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Original article

Tumor-infiltrating Lymphocytes in Breast Cancer: Prognostic Significance, Spatial Heterogeneity, And Emerging AI-based Assessment Approaches

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Abstract
Tumor-infiltrating lymphocytes (TILs) represent a crucial component of the tumor microenvironment in breast cancer (BC), playing a significant role in tumor progression, immune surveillance, and therapeutic response. This review provides a comprehensive synthesis of current evidence regarding the prognostic and predictive significance of TILs across breast cancer molecular subtypes, with particular emphasis on luminal breast cancer and recent advances in artificial intelligence (AI)-based assessment approaches. A narrative review with a structured literature search was conducted using major biomedical databases, including PubMed, Scopus, and Web of Science, to identify relevant studies published between 2014 and 2025. Current evidence demonstrates that high stromal TIL levels are strongly associated with improved overall survival, disease-free survival, and pathological complete response in triple-negative breast cancer (TNBC) and HER2-positive breast cancer. In contrast, the prognostic role of TILs in luminal breast cancer remains controversial, largely due to lower baseline immune infiltration, biological heterogeneity, and methodological variability across studies. Novel computational approaches, including deep learning-based TIL assessment systems, may provide more consistent quantification of TIL density and spatial distribution patterns, with potential to improve prognostic stratification. Emerging evidence suggests that not only TIL density but also spatial organization, including aggregated and diffuse immune infiltration patterns, significantly influences clinical outcomes. Compared with conventional histopathological evaluation, AI-driven models have shown potential to improve reproducibility, quantitative precision, and spatial characterization. This review highlights the need for standardized TIL assessment methodologies and supports the integration of digital pathology and AI-based tools into clinical practice to improve prognostic accuracy and facilitate personalized therapeutic decision-making in breast cancer.
Keywords. Breast Cancer, Tumor-infiltrating Lymphocytes, Artificial Intelligence, Tumor Microenvironment.

Introduction

Breast cancer remains one of the most prevalent malignancies worldwide [1]. Increasing evidence demonstrates that the tumor microenvironment (TME), particularly immune components such as TILs, plays a pivotal role in tumor behavior and patient outcomes. TILs consist mainly of CD8+, CD4+, and regulatory T cells, located predominantly in stromal regions adjacent to tumor cells. Their presence reflects host immune response against tumor antigens and influences both tumor suppression and progression [2–4].

Clinical studies have established the strong prognostic significance of TILs in triple-negative breast cancer (TNBC) and HER2-positive breast cancer. However, their role in luminal breast cancer remains controversial because of low baseline infiltration, tumor heterogeneity, and the lack of standardized evaluation methods. Recent advances in artificial intelligence (AI) and digital pathology have enabled objective quantification of TILs and introduced spatial pattern analysis as a novel biomarker [5–9].

Several studies have investigated the prognostic and predictive significance of tumor-infiltrating lymphocytes (TILs) in breast cancer. Early work by Salgado et al. established standardized guidelines for TIL evaluation and highlighted their importance as reproducible biomarkers in breast cancer pathology [10]. Denkert et al. subsequently demonstrated that increased stromal TIL levels were associated with improved survival outcomes and enhanced response to neoadjuvant therapy, particularly in triple-negative breast cancer (TNBC) and HER2-positive subtypes [11].

Loi et al. reported that high TIL infiltration was strongly correlated with a favorable prognosis and improved disease-free survival in TNBC [12,13], supporting the role of anti-tumor immune responses in disease control. Similarly, Adams et al. confirmed the prognostic value of TILs in large randomized clinical trials [14] and suggested their utility as predictive markers of treatment response.

In HER2-positive breast cancer, several studies demonstrated that elevated TIL levels were associated with improved outcomes among patients receiving HER2-targeted therapies, indicating a synergistic interaction between immune activation and therapeutic efficacy. However, findings regarding luminal breast cancer remain inconsistent. While some studies reported favorable prognostic associations, others observed limited or non-significant effects, reflecting the biological heterogeneity and lower immune infiltration characteristic of hormone receptor-positive tumors.

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Recent advances in digital pathology and artificial intelligence (AI) have introduced novel approaches for automated TIL quantification. Studies by Saltz et al., Campanella et al., and Bera et al. demonstrated that deep learning algorithms can accurately identify immune cells, quantify TIL density, and analyze their spatial distribution within the tumor microenvironment [5,6,9]. Furthermore, Cai et al. highlighted the prognostic importance of spatial TIL organization in luminal breast cancer [15], and showed that AI-based assessment may improve prognostic stratification beyond conventional density-based evaluation.

Despite these advances, significant variability remains regarding TIL assessment methodologies, spatial characterization, and subtype-specific interpretation. Therefore, the present review aims to integrate current evidence on the prognostic significance of TILs across breast cancer subtypes while emphasizing spatial heterogeneity and emerging AI-based assessment approaches that may enhance clinical decision-making and personalized oncology.

A structured literature search was conducted to identify relevant peer-reviewed studies investigating the role of TILs in breast cancer. Major biomedical databases, including PubMed, Scopus, and Web of Science, were explored to retrieve studies published between January 2014 and March 2025. Search terms included combinations of keywords related to tumor-infiltrating lymphocytes, breast cancer, immune microenvironment, prognosis, immunotherapy, spatial heterogeneity, digital pathology, and artificial intelligence. Reference lists of key publications were also manually screened to identify additional relevant studies.

Methods

Study Design

This study was conducted as a comprehensive narrative review aimed at critically evaluating current evidence regarding the prognostic and predictive significance of tumor-infiltrating lymphocytes (TILs) in breast cancer, with particular emphasis on molecular subtype variability, spatial heterogeneity, and emerging artificial intelligence (AI)-based assessment approaches. The review was designed to synthesize contemporary evidence from clinical, pathological, and computational studies to provide an integrated understanding of the biological and clinical relevance of TILs in breast cancer progression and therapeutic response.

Study Selection

Relevant articles were selected based on their scientific relevance, methodological rigor, and contribution to understanding the prognostic and predictive significance of TILs in breast cancer. Priority was given to original clinical studies, large cohort studies, high-impact translational research, studies evaluating AI-based TIL assessment, and publications addressing molecular subtype differences and spatial immune heterogeneity. Studies with insufficient methodological clarity, non-peer-reviewed evidence, or limited clinical relevance were deprioritized.

Data Extraction and Evidence Synthesis

Data were narratively synthesized with emphasis on breast cancer molecular subtype, TIL density and composition, the spatial organization of immune infiltrates, histopathological versus AI-based assessment methods, and associations with survival outcomes and treatment response. The findings were critically interpreted to identify consistencies, contradictions, and emerging trends across studies, while considering methodological variability and biological context.

Scope and Analytical Framework

This review emphasizes a multidimensional interpretation of TILs, integrating quantitative density, immune composition, and spatial heterogeneity to better understand their clinical relevance in breast cancer prognosis and therapeutic decision-making.

Results, evidence synthesis, and key findings

Overview of Current Evidence

Accumulating evidence demonstrates that tumor-infiltrating lymphocytes (TILs) constitute a critical component of the breast cancer tumor microenvironment and play a central role in shaping tumor progression, therapeutic responsiveness, and survival outcomes. However, the biological and clinical significance of TILs appears highly context-dependent, varying substantially according to molecular subtype, immune composition, and spatial organization.

Across contemporary studies, TILs emerged not merely as passive indicators of immune presence but as biologically active determinants of anti-tumor immunity. Nevertheless, considerable heterogeneity exists regarding assessment methodologies, cut-off values, and interpretation of immune infiltration patterns, contributing to variability in reported findings. Collectively, recent literature supports a multidimensional model in which TIL density, immune phenotype, and spatial heterogeneity jointly determine prognostic and predictive relevance.

Prognostic Significance of TILs Across Molecular Breast Cancer Subtypes

Triple-Negative Breast Cancer (TNBC)

The strongest evidence supporting the prognostic significance of TILs has consistently been reported in triple-negative breast cancer (TNBC), a subtype characterized by high immunogenicity and elevated mutational burden. Multiple cohort

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studies and pooled analyses have demonstrated that increased stromal TIL levels are associated with improved overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS) [11–14]. Moreover, elevated TIL infiltration has shown a strong correlation with higher rates of pathological complete response (pCR), particularly in patients receiving neoadjuvant chemotherapy [11,12,16]. These findings suggest that TILs may serve not only as prognostic biomarkers but also as predictive indicators of therapeutic responsiveness in TNBC, reflecting a biologically active anti-tumor immune microenvironment.

HER2-Positive Breast Cancer

In HER2-positive breast cancer, moderate-to-high TIL infiltration has similarly demonstrated favorable prognostic implications, particularly among patients receiving HER2-targeted therapies [1,11,17]. Evidence suggests that immune activation may synergistically enhance the efficacy of anti-HER2 agents, thereby improving treatment response and long-term survival outcomes. Studies increasingly indicate that patients with higher TIL levels exhibit greater therapeutic sensitivity, emphasizing the potential clinical utility of immune profiling in treatment stratification. Nevertheless, prognostic associations appear somewhat less consistent than those observed in TNBC, likely due to variability in therapeutic regimens and immune landscape heterogeneity.

Luminal Breast Cancer

Compared with immunologically active subtypes, luminal breast cancers generally demonstrate lower immune infiltration and greater biological complexity, resulting in less consistent findings regarding the prognostic role of TILs. Several studies reported modest survival benefits associated with increased TIL density, whereas others observed weak or statistically insignificant associations [1,15]. This inconsistency likely reflects differences in TIL quantification methods, subtype classification, and immune composition. Emerging evidence suggests that conventional density-based evaluation alone may inadequately characterize immune relevance in luminal tumors, highlighting the importance of spatial and compositional analyses for improved prognostic interpretation.

Spatial Heterogeneity as an Emerging Prognostic Dimension

Beyond quantitative density, recent studies increasingly emphasize the prognostic significance of spatial TIL organization within the tumor microenvironment. Artificial intelligence-driven analyses have identified distinct immune spatial phenotypes, most notably clustered (aggregated) and diffuse (distributed) infiltration patterns [9,15].

Aggregated TIL patterns are frequently associated with more favorable clinical outcomes, potentially reflecting localized immune activation and effective tumor containment. Conversely, diffuse immune infiltration demonstrates variable prognostic implications, possibly representing less coordinated anti-tumor responses. These findings indicate that tumors with comparable TIL density may exhibit substantially different biological behaviors depending on immune spatial organization, suggesting that spatial heterogeneity represents an independent and clinically meaningful prognostic layer [9,15].

Immune Cell Composition and Clinicopathological Associations

The prognostic significance of TILs extends beyond overall abundance to include immune cell composition. Increased CD8+ cytotoxic T-cell infiltration has consistently been associated with improved survival outcomes, reduced tumor burden, and enhanced anti-tumor immune activity [2,4]. Similarly, B-cell populations, particularly CD20+ lymphocytes, have demonstrated supportive immunological roles and associations with improved treatment responsiveness [2]. In contrast, immunosuppressive immune populations, including FOXP3+ regulatory T cells, may contribute to tumor immune evasion and unfavorable prognosis in selected contexts [2,18]. Several studies further reported inverse relationships between TIL density and adverse clinicopathological characteristics, including lymph node involvement and aggressive tumor progression.

Artificial Intelligence-Based Assessment Versus Conventional Evaluation

Conventional histopathological TIL assessment remains widely utilized but is frequently limited by inter-observer variability and restricted reproducibility. Recent advances in digital pathology and artificial intelligence have substantially transformed TIL evaluation through automated quantification, immune cell detection, and spatial pattern analysis [5–9].

Compared with visual scoring approaches, AI-based methods may improve reproducibility, quantitative precision, and spatial characterization while reducing observer-dependent variability; however, performance remains dependent on dataset quality and external validation [5–9]. Some AI-driven studies have reported more reproducible associations between TIL metrics and clinical outcomes, particularly in luminal breast cancer; however, these findings require broader external validation [5,6,9,15].

Integrated Interpretation of Current Evidence

Taken together, current evidence supports a paradigm shift in the interpretation of tumor-infiltrating lymphocytes in breast cancer. Rather than functioning as a single-dimensional biomarker based solely on density, TILs should be interpreted through an integrated framework encompassing immune cell abundance, cellular composition, spatial organization, and

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molecular subtype context. This multidimensional perspective improves prognostic precision and may ultimately facilitate more personalized therapeutic decision-making, particularly with the expanding role of immunotherapy and AI-assisted pathology.

Table 1. TILs Across Breast Cancer Subtypes [1,11–15,17]

Subtype	TIL Level	Prognostic Value
TNBC	High	Strong positive
HER2+	Moderate–High	Positive
Luminal A	Low	Weak/variable
Luminal B	Moderate	Context-dependent

Table 2. TIL Subtypes and Function [2,4,18]

Cell Type	Function
CD8+	Cytotoxic anti-tumor
CD4+	Immune regulation
FOXP3+	Immunosuppression
CD20+	B-cell mediated immunity

Table 3. Conventional and AI-Assisted TIL Assessment [5–9,15]

Feature	Conventional assessment	AI-assisted assessment
Quantification	Semi-quantitative	Automated; validation-dependent
Reproducibility	Observer-dependent	Potentially improved
Spatial analysis	Limited in routine scoring	Can quantify spatial patterns

Discussion

The findings of this review indicate that the clinical meaning of tumor-infiltrating lymphocytes (TILs) cannot be interpreted independently of breast cancer subtype, immune composition, spatial organization, and assessment method. The principal implication is therefore not simply that higher TIL levels are favorable, but that TILs should be evaluated as a multidimensional biomarker within the biological context of each tumor.

The subtype-specific differences summarized in the evidence synthesis likely reflect distinct levels of tumor immunogenicity and treatment-immune interaction. The reproducible value of TILs in TNBC and HER2-positive disease supports their use in risk and response stratification, whereas the variability observed in luminal tumors argues against applying a single density threshold across all molecular subtypes [1,11,15,17].

In luminal breast cancer, inconsistent associations should not be interpreted as evidence that immune infiltration is unimportant. Instead, they suggest that density-only scoring may overlook relevant differences in immune-cell composition and spatial organization. Subtype-specific thresholds and composite immune metrics may therefore provide more clinically meaningful interpretation than a universal TIL score.

Spatial analysis offers a plausible explanation for why tumors with similar TIL densities may have different clinical behavior. Clustered and diffuse immune patterns may represent distinct functional states rather than merely different visual arrangements, so spatial context could refine the biological interpretation of conventional percentage-based scores [9,15]. This issue is particularly relevant in luminal breast cancer, where low overall infiltration can obscure localized immune activity. Combining TIL density with cellular composition and spatial architecture may yield a more informative prognostic model, although standardized definitions and prospective validation are required before spatial metrics can be used routinely.

The principal contribution of artificial intelligence is not simply faster cell counting, but the potential to standardize measurements that are difficult to reproduce visually and to quantify spatial features at whole-slide scale [5–9]. AI should therefore be viewed as a complement to, rather than a replacement for, expert pathological assessment.

Clinical translation nevertheless depends on external validation across institutions, staining protocols, scanners, and patient populations. Transparent reporting, quality control, and demonstration of incremental prognostic value beyond established clinicopathological variables are necessary before AI-derived TIL metrics can support treatment decisions [5,6,9,15].

Clinically, a context-specific TIL framework could improve prognostic stratification and help identify tumors more likely to

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respond to neoadjuvant therapy or immunotherapy. Its value is expected to be greatest when immune measures are interpreted alongside molecular subtype and standard clinicopathological factors. Distinguishing immune-active from immune-poor tumors may also guide rational treatment combinations; however, TIL assessment alone should not be used as a standalone treatment-selection tool. Integration with molecular profiling, genomic signatures, and validated digital pathology models offers a more credible route toward personalized decision-making [16,19,20].

Despite growing evidence supporting the clinical value of TILs, several limitations remain. Considerable methodological heterogeneity persists across studies, including differences in TIL scoring systems, histopathological protocols, immune cell subtype characterization, spatial assessment methodologies, and threshold definitions. Additionally, many studies remain retrospective in nature and vary substantially in sample size and clinical context, limiting comparability. The lack of universally accepted standardized approaches continues to hinder clinical translation and may contribute to inconsistencies, particularly within luminal breast cancer studies.

Future Directions

Future research should prioritize the development of standardized and reproducible TIL assessment frameworks capable of integrating quantitative, compositional, and spatial immune characteristics. Large prospective multicenter studies are required to validate AI-based approaches and determine clinically meaningful thresholds for implementation in routine pathology practice. Furthermore, combining TIL assessment with molecular biomarkers, transcriptomic profiling, and therapeutic response prediction models may facilitate more precise and personalized treatment strategies in breast cancer. In this evolving landscape, TILs are increasingly positioned not merely as prognostic indicators but as integral components of precision oncology and immune-guided therapeutic decision-making.

Conclusion

Tumor-infiltrating lymphocytes (TILs) represent an increasingly important component of the breast cancer tumor microenvironment with substantial prognostic and predictive relevance. Current evidence demonstrates that the clinical significance of TILs is highly dependent on molecular subtype, immune composition, and spatial organization. The prognostic role of TILs appears most robust in triple-negative and HER2-positive breast cancer, whereas findings in luminal subtypes remain heterogeneous and require more refined interpretative frameworks. Emerging evidence suggests that spatial heterogeneity and immune architecture may substantially improve prognostic precision beyond conventional density-based assessment. Artificial intelligence-driven computational pathology has emerged as a promising approach that may improve the reproducibility, standardization, and clinical applicability of TIL evaluation. By enabling quantitative and spatially resolved immune profiling, AI-assisted methods may help address limitations associated with conventional histopathological assessment. Future research should focus on the standardization of TIL assessment methodologies, prospective validation of AI-based tools, and integration of immune biomarkers with molecular and genomic profiling to support precision oncology and personalized treatment strategies in breast cancer.

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Conflicts of Interest

The authors declare no conflicts of interest.

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