly related to the improvement of cancer prognosis. Under physiological status, CTLs will differentiate into multiple memory T cell subsets after clearing target cells, providing long-term anti-infection protection even after immune activity fades away. Such memory T cells have extremely heterogeneous phenotypes, gradually transition from an approximate initial T cell phenotype to an effector T cell phenotype, including stem cell-like memory T cells (TSCM), central memory T cells (TCM), effector memory T cells (TEM), and effector memory RA^+T cells (TEMRA). Among them, the phenotype of the TCM is called $CD45RA^-CD45RO^+$ [11], which can be used as a marker to monitor the activity of effector T cells and memory T cells. Studies have inferred that the higher the infiltration level of $CD45RO^+$TIL, the more sufficient the anti-tumor immune response induced by tumor induction. It can also form tumor-specific immune memory, remaining capable of eliminating residual tumor cells and micro-metastases even after surgery [13].

Therefore, the prognostic evaluation efficacy of $CD45RO^+$ TIL is long-term and can be used to predict the survival of patients. In addition, tissue-resident memory T cells (TRM) can express the classical marker CD103, also known as integrin αE. CD103 is usually combined with integrin β7 to form the αEβ7 complex, which interacts with the E-cadherin expressed on tumor cells, allowing TRM/$CD8^+$CTLs to stably reside in the tumor tissue, establishing a stable surveillance network for long-term anti-tumor immune monitoring [11]. Thus, monitoring TRM can effectively detect residual or recurrent tumor cells in patients, thereby effectively reducing the risk of tumor recurrence. However, it is still necessary to clarify the immune pathways connecting new antigen-activated T cells with TRM cells [9].

Apart from T cells, B cells, as specialized APCs, can trigger the primary activation of initial CD4+T cells [14]. Besides, research has proved that the $CD20^+$ B cells expressed by the plasma cell (PC) subset and $CD8^+$T cells can together amplify the prognostic benefits brought by $CD8^+$ T [15]. The role of B cells in prognosis has not been systematically and uniformly described in current studies,

so only part of them is mentioned here. Moreover, the number and functional activity of NK cells in the peripheral circulation also in tumor-infiltrating sites are also closely related to tumor progression, distant metastasis formation, and patient survival prognosis. NK cells are defined as those cells that lack the recombination activation genes and are unable to rearrange genes to produce antigen-specific B cell or T cell receptors. They can also exert anti-tumor effects indirectly by releasing granzymes, perforin, triggering tumor necrosis factor-related apoptotic signaling pathways, and activating T-helper cells through IFN-γsecretion [11]. Other immune cell subsets within TILs include DCs and macrophages. DCs can start the T cell activation process, playing a crucial role in mounting $CD8^+$T cells to drive anti-tumor immunity. Besides, higher levels of tumor-associated macrophages (TAMs) within tumor tissue correlate with poor survival outcomes among breast cancer patients [9].

3. The Clinical Assessment of TILs

3.1. The Evaluation System

The 2014 version of the International Tumor Immune Biomarker Working Group's guidelines adopted a quantitative evaluation based on the percentage of sTILs, and in 2016, it improved the clinical standardized reporting guidelines. This indicator can represent the expression level of sTILs. The calculation method is TILs area in the stroma divided by aggregate stromal area of the tumor, including the percentages of lymphocytes, plasma cells, and macrophages occupying the total stromal area of the tumor, rather than the stromal cells count [9]. The scoring range is limited within the tumor infiltration boundary to ensure the specificity of the assessment. In addition to focusing on the overall expression level of sTILs, the counting of immune cell areas is also of significant clinical research value. Subtypes such as $CD3^+$, $CD8^+$, and $CD45RO^+$ TIL have higher infiltration efficacy in TNBC, which can be incorporated into the TILs evaluation system [13].

Currently, with the development of clinical diagnosis interpretation, many studies have established algorithm models based on machine learning to address the shortcomings of traditional reliance on manual counting by pathologists, such as differences in personal interpretation and poor detection repeatability, which can promote the standardization of TILs assessment. In the future, the scoring system of TILs will be further improved in aspects such as expanding clinical validation, refining patient individual grouping, and expanding application scenarios [11]. Thus, sTILs have gradually become a quantifiable, assessable, and repeatable predictive marker in the NAC of TNBC.

3.2. Recent Clinical Studies on Assessment of TILs

The determination of the efficacy of NAC has several main indicators such as patient pCR and patient disease-free survival (DFS) [9]. Since Denkert's research team first proposed the association between TILs and breast cancer in 2010 [16], several studies have established the clear prognostic value of sTILs for early TNBC. The Loi research team summarized a large amount of individual data from 9 studies and constructed a multivariate regression model, finding that for every 10% increase in sTILs, the patient's invasive disease-free survival (iDFS), distant metastasis-free survival (D-DFS), and overall survival (OS) all improved. Patients with negative lymph nodes and sTILs > 30% had a 3-year iDFS rate, D-DFS rate, and OS rate of over 90% [17]. Geurts et al. explored the association between sTILs levels and patient prognosis, the results showed that there is a strong connection between sTILs levels and the patient's breast cancer-specific survival (BCSS), confirming that sTILs can be used as a biomarker for selecting patients with stage I TNBC, further improving the evidence for predicting the prognosis of early TNBC [18]. Most of the existing studies show the TILs value in early TNBC prognosis, while there are few systematic analyses for TNBC patients at other stages, and the correlation between sTILs infiltration and overall patient prognosis remains weak, making it difficult to accurately reflect its impact on clinical outcome. Moreover, it is worth noting that the sTILs infiltration levels in different studies have significant individual differences among the subjects, suggesting that personal factors are the key variables determining the anti-tumor effect under chemotherapy, which highlights the important value of TILs for individualized treatment again.

4. Clinical Applications of TILs

4.1. Prognostic Stratification

As markers, the infiltration levels of TILs in patients can distinguish those with poor prognosis from those with excellent prognosis. In clinical trial design, TILs can be used for patient stratification. For patients with high infiltration of TILs, avoiding the use of high-intensity NAC schemes can reduce the severity of adverse reactions. Relevant studies have included large sample cases to provide a basis for clinical trials on the optimized treatment of stage I TNBC, using sTILs as the core biomarker. Researchers proposed that patients with high sTILs infiltration can still achieve ideal long-term survival even without neoadjuvant or adjuvant chemotherapy, providing new ideas for the design of treatment regimens for early TNBC [18]. In addition, low infiltration levels of TILs can effectively select patients who receive standard treatment but still have poor prognosis. This provides important biological evidence for exploring new treatment strategies and individualized clinical trial stratification intervention for this group of patients, helping to achieve precise treatment. Currently, clinical treatment statistics mostly rely on baseline indicators to formulate treatment projects. It is still necessary to supplement standardized assessment stratification of TILs. Further research can also be conducted around dynamic monitoring indicators during NAC to achieve individualized adjustments during the treatment course based on prognostic indicators.

4.2. Assist in Evaluating the Efficacy of NAC New Drugs

Traditional unified chemotherapy regimens in neoadjuvant clinical trials have become inadequate for adapting to the current concepts of precision treatment. Therefore, research in the field of NAC has shifted its focus to using NAC efficacy as a predictive marker. By combining new drugs to increase the pCR rate, observing whether the combined treatment can translate into long-term survival benefits becomes an important indicator to evaluate the clinical feasibility of a new drug [4]. Given that TILs have clear value in patients with poor prognosis and residual tumor lesions after NAC, and in TNBC, the association between pCR and long-term survival is relatively strong, some studies have proposed adding TILs to observation indicators to assess pCR/residual lesion endpoint, they indicated that not all patients who do not achieve pCR will experience recurrence and held the view that optimize existing indicators such as tumor residual burden (RCB) to improve the prognosis prediction efficacy of evaluation system to evaluate the efficacy of new drugs objectively [7]. Thus, the application of TILs in NAC can help precisely screen the population who probably benefits from new drugs, clarify the target patients for new drugs, and assist in both the design and implementation of clinical trial schemes.

5. Conclusion

This article systematically reviews the composition of TILs in TME of TNBC, explains the mechanism by which each cell subset participates in the immune activity aiming at tumor, and also organizes the evaluation system of TILs, and summarizes the various clinical research results of these cells in NAC. Comprehensive analysis indicates that sTILs have relevance to patient prognosis undergoing NAC, which are reliable biomarkers for predicting the efficacy of NAC for TNBC and evaluating the clinical outcome. This article responds to the practical needs of achieving precise treatment goals in clinical practice, deepening the understanding of the immune characteristics of the TME in TNBC, and providing a solid theoretical support and practical references for clinical implementation of patient stratification management, formulation of individualized treatment plans, and evaluation of the efficacy of new combination drug schemes. Currently, there are still shortcomings in this field, with relatively scarce systematic studies on patients of different stages, and the specific mechanisms of some immune pathways have not yet been fully elucidated. In the future, by further optimizing the intelligent detection and scoring standards for TILs, dynamic monitoring studies throughout the treatment process can be carried out. In addition, clinical sample

sizes should be expanded to promote multi-center trials. With the continuous exploration of the application value of TILs, it is expected to be effectively used in the area of precise treatment of TNBC and the development of new drugs.

References

[1] Kashyap D, Pal D, Sharma R, et al. Global increase in breast cancer incidence: risk factors and preventive measures. BioMed Research International, 2022, 2022: 685–688.

[2] Navrátil J, Fabian P, Palácová M, et al. Triple negative breast cancer. Klin Onkologie, 2015, 28(6): 406–415.

[3] Thomas A, Reis-Filho JS, Geyer CE Jr, Wen HY. Rare subtypes of triple negative breast cancer: current understanding and future directions. npj Breast Cancer, 2023, 9(1): 55.

[4] Korde LA, Somerfield MR, Carey LA, et al. Neoadjuvant chemotherapy, endocrine therapy, and targeted therapy for breast cancer: ASCO guideline. Journal of Clinical Oncology, 2021, 39(13): 1485–1505.

[5] van den Ende NS, Nguyen AH, Jager A, et al. Triple-negative breast cancer and predictive markers of response to neoadjuvant chemotherapy: a systematic review. International Journal of Molecular Sciences, 2023, 24(3): 2969.

[6] Zhao Y, Jiang SJ, Wang JL, et al. Correlation study of tumor-infiltrating lymphocytes (TILs) and subtypes analysis before and after neoadjuvant chemotherapy in triple-negative breast cancer. Gland Surgery, 2025, 14(4): 628–645.

[7] Virassamy B, Salgado R, Loi S. Clinical validity and utility of tumor-infiltrating lymphocytes in routine clinical practice for breast cancer patients: current and future directions. Frontiers in Oncology, 2017, 7: 156.

[8] Mao X, Xu J, Wang W, et al. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. Molecular Cancer, 2021, 20: 131.

[9] Zhou Y, Tian Q, Wang BY, et al. The prognostic significance of TILs as a biomarker in triple-negative breast cancer. European Review for Medical and Pharmacological Sciences, 2021, 25(7): 2885–2897.

[10] Han JW, Su TT, Xu YB, Hu CH. Research progress of tumor infiltrating lymphocytes in predicting the efficacy of neoadjuvant chemotherapy for triple negative breast cancer. Chinese Journal of Clinical Oncology, 2023, 50(19): 1011–1016.

[11] Paijens ST, Vledder A, de Bruyn M, Nijman HW. Tumor-infiltrating lymphocytes in the immunotherapy era. Cellular and Molecular Immunology, 2021, 18(4): 842–859.

[12] Xu X, Yi C, Feng T, et al. Regulating tumor microenvironments by a lymph node-targeting adjuvant via tumor-specific CTL-derived IFNγ. Clinical Immunology, 2023, 253: 109685.

[13] Ahmadvand S, Faghih Z, Montazer M, et al. Importance of CD45RO+ tumor-infiltrating lymphocytes in post-operative survival of breast cancer patients. Cellular Oncology, 2019, 42(3): 343–356.

[14] Hong S, et al. B cells are the dominant antigen-presenting cells that activate naive CD4+ T cells upon immunization with a virus-derived nanoparticle antigen. Immunity, 2018, 49: 695–708.e4.

[15] Kroeger DR, Milne K, Nelson BH. Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, cytolytic T-cell responses, and superior prognosis in ovarian cancer. Clinical Cancer Research, 2016, 22: 3005–3015.

[16] Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. Journal of Clinical Oncology, 2010, 28: 105–113.

[17] Loi S, Drubay D, Adams S, et al. Tumor-infiltrating lymphocytes and prognosis: a pooled individual patient analysis of early-stage triple-negative breast cancers. Journal of Clinical Oncology, 2019, 37(7): 559–569.

[18] Geurts VCM, Balduzzi S, Steenbruggen TG, et al. Tumor-infiltrating lymphocytes in patients with stage I triple-negative breast cancer untreated with chemotherapy. JAMA Oncology, 2024, 10(8): 1077–1086.