

Evaluating the prognostic significance of tumor-infiltrating lymphocytes in solid tumor: practice of a standardized method from the International Immuno-Oncology Biomarkers Working Group

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Purpose: Tumor-infiltrating lymphocytes (TILs) become increasingly relevant to tumor progression. This study aims to evaluate (a) methods of TILs assessment and (b) their prognostic significance in gastric cancer (GC).

Methods: The percentage of stromal TILs (psTIL) was reported semi-quantitatively by H&E evaluation. Herein, we screened two independent cohorts of breast cancer (n=240) and GC (n=481) for psTIL characterization. Correlations between psTIL and clinic-pathological features, as well as overall survival (OS) were further explored. Additionally, the prediction role of psTIL in GC was evaluated by receiver operating characteristic curve (ROC) analysis.

Results: TILs could be demonstrably distinguished from other stromal areas and surrounding tumor nests according to the assessment method. More importantly, it is reproducible, easily to determine, and quickly performed. In GC, a two-grade scale for psTIL was appropriate to be divided into low and high subgroups by using the median value of 10% as the threshold. High psTIL was correlated with no serosa invasion, earlier TNM stage and better survival state ($P < 0.05$ for all), and identified as a favorable prognostic factor both by univariate (HR: 0.734, $P = 0.047$) and multivariate analyses (HR: 0.722, $P = 0.030$). A beneficial OS of high psTIL was found in a linear manner with increasing TILs infiltrates associated with improved survival by Kaplan–Meier survival curve ($P = 0.030$) and ROC analysis (AUC: 0.432, $P = 0.012$).

Conclusion: TILs provide a reproducible method for assessment that can potentially be used to guide management. The parameter psTIL could be served as an independent, favorable prognostic factor of GC.

Keywords: tumor-infiltrating lymphocytes, gastric cancer, prognosis, biomarker

Introduction

Gastric cancer (GC) is the fifth most common human malignant disease and the fourth leading cause of cancer-related death worldwide.¹ The survival of GC patients remains poor even after the surgery-centered multidisciplinary treatments. Large variations in clinical outcomes have been reported in patients with the same stage and similar treatment regimens.^{2,3} One of the key causes is that GC is a heterogeneous disease in its biologic behaviors, but the present GC staging system provides incomplete prognostic information; thus, additional information is necessary to improve tailored treatment for the individual patient.^{4,5} Notably, pathologists have long recognized the

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stromal, immune infiltration, nerves, and vasculature as integral parts of the tumor microenvironment, which often provide important information regarding tumor behavior, prognosis, and response to treatment.⁶

Tumor-infiltrating lymphocytes (TILs), represented by T cells, B cells, and NK cells, are the major type of infiltrating immune cells. TILs are considered a manifestation of the host immune response against tumor cells, and several studies have already reported the potential of TILs as a prognostic parameter for various human malignancies, such as colorectal cancer,⁷ non-small-cell lung cancer,⁸ oropharyngeal cancer,⁹ and breast cancer (BC).¹⁰ Taken together, current data suggest that stromal TILs may be an important biomarker to predict the survival and select patients with the highest likelihood of responding to therapy.¹¹ For TIL evaluation to be incorporated into research (ie, stratification for clinical trials) or routine clinical care, the approach to quantifying TIL within the stromal and the tumor needs to be standardized. According to the International TILs Working Group, a detailed description of how to evaluate stromal TILs instead of total TILs and intratumoral TILs has been recommended as a potential consensus.^{12,13}

Although such methods have also been recommended for other solid cancer,¹⁴ only a few studies have investigated the prognostic impact of TILs in GC.¹⁵ It is well acknowledged that good biomarkers in cancer must be validated refers to the robustness of the test, in terms of accuracy, reliability, and interobserver reproducibility.¹⁶ Herein, the recommended method has been validated in 240 BC tissues, and then percentage of stromal TILs (psTIL) in 481 GC tumors was evaluated according to the recommended method. This study was aimed to evaluate the feasibility and reproducibility of this method, and the possibility for TIL to be considered as a promising factor in terms of GC prognosis for clinical use.

Materials and methods

Patients and follow-up

There were 240 BC and 481 GC cases included in this study, all of which have received radical operation at the Department of Oncology, Zhongnan Hospital of Wuhan University (Wuhan, China) from December 2002 to February 2011. Major clinic-pathological characteristics including age, gender, tumor location, pathological types, lymph node metastasis, invasion depth, and distant metastasis were collected. In addition, the information of follow-

up was available. TNM stages were determined according to the UICC/AJCC 7th TNM staging system. The primary end point for this study was overall survival (OS), which was defined as the interval from the date of surgery to cancer-related death. Additionally, the 3-year disease-free survival (DFS) and 5-year DFS were also assessed in the BC analysis.

Ethics statement

All patients provided written informed consent for their information to be stored in the hospital database, and we obtained separate consent for use of research. Study approval was obtained from independent ethics committees from Zhongnan Hospital of Wuhan University. The study was undertaken in accordance with the ethical standards of the World Medical Association Declaration of Helsinki.

H&E staining and image acquisition

Serial tissue sections (4- μ m thickness) of BC and GC were prepared for H&E staining before proceeding to the following imaging studies (Figure 1A). Routine H&E staining was performed as previously published.¹⁷ The digital images of H&E staining were captured under Olympus BX51 fluorescence microscope equipped with Olympus DP72 camera (Olympus Optical Co., Ltd., Tokyo, Japan), at 100 \times or 200 \times magnification (Figure 1B). Identical settings were used to avoid selection bias.

Morphological evaluation and quantification of stromal TILs

A standardized method for stromal TILs morphological evaluation was conducted according to the International Immuno-Oncology Biomarkers Working Group. The exclusion criteria were as follows: TILs outside of the borders of tumor invasive front, TILs in tumor zones with crush artifacts, necrosis, and regressive hyalinization (Figure 1C). In the selected areas, stromal TILs were considered no co-evolution with cancer if not containing any tumor cells. Therefore, tumor cells should be present at all borders of the image field. To minimize selection bias, one section with at least 4 standard-compliant vision fields per patient was randomly selected and considered as adequate, with no focus on hotspots.

Then, the percentage of TILs (psTIL) were obtained from selected images. The clinical information was unknown by two independent pathologists in advance.

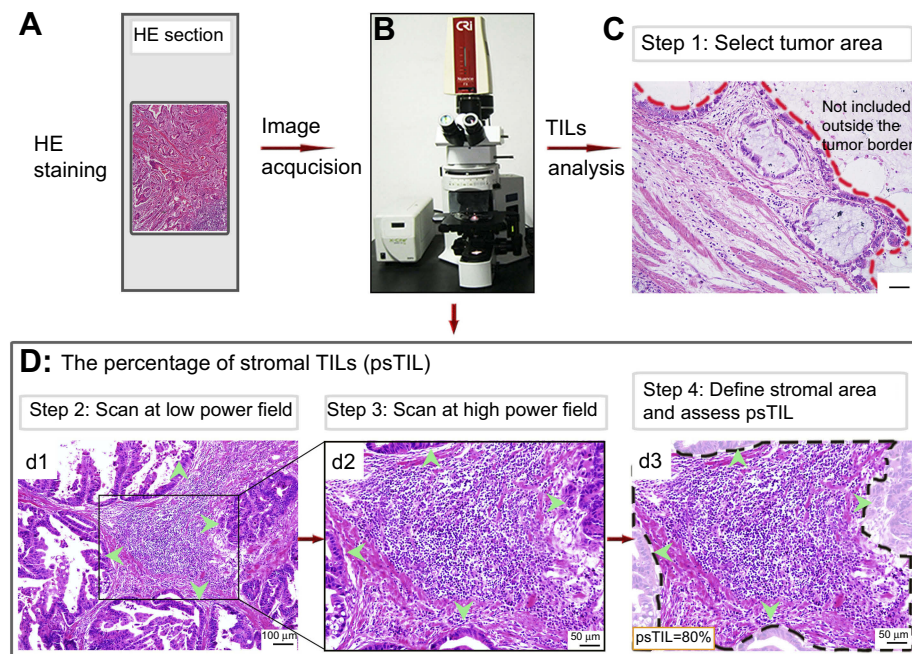


Figure 1 The flowchart of study design and stromal TILs evaluation.

Notes: (A) Routine H&E staining method was adopted to assess TILs. (B) An Olympus BX51 fluorescence microscope was used for image acquisition at $\times 100$ and $\times 200$ magnification, respectively. Then, TILs were visually estimated on the morphological characteristics following a standardized methodology. (C) TILs should be assessed within the tumor borders of the invasive tumor. (D) Stromal TILs were defined and assessed according to steps shown from d1 ($\times 100$) to d2 and d3 ($\times 200$). In the image field, tumor cells should be present at all borders, which was indicated by green arrowheads. On this basis, different TILs areas should be evaluated at higher magnification ($\times 200$ in this study).

Abbreviation: TILs, tumor-infiltrating lymphocytes.

The parameter psTIL was reported for the stromal compartment and calculated by the area occupied by stromal TILs over the total intratumoral stromal area, which was round up to the nearest 5–10% in this practice for as much detailed information. A magnification of $\times 100$ or $\times 200$ was optional if psTIL value was 0–10%, and 50–100%, while a higher magnification of $\times 200$ was optimized if psTIL value was located in the intermediate group (Figure 1D). Then, a full assessment of average psTIL was determined. In this study, levels of psTIL were categorized according to the median value, which was determined as a threshold for survival analysis.

Statistical analysis

Statistical analyses were performed with IBM SPSS 19.0 software (SPSS Institute, Chicago, IL, USA). Correlations between psTIL and clinic-pathological parameters were calculated with the chi-square test. Survivals were analyzed with the Kaplan–Meier method, by the use of the log-rank test for univariate analyses, and by use of Cox regression model for multivariate analyses. Receiver operating characteristic curve (ROC) analysis was used to determine the predictive value of the parameters. Two-

sided P -value of <0.05 was considered to be statistically significant.

Results

Prognostic values of psTIL in BC

A total of 240 BC patients were included in the present study. Baseline characteristics were summarized in Table 1. The median age was 48.0 years, ranging from 29 to 78 years. According to the recommended methods, TILs have been evaluated in all patients. TILs were distributed in both intratumoral and stromal areas within tumor borders, whereas were more frequent and abundant in the stromal area through direct visualization of H&E staining results, as shown in Figure 2A1. The median psTIL value was 5%, ranging from 0% to 100%. There were 142 (59.2%) and 98 (40.8%) cases divided into psTIL-Low (L) and psTIL-High (H) subgroups, respectively. Representative views of psTIL-L and psTIL-H are shown in Figure 2A2 and A3. Consequently, no statistically significant correlations could be found between psTIL and age, menopausal status, tumor size, clinical nodal status, TNM stage, histological grade, and OS status ($P>0.05$ for all). Kaplan–Meier survival analysis was conducted according to OS (Figure 2B),

Table 1 Patients' demographics and correlations between psTIL and major clinic-pathological features in breast cancer

Variables	Value (%)	psTIL			
		Low ($\leq 5\%$) (n=142) n (%)	High ($\leq 5\%$) (n=98) n (%)	χ^2	P
Age at diagnosis, years					
Median age	48.0 (Range 29–78)			0.002	1.000
≤ 50	149 (62.1)	88 (62.0)	61 (62.2)		
> 50	91 (37.9)	54 (38.0)	37 (37.8)		
Menopausal status					
Pre	134 (55.8)	81 (57.0)	53 (54.0)	0.206	0.692
Post	106 (44.2)	61 (43.0)	45 (46.0)		
Clinical tumor size					
≤ 2 cm	35 (14.6)	20 (14.1)	15 (15.3)	0.378	0.828
2 cm < d ≤ 5 cm	162 (67.5)	98 (69.0)	64 (65.3)		
> 5 cm	43 (17.9)	24 (16.9)	19 (19.4)		
Clinical nodal status					
N0	109 (45.4)	64 (45.1)	45 (45.9)	1.789	0.617
N1	66 (27.5)	37 (26.1)	29 (29.6)		
N2	30 (12.5)	21 (14.8)	9 (9.2)		
N3	35 (14.6)	20 (14.0)	15 (15.3)		
TNM stage at diagnosis					
I	18 (7.5)	10 (7.0)	8 (8.2)	0.158	0.924
II	151 (62.9)	89 (62.7)	62 (63.3)		
III	71 (29.6)	43 (30.3)	28 (28.5)		
IV	0 (0)	0 (0)	0 (0)		
Recurrence state					
No	141 (58.8)	82 (57.7)	59 (60.2)	0.688	0.709
Local regional	24 (10.0)	13 (9.2)	11 (11.2)		
Distant	75 (31.3)	47 (33.1)	28 (28.6)		
Histological grade					
I	40 (16.7)	30 (21.2)	10 (10.2)	5.088	0.079
2	141 (58.8)	80 (56.3)	61 (62.2)		
3	59 (24.6)	32 (22.5)	27 (27.6)		
Overall survival					
Yes	193 (80.4)	112 (78.9)	81 (82.7)	0.526	0.511
No	47 (19.6)	30 (21.1)	17 (17.3)		

Abbreviations: psTIL, the percentage of stromal tumor infiltrating lymphocytes; TNM, tumor-node-metastasis.

3-year (DFS (Figure 2C), and 5-year DFS (Figure 2D), respectively. Among 98 patients in psTIL-H subgroup, no significant difference was observed ($P > 0.05$ for all).

Stromal TILs distribution features in GC

TILs could be evaluated in all GC patients (n=481). As shown in Figure 3, TILs were frequently distributed in

stromal compartments within tumor borders. Through direct visualization of H&E staining results, TILs were infrequently scattered in stromal areas in some GC cases (Figure 3A, psTIL=1%), whereas abundantly accumulated in others (Figure 3B, psTIL=70%). All psTIL values were determined and included for next-step analyses. The potential relationship of psTIL and histological grade seemed to

A Representative H&E images of TILs features in BC tissues

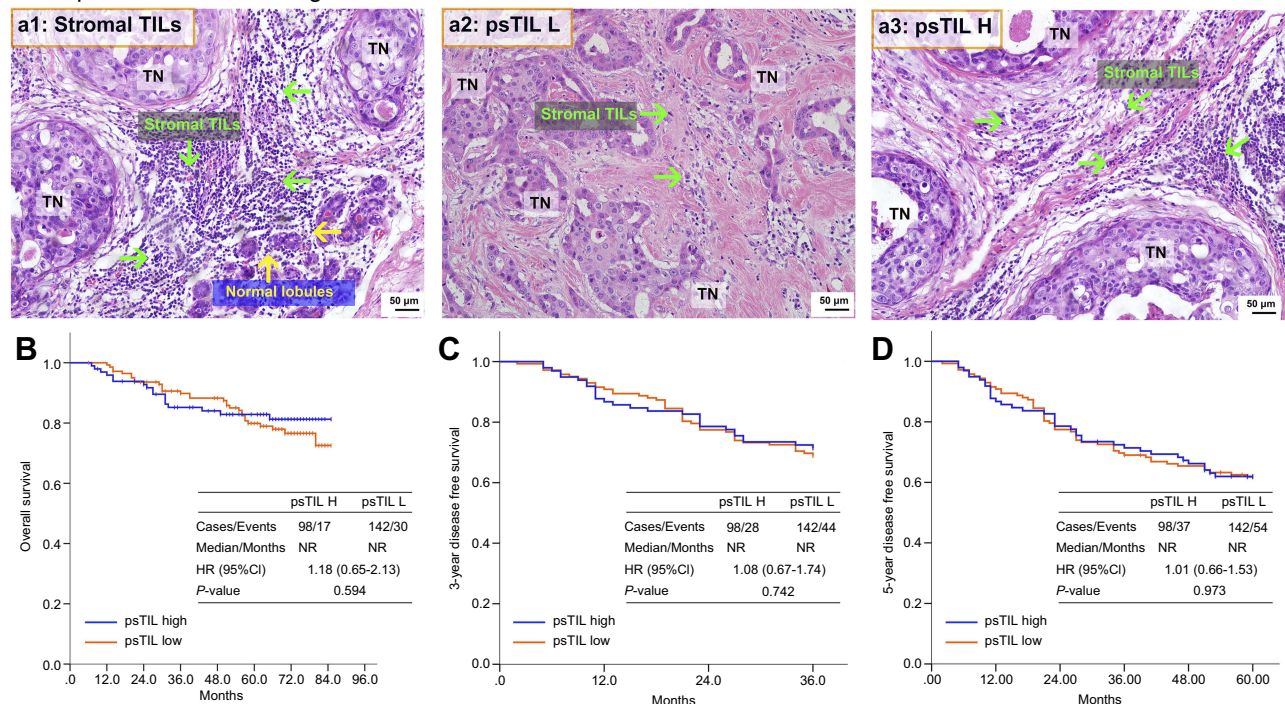


Figure 2 The standardized practice of psTIL in BC.

Notes: (A) Representative images of BC TILs. TILs groups could be observed as stromal TILs (green arrows) and TILs in normal lobes (yellow arrows) (A1). Herein, only stromal TILs were investigated for the psTIL parameter. According to the median value, BC cases with different psTIL values were divided into psTIL-L, an example of a case with few TILs (A2) and psTIL-H (A3) subgroups (magnification: $\times 200$, scale bar: 50 μm); Then survival analysis was based on a two-scale categorized method. No significant prognostic differences were found in (B) overall survival (OS), (C) 3-year DFS, as well as in (D) 5-year DFS.

Abbreviation: TILs, tumor-infiltrating lymphocytes.

be found by qualitative analysis. Compared with poorly differentiated adenocarcinoma (Figure 3A) and moderately differentiated adenocarcinoma (Figure 3C, psTIL=30%), highly differentiated cases (Figure 3D, psTIL=100%) had a tendency of higher psTIL values, which should be further confirmed by statistical analysis.

Correlations between psTIL and clinic-pathological characteristics in GC

Among the 481 GC cases included in this study were 338 (70.3%) males and 143 (29.7%) females, ranging in age from 25 to 87 (mean \pm SD: 59.0 \pm 11.9) years. According to the 7th UICC/AJCC TNM staging system for GC, there were 67 (13.9%), 108 (22.5%), 286 (59.5%), and 20 (4.2%) patients with stage I, stage II, stage III, and stage IV, respectively. The main demographic and clinic-pathological characteristics are presented in Table 2.

The median value of psTIL in GC was 10%, ranging from 0% to 100%. The threshold was determined according to the median value. Thus, psTIL-L ($\leq 10.0\%$) and psTIL-H ($>10.0\%$) subgroups were observed in 269 (55.9%) and 212

(44.1%) patients, respectively. Correlations between psTIL and major clinic-pathological features in GC were summarized in Table 2. psTIL-L was correlated with serosa invasion ($P<0.001$), advanced TNM stage ($P=0.028$), and poorer survival state ($P=0.004$). No statistically significant correlations could be observed with age, gender, tumor location, histological grade, and pN status ($P>0.05$ for all).

Survival analysis of psTIL in GC

For 481 GC cases, the median OS was 42.6 (95%CI: 32.7–52.5) months, and the 1-, 3-, and 5-year survival rate was 83.84%, 56.26%, and 43.35%, respectively. As expected, the traditional factors were associated with GC patients' OS, such as histological grade (Figure 4A), serosa invasion (pT) status, lymph node (pN) status (Figure 4B), distant metastasis, and TNM stage (Figure 4C) ($P<0.05$ for all).

There were 123 (45.7%) and 69 (32.5%) patients dead in psTIL-L and psTIL-H subgroups, respectively. The estimated 3-year survival rates (%) in the above 2 subgroups were 53.89% and 59.77%, respectively. The estimated 5-year survival rate (%) was 36.81% and 52.10%,

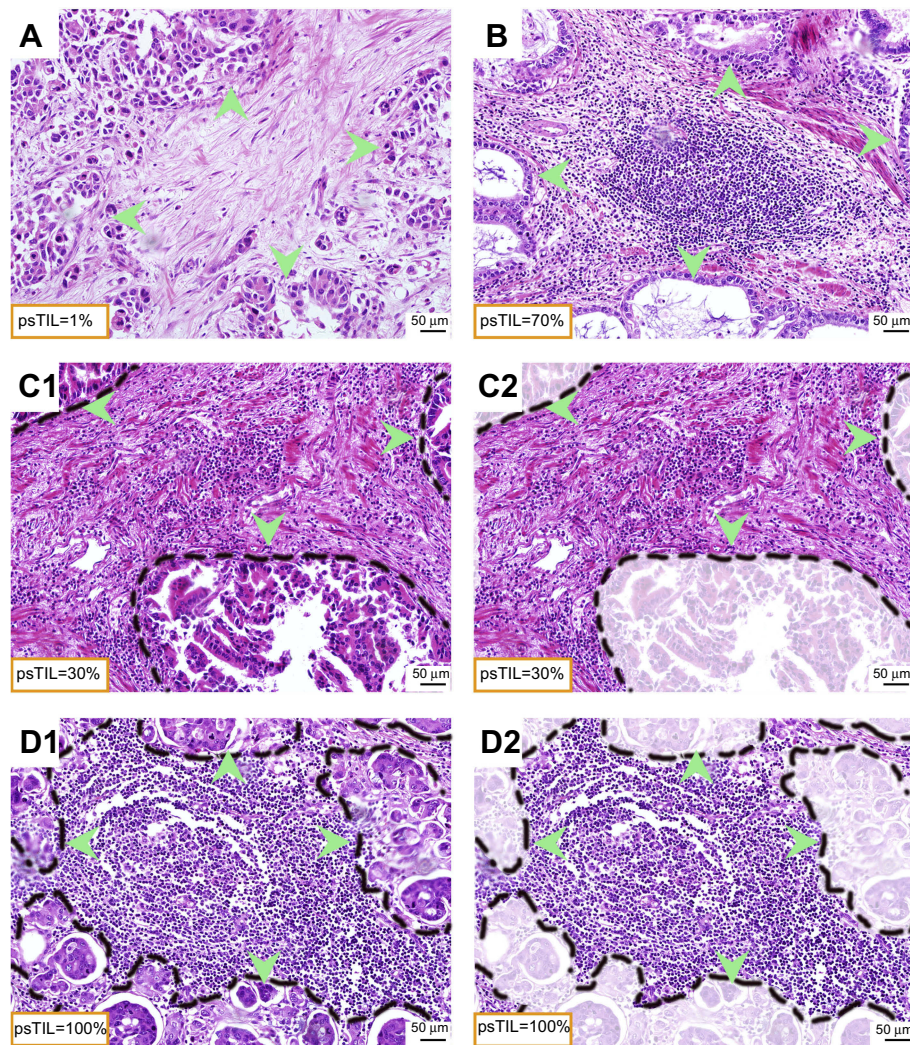


Figure 3 Representative views of TILs in GC cases.

Notes: (A) psTIL=1% ($\times 200$), poorly differentiated adenocarcinoma; (B) psTIL=70% ($\times 200$), mucinous adenocarcinoma; (C) psTIL=30% ($\times 200$), moderately differentiated adenocarcinoma; (D) psTIL=100% ($\times 200$), highly differentiated adenocarcinoma; stromal TILs were defined and assessed according to steps shown from **c1** to **c2**, and from **d1** to **d2**. Tumor cells presented at all borders were indicated by green arrowheads.

Abbreviations: TILs, tumor-infiltrating lymphocytes; GC, gastric cancer; psTIL, the percentage of stromal tumor-infiltrating lymphocytes.

respectively. The difference between psTIL-L and psTIL-H groups was statistically significant in terms of OS (Figure 4D, $P=0.030$). GC cases with high psTIL tended to have longer OS. Detailed data are shown in Table 3.

Multivariate and ROC analyses

To further clarify whether psTIL could have a prognostic value, univariate and multivariate survival analyses were performed. The univariate analyses revealed that high psTIL ($>10\%$), histological grade, serosa invasion, lymph node metastasis, distant metastasis, and TNM stage were significantly associated with postoperative outcome ($P<0.05$ for all). In multivariate analyses, high psTIL (HR=0.734, $P=0.047$) was identified as an independent

better prognostic factor. On the contrary, characteristics included in the TNM system (pT, pN status, and distant metastasis) and histological grade were identified as independent worse prognostic factors (Table 4).

Predictive values of the above-mentioned 5 independent factors (histological grade, pT, pN status, distant metastasis, and psTIL) were further studied by ROC analysis. TNM stage was also compared. Among the tested factors, distant metastasis was the weakest risk factor for death. psTIL performed well in predicting the clinical outcomes of GC patients. Compared with other factors, psTIL was a beneficial prognostic factor, in a linear manner with increasing TILs infiltrates associated with improved survival (area under the curve: 0.432 [95%CI: 0.380–0.484], $P=0.012$) (Figure 5).

Table 2 Patients' demographics and correlations between psTIL and major clinic-pathological features in gastric cancer

Variables	Value (%)	psTIL			
		Low (n=269) n (%)	High (n=212) n (%)	χ^2	P
Age (mean \pm SD, yr)	59.0 \pm 11.9				
\leq 59	243 (50.5)	138 (51.3)	105 (49.5)	0.149	0.714
>59	238 (49.5)	131 (48.7)	107 (50.5)		
Gender				0.043	0.842
Male	338 (70.3)	188 (69.9)	150 (70.8)		
Female	143 (29.7)	81 (30.1)	62 (29.2)		
Tumor location				0.067	0.854
Distal stomach	221 (45.9)	125 (46.5)	96 (45.3)		
Non-distal stomach	260 (54.1)	144 (53.5)	116 (54.7)		
Histological grade				1.548	0.241
1/2	158 (32.8)	82 (30.5)	76 (35.8)		
3/4	323 (67.2)	187 (69.5)	136 (64.2)		
Serosa invasion (pT status)				19.260	<0.001
No (T0, T1, T2)	103 (21.4)	38 (14.1)	65 (30.7)		
Yes (T3, T4)	378 (78.6)	231 (85.9)	147 (69.3)		
Lymph node metastasis (pN status)				1.694	0.208
No (N0)	164 (34.1)	85 (31.6)	79 (37.3)		
Yes (N1, N2, N3)	317 (65.9)	184 (68.4)	133 (62.7)		
Distant Metastasis				0.013	1.000
M0	460 (95.6)	257 (95.5)	203 (95.8)		
M1	21 (4.4)	12 (4.5)	9 (4.2)		
TNM stage				5.134	0.028
Early (stages I, II)	175 (35.8)	86 (32.0)	89 (42.0)		
Advanced (stages III, IV)	306 (64.2)	183 (68.0)	123 (58.0)		
Survival				8.585	0.004
Yes	289 (60.1)	146 (54.3)	143 (67.5)		
No	192 (39.9)	123 (45.7)	69 (32.5)		

Abbreviation: psTIL, the percentage of stromal tumor infiltrating lymphocytes; TNM, tumor-node-metastasis.

Discussion

The assessment of immune infiltrate in tumors, most commonly referred to as TILs, is gaining importance in the current quest for optimal biomarkers.¹⁸ TILs are mononuclear immune cells that infiltrate into tumor tissues. The presence of TILs reflects an individual immunological response state and contains potential clinical validity.¹⁹ In this study, we validated the usage of the TILs evaluating methods in BC, as well as the appropriate implementation in clinical practice for GC. In addition, the prognostic effects of psTIL were explored and stratified using the median value were further investigated in GC patients.

Theoretically, the complexity and heterogeneity of TILs originate from specific phenotypes of the cellular surface molecules.^{20,21} Hence, TILs can be evaluated by analyzing various subgroups through immunohistochemistry (IHC) methods.^{22,23} However, it remains unclear whether IHC method allows more accurate analysis, and different TILs subtypes may indicate controversial prognostic relevance.²⁴ In research settings, many different methods are being used to investigate the host immune response to tumors. IHC and multiplexed fluorescent IHC with multispectral imaging allow the definition of the majority of immune cell subsets that can be refined by combinations of markers, including CD8

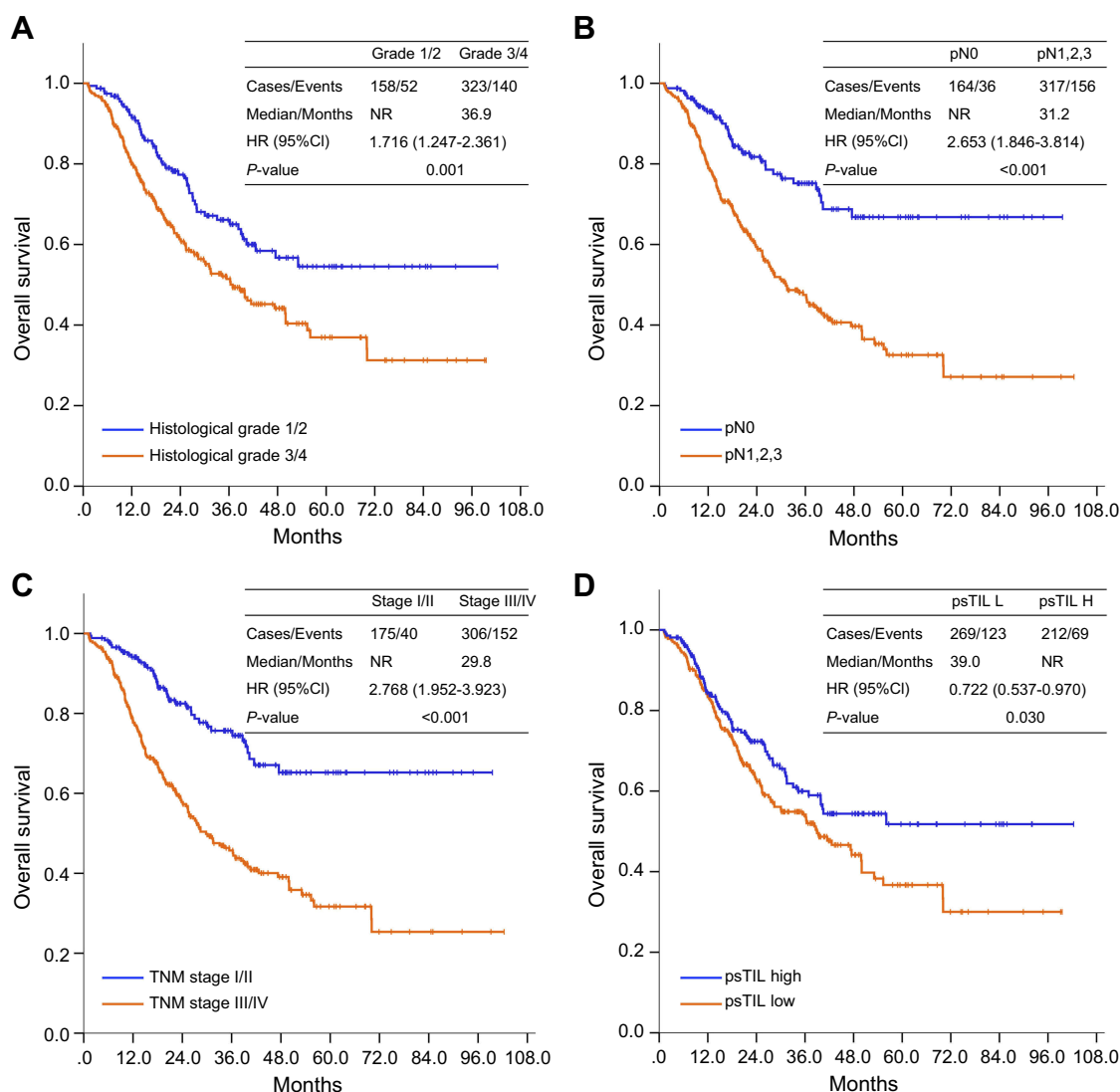


Figure 4 Correlations between psTIL and OS of GC patients.

Notes: Traditional clinic-pathological features were associated with OS such as (A) histological grade ($P=0.001$), (B) pN status ($P<0.001$), and (C) TNM stage ($P<0.001$). (D) Using 10% as the threshold, the psTIL value significantly correlated with GC patients' OS ($P=0.030$). GC cases with high psTIL tended to have longer OS.

Abbreviations: psTIL, the percentage of stromal tumor-infiltrating lymphocytes; OS, overall survival; GC, gastric cancer.

+ cytotoxic T cells, CD4+ T helper cells, FOXP3+ Tregs, B cells, macrophages, and dendritic cells. But those methods require cell surface identification and a significant investment in initial optimization, has complex technology, analysis and storage requirements, and is yet to become routine. Compared with the IHC method, H&E staining-based TILs evaluation has been demonstrated to be standardized and reproducible.¹³ Because of its low cost, availability, convenience, and importance in prognosis, H&E-based evaluation method is more suitable in clinical practice and could easily be implemented in routine pathology diagnostics. In this study, H&E images were with vibrant colors, excellent contrast, and appropriate for direct visualization and measurement. In this study, our

results showed that TILs could be demonstrably distinguished from other stromal areas and surrounding tumor nests, either in BC or in GC, which was essential for subsequent comparability. More importantly, it is reproducible, easy to determine, and quickly performed.

Before the recommendation has been proposed, the extent of TILs infiltration assessed by simple morphological evaluation of H&E-stained views has been shown to have predictive and prognostic values for BC.^{25,26} Luen and his colleagues showed that patients in certain subtype with advanced HER2-positive BC treated with docetaxel, trastuzumab, and pertuzumab or placebo should consider TILs as a stratification factor and investigate whether

Table 3 Analyses of major clinic-pathological factors and psTIL regarding GC OS

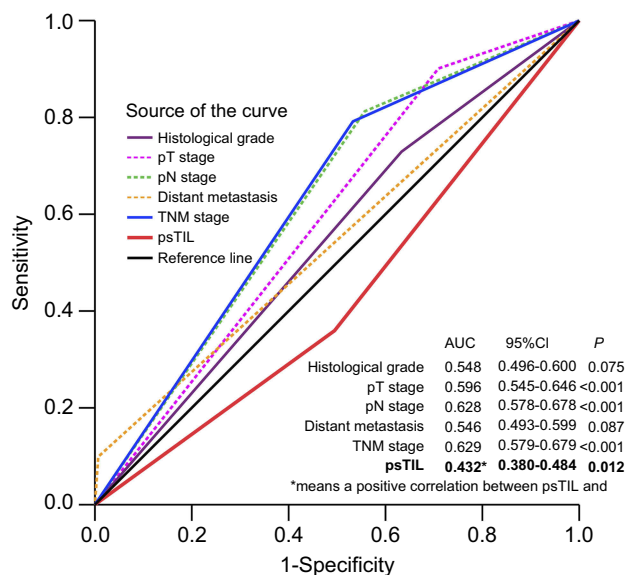
Variables	N (%)	N of death (%)	3-year survival rate (%)	5-year survival rate (%)	Log-rank test χ^2 value	P
Age (years)						
≤59	243 (50.5)	86 (35.4)	63.11	52.58	7.737	0.005
>59	238 (49.5)	106 (44.5)	48.60	33.25		
Gender						
Male	338 (70.3)	130 (38.5)	58.78	46.71	3.908	0.048
Female	143 (29.7)	62 (43.4)	49.75	34.40		
Histological grade						
I/2	158 (32.8)	52 (32.9)	65.35	54.08	11.255	0.001
3/4	323 (67.2)	140 (43.3)	51.58	37.36		
Serosa invasion (pT status)						
T1-2	103 (21.4)	19 (18.4)	75.99	70.75	21.628	<0.001
T3-4	378 (78.6)	173 (45.8)	51.25	36.64		
Lymph nodes metastasis (pN status)						
No	164 (34.1)	36 (22.0)	74.82	66.95	30.053	<0.001
Yes	317 (65.9)	156 (49.2)	47.23	32.21		
Distant metastasis						
M0	460 (95.6)	173 (37.6)	59.84	45.30	52.960	<0.001
M1	21 (4.4)	19 (90.5)	8.16	8.16		
TNM stage						
Early (stages I, II)	175 (36.4)	40 (22.9)	75.19	65.07	35.592	<0.001
Advanced (stages III, IV)	306 (63.6)	152 (49.7)	45.63	31.56		
psTIL						
Low (≤10%)	481 (100)	192 (39.9)	56.26	43.35	4.729	0.030
High (>10%)	269 (55.9)	123 (45.7)	53.89	36.81		
	212 (44.1)	69 (32.5)	59.77	52.10		

Abbreviations: psTIL, the percentage of stromal tumor infiltrating lymphocytes; GC, gastric cancer; OS, overall survival; TNM, tumor-node-metastasis.

Table 4 Univariate and multivariate analyses of factors associated with GC OS

Factors	Univariate analysis			Multivariate analysis		
	HR	95%CI	P	HR	95%CI	P
Histological grade			0.001			0.048
1/2	1.000			1.000		
3/4	1.716	1.247–2.361		1.392	1.003–1.930	
Serosa invasion (pT status)			<0.001			0.012
T0, 1, 2	1.000			1.000		
T3, 4	2.925	1.820–4.700		2.200	1.188–4.072	
Lymph node metastasis (pN status)			<0.001			0.038
N0	1.000			1.000		
N1, 2, 3	2.653	1.846–3.814		2.024	1.041–3.934	
Distant metastasis			<0.001			<0.001
M0	1.000			1.000		
M1	4.967	3.076–8.019		4.721	2.858–7.799	
TNM stage			<0.001			0.818
Stage I/II	1.000			1.000		
Stage III/IV	2.768	1.952–3.923		0.916	0.436–1.925	
psTIL			0.030			0.047
Low ($\leq 10.0\%$)	1.000			1.000		
High ($> 10.0\%$)	0.722	0.537–0.970		0.734	0.541–0.995	

Abbreviation: HR, hazard ratio; psTIL, the percentage of stromal tumor infiltrating lymphocytes; TNM, tumor-node-metastasis.

**Figure 5** ROC analysis.

Notes: Compared with other factors, psTIL was prognostically favorable, in a linear manner with increasing TILs infiltrates associated with improved survival (area under the curve: 0.432 [95%CI: 0.380–0.484], $P=0.012$).

Abbreviations: ROC, receiver operating characteristic curve; psTIL, the percentage of stromal tumor-infiltrating lymphocytes; TILs, tumor-infiltrating lymphocytes.

therapies that can augment immunity could potentially further improve survival.²⁷ In addition, further studies have shown that there is a strong linear relationship between the increase in TILs and improved DFSI for triple-negative and HER2-positive disease.²⁸ In this study, the prognostic value of TILs in BC has been explored, which is similar to the previous studies across all BC subtypes that have uncovered the prognostic significance of TILs.²⁹ All results revealed the high heterogeneous of BC, suggesting different biology of the immunological infiltration in different BC subtypes.³⁰

Interesting, this study showed the prognostic value of psTIL in all GC but not certain subtypes by the H&E-based evaluation. Considering the higher morbidity and mortality of GC in China, a total of 4,185 patients were enrolled in a recent meta-analysis to evaluate the prognostic significance of TILs subsets in GC.³¹ TILs were summarized as potential prognostic biomarkers in GC; however, contradictory findings existed. Some studies had shown associations between higher quantities of TILs and greater proportions of patients achieving longer survival time,^{32,33} whereas some subtypes showed a

bidirectional prognostic role or predicted worse prognosis.³⁴ As illustrated early, the difficulty of comparability among previous studies may be attributable to different methods, TILs subsets, and stratification decisions. In fact, few studies were conducted from the aspect of the percentage index according to the recommendation. However, further work is needed to ensure that the valuable information that could be obtained from TILs assessment is not lost due to issues of resource commitments, methodology, or lack of standardization. Notwithstanding, there is no current consensus on the best TILs distribution for predicting survival in GC.³⁵

In this study, GC cases with low psTIL were at high risk of serosa invasion, advanced stages, and poor survival. This result is in agreement with a retrospective analysis of a randomized phase 3 study,²⁷ suggesting TILs as a stratification parameter in clinical practice. Furthermore, psTIL was associated with GC OS in a linear manner with increasing TILs infiltrates associated with improved survival. Some studies observed TILs as a better predictor of patient survival at earlier stages or advanced stages of lung cancer.^{36,37} This result might originate from differing biological behaviors during GC progression, which was best conceptualized as cancer immunoediting.³⁸ During this process, TILs subgroups may play two-way regulating roles, with a predominant contribution to either protumor or antitumor.¹² Generally, TILs exist in intratumoral areas recruited by critical factors,³⁹ exerted antitumor immunity and thus high psTIL exhibited favorable prognosis. However, recent evidence also suggested that TILs subgroups like CD4+ regulatory T cells (Treg) might be responsible for the failure of host immunological surveillance by inhibiting local inflammatory processes.⁴⁰ Therefore, the functional role of psTIL differed in terms of GC clinical status.

Our study has some limitations. It was a retrospective study with limited generalizability as all specimens were obtained from patients in China, and the distribution of clinical characteristics might be different in other areas. However, the significant contribution of China to the worldwide burden of GC supports the performance of an initial investigation of the importance of H&E-based TILs evaluation in a Chinese population. Thus, a prospective, international, multicenter clinical trial will be needed to further validate our findings.

Conclusions

In summary, this study comprehensively analyzed the roles of psTIL in solid tumor and demonstrated that H&E staining–

based semi-quantitative evaluation obtains a pragmatic, reproducible and easy assessment of stromal TILs. To our knowledge, the parameter psTIL could be served as an independent, favorable prognostic factor of GC. The classification of GC via stratification of psTIL could help predicting clinical outcomes and fine-tuning therapies in future practice.

Synopsis

In this study, the percentage of stromal TILs (psTIL) was reported semi-quantitatively by H&E staining-based evaluation. Two independent cohorts of BC (n=240) and GC (n=481) were included for evaluating the relationship between psTIL and clinic-pathological features, as well as OS. A beneficial OS of high psTIL was found in a linear manner with increasing TILs infiltration associated with improved OS [Kaplan–Meier survival curve: $P=0.030$; univariable (HR: 0.734, $P=0.047$) and multivariate analyses (HR: 0.722, $P=0.030$)]. Therefore, the parameter psTIL could be served as an independent, favorable prognostic factor of GC and certain subtypes of BC.

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Disclosure

The authors declare no conflict of interest in this work.

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