



Clinicopathological Correlates of High Stromal Tumor-infiltrating Lymphocytes in Luminal Breast Cancer: A Real-world Study

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ABSTRACT

Objective: The prognostic role of stromal tumor-infiltrating lymphocytes (sTIL) is well recognized in triple-negative and human epidermal growth factor receptor 2 (HER2)-positive breast cancer (BC), but their meaning in hormone receptor-positive, HER2-negative tumors is still debated. In luminal BC, lymphocytic infiltration may reflect biologically aggressive disease rather than an effective antitumor response. We investigated clinicopathological factors associated with high sTIL and examined their relationships with disease-free survival (DFS) in an early-stage luminal BC cohort treated in routine clinical practice.

Material and Methods: This retrospective single-center study included 120 patients with early-stage estrogen receptor-positive, HER2-negative BC who were treated surgically between 2017 and 2021. Hematoxylin and eosin-stained slides were used to evaluate sTIL in accordance with International TILs Working Group recommendations. A 10% cut-off was used to define low-sTIL and high-sTIL categories. The relationship between sTIL category and clinicopathological variables was tested in univariable analyses and then explored using multivariable logistic regression. DFS was analyzed using the Kaplan-Meier method and groups were compared with the log-rank test.

Results: Twenty-three patients (19.2%) were classified as having high sTIL. In univariable analysis, high sTIL status was associated with grade 3 tumors, lymphovascular invasion, and Ki-67 $\geq 20\%$. In the adjusted model, Ki-67 $> 20\%$ remained the only independent factor associated with high sTIL (odds ratio: 8.68; 95% confidence interval: 1.07-70.45; $p=0.043$). During a median follow-up of 56.5 months, DFS did not differ significantly between patients with low and high sTIL ($p=0.37$); however, the high-sTIL group showed a numerically longer DFS.

Conclusion: In this real-world cohort of early-stage luminal BC, high sTIL density was most strongly associated with proliferative activity, but not with a statistically significant DFS benefit. These results suggest that immune infiltration in luminal tumors, when assessed by conventional pathology, may be interpreted primarily as a feature of aggressive tumor biology rather than a consistent marker of effective antitumor immunity.

Keywords: Breast cancer; disease-free survival; Ki-67; stromal tumor-infiltrating lymphocytes; tumor microenvironment

INTRODUCTION

Breast cancer (BC) represents a biologically diverse group of tumors characterized by distinct molecular profiles, clinical behaviors and patient outcomes.¹ Tumor-immune interactions are increasingly recognized as key determinants of disease progression and treatment response. Among immune-related markers, stromal tumor-infiltrating lymphocytes (sTIL) have demonstrated reproducible prognostic and predictive value

in early triple-negative BC (TNBC) and human epidermal growth factor receptor 2 (HER2)-positive disease, where greater lymphocytic infiltration is generally linked to more favorable treatment outcomes.²⁻⁵ Retrospective datasets have also suggested that incorporating sTIL may improve risk stratification in TNBC.⁶

The clinical significance of sTIL in hormone receptor (HR)-positive/HER2-negative BC remains controversial. In this

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subtype, several reports have described a paradoxical association between high sTIL levels and poorer outcomes, challenging the conventional view that immune infiltration consistently indicates a favorable prognosis.⁷ These findings indicate that in luminal BC, sTIL may represent subtype-specific biological activity rather than a direct marker of effective antitumor immune response.

In luminal BC, increased sTIL density has been associated with unfavorable tumor characteristics, including higher grade, greater proliferative activity and activation of oncogenic signaling pathways such as PI3K/AKT.^{8,9} The immune contexture of luminal tumors may also be shaped by immunosuppressive lymphocyte subsets, including regulatory T-cells (Tregs) and Th17 subsets.^{10,11} These findings suggest that immune infiltration in luminal BC may represent a surrogate marker of tumor aggressiveness rather than a protective immune phenotype. In addition, higher sTIL levels and immune-related gene expression have been linked to weaker endocrine therapy response, especially to aromatase inhibitors, suggesting a possible connection between immune activation and endocrine resistance.¹²

Despite growing interest in this topic, much of the existing evidence comes from mixed cohorts, post-hoc analyses, or selected trial populations. Data derived from unselected clinical practice and specifically focused on sTIL correlates in luminal BC remain scarce. For this reason, we aimed to determine which clinicopathological variables were associated with high sTIL levels in a real-world early-stage luminal BC cohort. We also assessed Disease-free survival (DFS) by sTIL status to determine whether routine visual sTIL scoring provides clinically relevant prognostic information in this setting.

MATERIAL AND METHODS

Study Design and Patient Population

This retrospective study was performed at a single center and included 120 patients with early-stage HR-positive/HER2-negative BC who underwent surgery at Ankara University Faculty of Medicine between 2017 and 2021. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ankara University Faculty of Medicine Human Research Ethics Committee (approval number: İ07-464-22, date: 02.09.2022). The requirement for informed consent was waived because of the retrospective design. Patients were eligible if they were at least 18 years old, had histologically confirmed primary invasive breast carcinoma, HR-positive status [estrogen receptor (ER) and/or progesterone receptor (PR) $\geq 1\%$ by immunohistochemistry (IHC)], HER2-negative status (IHC 0/1+ or IHC 2+ with

negative *in situ* hybridization), and available surgical tumor tissue adequate for sTIL evaluation on hematoxylin and eosin (H&E)-stained slides. Exclusion criteria were: sTIL level $<1\%$; receipt of neoadjuvant systemic therapy or radiotherapy; *de novo* metastatic disease; or missing clinicopathological or follow-up information required for classification or outcome analyses.

Evaluation of sTIL levels was performed on H&E-stained slides in accordance with the guidelines of the International TILs Working Group.¹³ TIL evaluation was independently performed by experienced breast pathologists who were not involved in patient management. In accordance with the International TILs Working Group's recommendations, sTIL were analyzed primarily as a continuous variable. For categorical analyses, patients were grouped using a 10% threshold, defining low sTIL as 1-10% and high sTIL as $>10\%$, a commonly used cut-off in luminal BC cohorts to ensure adequate group sizes. Menopausal status was defined clinically; patients were considered postmenopausal if they had ≥ 12 months of amenorrhea not attributable to pregnancy, lactation, or hormonal treatment or if they had undergone bilateral oophorectomy; all others were classified as premenopausal. Luminal subtypes were assigned using clinicopathologic surrogate definitions in HR+/HER2- tumors: luminal A-like tumors were defined as Ki-67 $<14\%$ or Ki-67 14-19% with PR $\geq 20\%$, whereas luminal B-like tumors were defined as Ki-67 $\geq 20\%$ or Ki-67 14-19% with PR $<20\%$.¹⁴ Variables associated with sTIL status in unadjusted analyses were first explored using chi-square or Mann-Whitney U tests, as appropriate. Factors showing significant or borderline associations were subsequently entered into multivariable logistic regression models. Given the limited number of cases with high sTIL, multivariable models were restricted to a small number of covariates to avoid overfitting. Variables reflecting overlapping biological information (e.g., grade and Ki-67) were not included simultaneously. DFS was defined as the time from surgery to disease recurrence, death from any cause, or last follow-up. DFS outcomes were compared between patients with low and high sTIL using the log-rank test.

Statistical Analysis

All statistical analyses were performed using SPSS version 25.0 (IBM Inc., Armonk, NY, USA), and a two-sided p-value of <0.05 was considered statistically significant.

RESULTS

A total of 120 patients diagnosed with early-stage luminal-type BC were included in the study. The median age was 53 years (interquartile range, 45-64), and 52.9% of the patients were postmenopausal. The predominant histological subtype was invasive (ductal) carcinoma of no special type

(76.7%), followed by invasive lobular carcinoma (10%) and other special types (13.3%). At diagnosis, 57.1% of patients presented with stage II disease, while stages I and III accounted for 21.8% and 21.0%, respectively. The majority of tumors were T2 (56.3%), and 47.5% of patients were node-negative. Regarding histologic grade, 5.8% of tumors were grade 1, 52.5% were grade 2, and 41.7% were grade 3. Lymphovascular invasion (LVI) and perineural invasion (PNI) were observed in 38.1% and 13.3% of cases, respectively. All tumors were ER-positive with 94.2% exhibiting ER expression >50% and 66.4% exhibiting PR expression >50%. The entire cohort consisted of HER2-negative tumors (IHC 0-2+). The Ki-67 proliferation index exceeded 20% in 66% of patients (Table 1).

TABLE 1: Baseline clinicopathological characteristics of the study population (n=120).

Variables	n (%)	Variables	n (%)
Age, years (median, IQR)	53 (45-64)	Grade	
Menopausal status		G1	7 (5.8)
Pre-menopausal	56 (47.1)	G2	63 (52.5)
Post-menopausal	63 (52.9)	G3	50 (41.7)
Histological type		LVI	
IC-NST	92 (76.7)	Yes	43 (35.8)
ILC	12 (10.0)	No	70 (58.3)
Others	16 (13.3)	Unknown	7 (5.9)
Stage at diagnosis		PNI	
Stage 1	26 (21.8)	Yes	16 (13.3)
Stage 2	68 (57.1)	No	83 (69.2)
Stage 3	25 (21.0)	Unknown	21 (17.5)
T stage		ER status, %	
T1	41 (34.5)	ER ≤50	7 (5.8)
T2	67 (56.3)	ER >50	113 (94.2)
T3	11 (9.2)	PR status, %	
N stage		PR ≤50	40 (33.6)
N0	56 (46.6)	PR >50	79 (66.4)
N1	37 (30.8)	HER2 status, IHC	
N2	15 (12.5)	HER2 0	56 (46.7)
N3	10 (8.3)	HER2 1-2	64 (53.3)
Unknown	2 (1.6)	Ki-67, %	
		<20	32 (26.7)
		≥20	62 (51.6)
		Unknown	26 (21.7)

Values are median (IQR) or n (%). "Unknown" indicates missing data. Analyses were performed using available cases; evaluable n varied by variable (Ki-67 n=94; PNI n=99; LVI n=113). IQR: Interquartile range; IC-NST: Invasive carcinoma of no special type; ILC: Invasive lobular carcinoma; LVI: Lymphovascular invasion; PNI: Perineural invasion; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry.

When stratified by sTIL density, 97 patients (80.8%) were categorized as having low sTIL and 23 (19.2%) as having high sTIL. High sTIL were significantly associated with higher histologic grade (G3) ($p=0.011$), LVI ($p=0.046$), and elevated Ki-67 index ($\geq 20\%$) ($p=0.010$). Although the difference did not reach statistical significance, sTIL density tended to be higher in younger patients and in tumors with a luminal B-like subtype. No significant associations were found between sTIL levels and menopausal status, tumor stage, nodal status, PNI, HR expression, and HER2 status (all $p>0.05$) (Table 2).

In multivariable logistic regression analysis, high proliferative activity (Ki-67 $\geq 20\%$) was independently associated with high sTIL, with an approximately 8.7-fold increased odds [odds ratio: 8.68; 95% confidence interval (CI): 1.07-70.45; $p=0.043$]. LVI was not independently associated with high sTIL (Table 3).

At a median follow-up of 56.5 months, disease recurrence occurred in 12 of 120 patients (10%). Although the median DFS was not reached, stratification by sTIL status revealed no statistically significant difference between the groups ($p=0.37$). However, a numerically longer DFS was observed in patients with high sTIL levels (Figure 1).

DISCUSSION

In this real-world cohort of early-stage luminal BC, we demonstrate that elevated sTIL are predominantly associated with markers of aggressive tumor biology—most notably high proliferative activity—rather than with a clear survival

TABLE 2: Comparison of clinicopathological characteristics between low and high sTIL groups.

Characteristics	sTIL low, n=97	sTIL high, n=23	p-value
Age, years (median, IQR)	55 (46-64)	47 (41-62)	0.1
Menopausal status (pre; post)	43/53	13/10	0.31
Stage at diagnosis (3; 1-2)	20/76	5/18	0.92
T stage (T1; T2-3)	35/61	6/17	0.34
N stage (positive, negative)	50/45	12/11	0.96
Grade (3; ≤1-2)	35/62	15/8	0.01
LVI (positive, negative)	31/61	12/9	0.04
PNI (positive, negative)	13/67	3/16	0.96
ER status (≤50; >50)	4/93	3/20	0.10
PR status (≤50; >50)	31/66	9/13	0.42
HER2 status (IHC 0; IHC+1-2)	46/51	10/13	0.73
Ki-67 (<20; ≥20)	31/47	1/15	0.01
Luminal A/B like subtype	20/57	1/15	0.08

Analyses were performed using available cases; evaluable n varied by variable. sTIL: Stromal tumor-infiltrating lymphocytes; IQR: Interquartile range; LVI: Lymphovascular invasion; PNI: Perineural invasion; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry.

TABLE 3: Multivariable analysis of clinicopathological determinants of high sTIL.			
Variable	OR	95% CI	p-value
Ki-67 ≥20%	8.68	1.07-70.45	0.043
LVI (yes)	1.96	0.61-6.31	0.260

OR: Odds ratio; CI: Confidence interval; sTIL: Stromal tumor-infiltrating lymphocytes; LVI: Lymphovascular invasion.

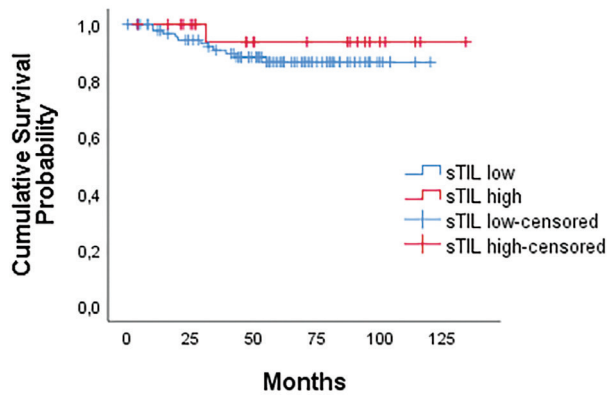


FIGURE 1: Kaplan-Meier curves for DFS according to sTIL status (low vs. high). Kaplan-Meier curves showing DFS according to sTIL status (low: 0-10% vs. high: >10%). Tick marks indicate censored observations.

DFS: Disease-free survival; sTIL: Stromal tumor-infiltrating lymphocytes

advantage. Among all clinicopathological variables examined, Ki-67 ≥20% emerged as the sole independent determinant of high sTIL density, whereas classical adverse features such as LVI lost significance in multivariable analysis.

Luminal BC is generally characterized by low baseline immune infiltration, a finding consistently reported across large pooled analyses and systematic reviews. Stanton et al.¹⁵ demonstrated that HR+/HER2-tumors exhibit the lowest incidence of lymphocyte-predominant BC among all molecular subtypes, underscoring the intrinsically “immune-desert” nature of luminal disease. Our observation that only 19.2% of patients displayed high sTIL levels is, therefore, consistent with the existing literature and supports the biological plausibility of our cohort. Although this proportion may appear relatively high compared with previous reports, patients without sTIL were not included in our cohort. The overall low prevalence of elevated sTIL in luminal disease is likely related to ER-mediated immune modulation, as ERα signaling has been shown to impair CD8⁺ T-cell-mediated cytotoxicity and reduce interferon-γ-dependent antigen presentation, thereby facilitating immune escape.^{16,17} Higher sTIL levels observed in highly proliferative tumors may reflect an increased antigenic burden and a relative reduction in

ER-mediated immunosuppression, potentially resulting in a more immune-infiltrated tumor microenvironment.¹⁸

While high sTIL levels correlated with adverse pathological features, no statistically significant difference in DFS was observed between groups. In line with this finding, Criscitiello et al.⁸ reported no overall association between TIL levels and distant DFS in luminal BC; however, subgroup analyses suggested a favorable impact of high TIL levels among patients with Ki-67 ≥20%. In contrast, Fujimoto et al.¹⁸ identified higher TIL levels as a marker of worse DFS in luminal BC with low Ki-67 expression. Similarly, in our cohort, elevated sTIL levels were observed almost exclusively among patients with high Ki-67 expression and were associated with a numerical improvement in DFS. These data support the notion that the prognostic relevance of TILs is influenced by tumor biological features—especially proliferative activity—and appears to be most evident in ER+/HER2-negative BC.¹⁹

Recent artificial intelligence-based studies have suggested that more sophisticated computational approaches, particularly those incorporating the spatial distribution of TILs, may enhance the prognostic value of immune infiltration in luminal BC. By moving beyond simple quantification and capturing aggregated versus distributed immune patterns, these artificial intelligence-driven analyses have reported favorable survival associations in selected luminal subgroups, implying that spatial immune architecture may reflect biologically meaningful tumor–immune interactions that are not fully appreciated by conventional visual assessment.²⁰ These findings have generated significant interest, especially given the longstanding uncertainty surrounding the prognostic role of TILs in HR+ disease.

Study Limitations

Several limitations merit consideration. The retrospective, single-center design and the limited number of survival events restricted the statistical power of survival analyses and precluded more complex multivariable modeling for DFS. Moreover, the high-sTIL group comprised only 23 patients, which likely contributed to imprecision in the effect estimates; accordingly, the association observed for Ki-67 was accompanied by a wide CI, reflecting the limited sample size and number of events. In addition, the lack of assessment by a single dedicated pathologist and the lack of analyses distinguishing between effector and suppressive immune cell subsets represent major limitations of the present study. Finally, the median follow-up of 56 months may be relatively short for HR-positive BC, in which late recurrences are common, and may therefore underestimate long-term DFS differences. Nevertheless, the consistency of our findings

with those from large real-world cohorts and pooled analyses strengthens the validity of our conclusions.

CONCLUSION

The present study was designed to reflect real-world clinical practice. Strictly according to the International TILs Working Group recommendations, sTIL were assessed using standard visual evaluation without computational or spatial augmentation. Our results demonstrate that high sTIL density in early-stage luminal BC predominantly mirrors increased tumor proliferative activity—most notably high Ki-67 expression—rather than conferring an independent survival advantage. This observation suggests that, when evaluated using conventional pathology-based methods, immune infiltration in luminal tumors may function more as a surrogate marker of aggressive tumor biology than as an indicator of effective antitumor immunity.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ankara University Faculty of Medicine Human Research Ethics Committee (approval number: İ07-464-22, date: 02.09.2022).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.S., Concept: B.D., H.A.Y., Design: B.D., H.A.Y., Data Collection or Processing: B.D., E.K., S.B., I.A., Analysis or Interpretation: B.D., E.K., S.B., I.A., S.S., Literature Search: B.D., Writing: B.D., S.S., H.A.Y.

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