

Correlation of Tumor-Infiltrating Lymphocytes with Histological Grade and Stage in Breast Carcinoma

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Abstract

Background: Tumor-infiltrating lymphocytes (TILs) are a reflection of host antitumor immunity and are now being identified as a prognostic and predictive biomarker in certain breast cancer subtypes. They are still useful in routine pathological practice in relation to conventional histopathological variables.

Objective: To measure the number of stromal TILs in invasive breast carcinoma and to assess their relationship to histological grade, pathological stage, nodal status, and receptor profile and proliferation activity.

Methods: A cross sectional histopathological study was conducted on 168 primary invasive breast carcinomas that were resected without systemic treatment. Hematoxylin-eosin sections were used to score stromal TILs following the principles of the International TILs Working Group and classify them as low (less than 10%), intermediate (10-49%) or high (50 or more). Histological type, Nottingham grade, tumor size, nodal status, pathologic stage, lymphovascular invasion, ER, PR, HER2, and Ki-67 were recorded.

Results: Low, intermediate, and high TIL levels were observed in 74 (44.0%), 71 (42.3%), and 23 (13.7%) tumors, respectively. The median stromal TILs were higher at Nottingham grades: 7% at grade 1, 16% at grade 2 and 35% at grade 3 ($p < 0.001$). The incidence of high TILs was 26.4% in grade 3 tumors, and 2.9% in grade 1 tumors. TIL level showed a moderate positive correlation with grade (Spearman $r = 0.46$, $p < 0.001$) and Ki-67 ($r = 0.41$, $p < 0.001$). Triple-negative (38%) and HER2-positive (30%) carcinomas had higher median TILs compared to hormone receptor-positive/HER2-negative (12%) carcinomas ($p < 0.001$). There was only a weak correlation between TILs and pathologic stage ($r = 0.17$, $p = 0.031$) and no independent association between stage and high TILs after adjusting for grade and molecular phenotype.

Conclusion: Stromal TILs are strongly correlated with higher histological grade, high proliferation and aggressive immunophenotypic subtypes, and less so with anatomic stage. Biologically relevant information can be added to the standard breast carcinoma report with routine standardized TIL assessment.

Keywords: Breast Carcinoma; Tumor-Infiltrating Lymphocytes; Histological Grade; Pathologic Stage; Triple-Negative Breast Cancer; HER2.

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Introduction

Breast carcinoma is a highly heterogeneous disease and prognosis and treatment are influenced by tumor size, nodal status, histological grade, hormone receptor expression, HER2 status, proliferative activity and more recently by the immune microenvironment. Tumor-infiltrating lymphocytes (TILs) can be used to give a morphologic estimate of the host immune response to neoplastic cells and are not an additional laboratory expense. The International TILs Working Group set the recommendations for the

scoring of stromal TILs in the invasive tumour border, which offers a practical framework for reproducible assessment [1]. TILs are especially clinically significant in triple-negative breast cancer and in HER2-positive breast cancer. In TNBC, higher stromal TILs have been correlated with better prognosis and with higher likelihood of response to systemic therapy in various treatment settings. A pooled individual-patient analysis of early TNBCs showed that higher stromal TILs are an independent prognostic factor in addition to the

clinicopathological factors [2]. These observations have sparked interest in using immune morphology in research studies and some clinical decision making processes. Breast cancer does not have a uniform distribution of TILs. High histological grade, hormone receptor negativity, HER2 amplification, high Ki-67, and triple-negative phenotype are associated with more prominent lymphocytic infiltration. This seemingly paradoxical association of aggressive morphology and strong immune infiltration is related to the relationship between tumor immunogenicity and host response. Higher TILs were prognostic in triple-negative disease and also were associated with trastuzumab benefit in HER2-positive cancer in the FinHER trial [3].

While there are many prognostic studies, a more common question in routine pathology laboratories is how is the density of TILs correlated with the conventional histological grade and anatomic stage that are routinely reported in breast carcinoma? The present study examined the stromal TILs in a consecutive series of untreated invasive breast carcinomas and investigated their association with Nottingham grade, pathologic stage, nodal status, receptor phenotype and proliferative activity.

Materials and Methods

The present study is a cross-sectional histopathological analysis of 168 consecutive primary invasive breast carcinomas that were resected during a specific study period by either breast-conserving surgery or mastectomy. Cases were included if sufficient surgical tissue for pretreatment was available and complete clinicopathological information was available. Patients who had received neoadjuvant chemotherapy, endocrine therapy, anti-HER2 therapy or immunotherapy prior to surgery were excluded as these treatments can significantly affect the infiltration of lymphocytes. In addition, cases in which the tissue artifact was extensive and there was not enough invasive tumor to assess TILs were excluded.

Formalin fixed paraffin embedded blocks were retrieved and representative hematoxylin-eosin slides were independently reviewed by two pathologists. The histological type was determined by the current World Health Organization classification, with invasive carcinoma of no special type being the most common type. Histological grade was determined by Nottingham modification of Scarff-Bloom-Richardson system according to tubule formation, nuclear pleomorphism and mitotic activity. The size of the tumour, presence of lymphovascular invasion and axillary nodal status were retrieved from the pathological report. Pathologic stage was classified

as stage I, II or III based on the relevant TNM system.

The principles recommended by the International TILs Working Group were used to evaluate Stromal TILs. The proportion of intratumoral stromal area that was filled with mononuclear inflammatory cells was estimated throughout the invasive tumor, excluding areas outside the tumor border, necrosis, crush artifact, and inflammation around in situ carcinoma or biopsy-related change. The percentage of TILs was recorded continuously and also classified as low (<10%), intermediate (10-49%) or high (≥50%) for clinicopathological comparisons. When there was a difference of more than 10 percentage points between assessments, they were discussed together to achieve consensus.

Estrogen receptor (ER) and progesterone receptor (PR) were deemed positive by laboratory adopted guideline thresholds. HER2 was assessed by immunohistochemistry with reflex in situ hybridization (ISH) if necessary. Ki-67 was reported as the percentage of positive stained tumour nuclei and was divided into low and high proliferation groups based on the institutional cut-off value. Tumors were simply classified as hormone receptor positive/HER2 negative, HER2 positive, or triple negative for comparison.

The distribution of TILs was right skewed and continuous TIL percentages were compared by Mann-Whitney U or Kruskal-Wallis tests by grade, stage, and molecular phenotype. Chi-square tests were used to evaluate the associations between categorical TIL groups and clinicopathological variables. Spearman correlation was used to assess the correlation between TIL percentage and ordinal grade, stage and Ki-67. Multivariable logistic regression was used to determine factors independently associated with high TILs (TILs ≥ 50%), which included age, grade, stage, nodal status, lymphovascular invasion, and molecular phenotype. Odds ratios with 95% confidence intervals are presented. The p value of < 0.05 was considered statistically significant.

Results

The study included 168 invasive breast carcinomas from women with a mean age of 53.6 ± 11.8 years. Invasive carcinoma of no special type accounted for 148 cases (88.1%), while the remainder included lobular and other special types. Nottingham grade distribution was 34 grade 1 (20.2%), 62 grade 2 (36.9%), and 72 grade 3 (42.9%). Pathologic stage was stage I in 39 cases (23.2%), stage II in 92 (54.8%), and stage III in 37 (22.0%). Low, intermediate, and high stromal TIL categories were observed in 74 (44.0%), 71 (42.3%), and 23 (13.7%) cases, respectively.

Clinicopathological characteristics by TIL category are shown in Table 1.

Table 1: Clinicopathological characteristics by stromal TIL category

Characteristic	Low <10% (n=74)	Intermediate 10-49% (n=71)	High ≥50% (n=23)	p-value
Mean age, years	55.4 ± 11.7	52.6 ± 11.5	50.8 ± 12.0	0.118
Grade 1	24 (32.4%)	9 (12.7%)	1 (4.3%)	<0.001
Grade 2	34 (45.9%)	25 (35.2%)	3 (13.0%)	<0.001
Grade 3	16 (21.6%)	37 (52.1%)	19 (82.6%)	<0.001
Node positive	34 (45.9%)	38 (53.5%)	14 (60.9%)	0.382
Lymphovascular invasion	22 (29.7%)	27 (38.0%)	12 (52.2%)	0.104
HR+/HER2–	58 (78.4%)	38 (53.5%)	5 (21.7%)	<0.001
HER2 positive	9 (12.2%)	15 (21.1%)	7 (30.4%)	0.071
Triple negative	7 (9.5%)	18 (25.4%)	11 (47.8%)	<0.001
High Ki-67	25 (33.8%)	40 (56.3%)	19 (82.6%)	<0.001

TIL density increased substantially with histological grade (Table 2). Median stromal TILs were 7% (IQR 4-12) in grade 1, 16% (IQR 8-28) in grade 2, and 35% (IQR 15-55) in grade 3 tumors ($p<0.001$). High TILs ($\geq 50\%$) occurred in only one grade 1 carcinoma, three grade 2 carcinomas, and 19 grade 3 carcinomas. Spearman analysis demonstrated a moderate positive correlation

between continuous TIL percentage and grade ($r=0.46$, $p<0.001$). TILs also correlated with Ki-67 ($r=0.41$, $p<0.001$).

Triple-negative tumors had the highest median TIL level (38%), followed by HER2-positive tumors (30%) and hormone receptor-positive/HER2-negative tumors (12%; $p<0.001$).

Table 2: Stromal TIL percentage according to histological grade and stage

Group	n	Median TIL %, IQR	High TIL ≥50%	p-value
Grade 1	34	7 (4-12)	1 (2.9%)	<0.001
Grade 2	62	16 (8-28)	3 (4.8%)	
Grade 3	72	35 (15-55)	19 (26.4%)	
Stage I	39	15 (6-26)	3 (7.7%)	0.046
Stage II	92	20 (8-38)	13 (14.1%)	
Stage III	37	24 (10-45)	7 (18.9%)	

The relationship between TILs and anatomic stage was weaker. Median TILs were 15% in stage I, 20% in stage II, and 24% in stage III disease ($p=0.046$), corresponding to a weak positive ordinal correlation ($r=0.17$, $p=0.031$).

High TIL frequency increased from 7.7% in stage I to 14.1% in stage II and 18.9% in stage III, but this pattern was partly explained by the higher

prevalence of grade 3 and receptor-negative tumors in advanced stages.

On multivariable analysis (Table 3), grade 3 histology, triple-negative phenotype, HER2-positive phenotype, and high Ki-67 remained independently associated with high TILs. Pathologic stage and nodal positivity did not remain significant after adjustment.

Table 3: Multivariable factors associated with high stromal TILs ($\geq 50\%$)

Factor	Adjusted OR	95% CI	p-value
Grade 3 vs grade 1/2	4.18	1.54-11.36	0.005
Stage III vs I/II	1.32	0.46-3.80	0.607
Node positive	1.21	0.46-3.18	0.700
Lymphovascular invasion	1.43	0.54-3.81	0.472
HER2 positive vs HR+/HER2–	3.12	1.01-9.67	0.048
Triple negative vs HR+/HER2–	5.46	1.88-15.88	0.002
High Ki-67	2.94	1.05-8.23	0.040

Discussion

The results of this study clearly show that there is a relationship between stromal TIL density and histological grade in untreated invasive breast carcinoma. The median TIL percentage increased with increasing grade of tumour and over four-

fifths of high-TIL cancers were grade 3. High Ki-67, triple-negative phenotype and HER2-positive disease were also highly correlated with TILs. The association with anatomic stage was weak and was not observed in multivariable analysis, indicating

that immune infiltration is more closely associated with tumor biology than disease extent.

The clinicopathological correlation of stromal and intratumoral TILs with tumor grade, molecular subtype, nodal status and mitotic activity were also reported by Pujani et al. with significant correlation [4]. Both datasets showed a strong grade-TIL association, which is biologically plausible as high-grade carcinomas are likely to have higher genomic instability and neoantigen burden, which may facilitate the recruitment of immune cells into the tumor microenvironment. Concurrently, the fact that TIL values are similar across clinicopathological categories highlights that morphology is not sufficient to fully define antitumor immunity.

TILs have been shown to be prognostic in large clinical trial cohorts of triple-negative breast cancer. Adams et al. showed in ECOG 2197 and ECOG 1199 that for every 10% increase in stromal TILs, there was an improvement in disease-free and overall survival after adjusting for conventional prognostic factors [5]. This study was not conducted as a survival study, but the close relationship between TILs and triple-negative phenotype is similar to the tumor biology of those trials. Importantly, high TILs are not necessarily a marker of aggressive disease, but may reflect a more active antitumor host response and treatment sensitivity in immune-responsive subtypes.

The association between immune infiltration and biologic responsiveness is also supported by neoadjuvant studies. Denkert et al. demonstrated that tumor-associated lymphocytes were an independent predictor of pathologic complete response to anthracycline/taxane chemotherapy [6]. This discovery may account for the dual nature of high grade and good response to treatment in TIL-rich tumors. The present correlation with Ki-67 and grade is related to the proliferative aggressiveness, and the prognostic significance of TILs is dependent on the subtype, the treatment setting, and the endpoint of the prognosis.

Large retrospective cohorts have also been shown to be clinically valid. Increased TILs independently associated with improved disease-free and overall survival in triple-negative breast cancer, suggesting analytical validity of standardized TIL scoring, as reported by Pruneri et al. [7]. In the same way, Pruneri et al. who analysed the IBCSG 22-00 trial, reported that TILs were a powerful prognostic factor, with higher TIL levels correlating with lower risk of recurrence and death [8]. The observations confirm the importance of reporting TILs as a continuous variable in research, even if categorical thresholds are used for descriptive purposes.

The International Immuno-Oncology Biomarker Working Group then extended the recommendations to post-neoadjuvant residual disease and carcinoma in situ [9]. These updates emphasize the transition of TIL assessment from a research tool to a morphologic biomarker that is widely harmonized. However, these percentages (10%, 50% or the term lymphocyte-predominant breast cancer) should be understood in context. Continuous percentage scoring retains more information and prevents artificial separation of tumors around a cut-off.

It is important to note that there was a poor correlation between TILs and stage in the current study. Stage is a mostly anatomic term that indicates the size of the tumor and the presence of regional or distant spread, while TILs measure the immune response locally. Depending on the growth rate, the time of detection, the dissemination of the tumor in the lymphatics and the availability of treatment, the tumor can be highly infiltrated and in an early or late stage. The distribution of TILs has been studied and has been found to be correlated with grade, receptor status, and proliferative features, which suggests that immune infiltration is a complementary, not redundant, parameter.

The enrichment of high TILs in triple-negative and HER2-positive cancers is consistent with other clinical studies that have shown that immune infiltration is most relevant in these biologically aggressive cancers [10]. In our cohort, the level of TILs was lower in hormone receptor-positive/HER2-negative tumors. This is significant since the prognostic significance and magnitude of TILs is not the same for all breast cancer phenotypes, and therefore, routine interpretation should be combined with receptor status, not as a standalone marker.

More general evidence also suggests that the interpretation of TILs should be done in the context of the tumor type and treatment. Pruneri et al. reviewed the biologic and clinical significance of TILs in early breast cancer and emphasized their most proven role in triple-negative and HER2-positive breast cancer [11]. Denkert et al. showed that the level of TILs correlated with treatment response and had subtype-dependent prognostic significance in a pooled analysis of 3,771 patients who received neoadjuvant therapy [12]. Mao et al. found that TILs were prognostic in all breast cancer studies via meta-analysis and that there was heterogeneity in the prognostic value of TILs based on their location and molecular subtype [13].

In a systematic review and meta-analysis, Yu et al. also found prognostic and predictive associations, which also argues for the inclusion of immune infiltration with existing clinicopathological variables [14]. Heppner et al. defined TILs as a

promising, easily accessible biomarker and stressed the need for a standardised scoring system for reproducible clinical research [15]. These studies collectively support the concept that high TIL density is a reflection of active tumor-host immune interaction and not just a reflection of anatomic stage. There are some limitations in this study. It was cross-sectional and lacked survival and treatment response endpoints. The number of high TIL cases was small, which restricted precision of the subgroups. The results were only from pretreatment surgical specimens and cannot be directly applied to post-neoadjuvant residual tumors. The interobserver variability was minimized by consensus review, but cannot be eliminated in semiquantitative scoring. The molecular classification was made using the traditional surrogate immunohistochemical categories, not gene-expression profiling. Nevertheless, the study offers a practical clinicopathological proof that the standardized evaluation of stromal TILs is a good surrogate for grade and biologic subtype and has only a modest independent association with anatomic stage.

Conclusion

There was a strong positive correlation between stromal TIL density and histological grade, proliferative activity, triple-negative and HER2-positive status in invasive breast carcinoma. The association with pathologic stage was relatively weak and did not remain independent of tumor biology in the multivariable analysis. These results provide evidence for the use of standardized TIL assessment as a low-cost morphologic biomarker that can be used in addition to grade, stage and receptor profiling. Future clinical trials that include treatment response and survival would be useful to determine its clinical value in different breast cancer subtypes.

References

- Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol.* 2015;26(2):259-271. DOI: 10.1093/annonc/mdl450. PMID: 25214542.
- 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. *J Clin Oncol.* 2019;37(7):559-569. DOI: 10.1200/JCO.18.01010. PMID: 30650045.
- Loi S, Michiels S, Salgado R, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. *Ann Oncol.* 2014;25(8):1544-1550. DOI: 10.1093/annonc/mdl112. PMID: 24608200.
- Pujani M, Jain H, Chauhan V, Agarwal C, Singh K, Singh M. Evaluation of tumor infiltrating lymphocytes in breast carcinoma and their correlation with molecular subtypes, tumor grade and stage. *Breast Dis.* 2020;39(2):61-69. DOI: 10.3233/BD-200442. PMID: 32310155.
- Adams S, Gray RJ, Demaria S, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. *J Clin Oncol.* 2014;32(27):2959-2966. DOI: 10.1200/JCO.2013.55.0491. PMID: 25071121.
- Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol.* 2010;28(1):105-113. DOI: 10.1200/JCO.2009.23.7370. PMID: 19917869.
- Pruneri G, Vingiani A, Bagnardi V, et al. Clinical validity of tumor-infiltrating lymphocytes analysis in patients with triple-negative breast cancer. *Ann Oncol.* 2016;27(2):249-256. DOI: 10.1093/annonc/mdv571. PMID: 26598540.
- Pruneri G, Gray KP, Vingiani A, et al. Tumor-infiltrating lymphocytes are a powerful prognostic marker in patients with triple-negative breast cancer enrolled in the IBCSG phase III randomized clinical trial 22-00. *Breast Cancer Res Treat.* 2016;158(2):323-331. DOI: 10.1007/s10549-016-3863-3. PMID: 27372069.
- Dieci MV, Radošević-Robin N, Fineberg S, et al. Update on tumor-infiltrating lymphocytes in breast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma in situ. *Semin Cancer Biol.* 2018;52(Pt 2):16-25. DOI: 10.1016/j.semcancer.2017.10.003. PMID: 29024776.
- De Melo Gagliato D, Cortes J, Curigliano G, et al. Tumor-infiltrating lymphocytes in breast cancer and implications for clinical practice. *Biochim Biophys Acta Rev Cancer.* 2017;1868(2):527-537. DOI: 10.1016/j.bbcan.2017.10.003. PMID: 29061314.
- Pruneri G, Vingiani A, Denkert C. Tumor infiltrating lymphocytes in early breast cancer. *Breast.* 2018;37:207-214. DOI: 10.1016/j.breast.2017.03.010. PMID: 28363679.
- Denkert C, von Minckwitz G, Darb-Esfahani S, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet*

- Oncol. 2018;19(1):40-50. DOI: 10.1016/S1470-2045(17)30904-X. PMID: 29233559.
13. Mao Y, Qu Q, Chen X, Huang O, Wu J, Shen K. The prognostic value of tumor-infiltrating lymphocytes in breast cancer: a systematic review and meta-analysis. PLoS One. 2016;11(4):e0152500. DOI: 10.1371/journal.pone.0152500. PMID: 27073890.
 14. Yu X, Zhang Z, Wang Z, Wu P, Qiu F, Huang J. Prognostic and predictive value of tumor-infiltrating lymphocytes in breast cancer: a systematic review and meta-analysis. Clin Transl Oncol. 2016;18(5):497-506. DOI: 10.1007/s12094-015-1391-y. PMID: 26459255.
 15. Ingold Heppner B, Loibl S, Denkert C. Tumor-infiltrating lymphocytes: a promising biomarker in breast cancer. Breast Care (Basel). 2016;11(2):96-100. DOI: 10.1159/000444357. PMID: 27239170.