Histol Histopathol (2025) 40: 881-889

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE Open Access

Intratumoral T cells are associated with prognosis and chemotherapy benefit in gastric cancer

Lu Liu[1], Qianwen Chen[2], Chen He[3], Feilan Xie[1], Shengyuan Su[1], Lijun Wang[4] and Jintao Liu[1,5]

[1]Department of Critical Care Medicine, People's Hospital of Baoan Shenzhen, The 8th People's Hospital of Shenzhen, Shenzhen, [2]Department of Gastroenterology, The Second Affiliated Hospital of Guangdong Medical University, Zhanjing, [3]Department of Ophthalmology, Shenzhen Second People's Hospital, First Affiliated Hospital of Shenzhen University, [4]Department of Critical Care Medicine, Shenzhen FuYong People's Hospital and [5]Department of Gastroenterology, Shenzhen Baoan Traditional Chinese Medicine Hospital Group, Shenzhen, Guangdong, PR China

Summary. Tumor-infiltrating lymphocytes (TILs) have been described in various malignancies and viewed as a sign of anti-tumor immunity, so they are frequently thought to be implicated in the prognosis of cancers. However, little information is available on the association of the distribution pattern of TILs with clinical outcomes in gastric cancer (GC). TIL densities at different regions were assessed immunohistochemically in 59 GC patients to analyze their relationship with clinicopathological characteristics. We found that GC patients in the high-density TIL group were significantly associated with reduced tumor invasion depth, absence of lymph node metastasis, earlier TNM stage, and improved progression-free survival (PFS). Both intratumoral CD3+ TILs and pathological T stage were identified as having an independent prognostic value. Additionally, GC patients with a high density of intratumoral CD3+ TILs were found to gain more benefit from chemotherapy. Overall, these results underscored the predictive power of intratumoral TILs in survival prognosis and chemotherapy benefit for GC.

Key words: GC, TILs, Prognosis, PFS, Chemotherapy

Introduction

Gastric cancer (GC) is the fifth most common cancer worldwide and the third leading cause of cancer-related death, accounting for approximately 10% of cancer morbidity (Van Cutsem et al., 2016; Eusebi et al., 2020; Siegel et al., 2022). Over the past two decades, considerable progress in screening programs and diagnostic techniques has been made, leading to a high increase in the proportion of GC patients diagnosed in the early stages (Gambardella and Cervantes, 2018; Machlowska et al., 2020). Early GC now accounts for more than 50% of all cases. However, the cure rate and long-term survival of early GC patients have only marginally improved, largely due to traditional TNM classification and molecular biomarkers failing to identify early-stage patients who had progression after curative resection (Sumiyama, 2017; Katai et al., 2018). Therefore, additional prognostic indicators are needed to guide therapeutic decisions and improve the long-term survival of GC patients.

Traditionally, the course of tumor development has been considered to be governed by the epigenetic and genetic modifications of neoplastic cells (Joyce and Pollard, 2009; Pietras and Ostman, 2010; McAllister and Weinberg, 2014). Accordingly, current prognostic or predictive strategies have mostly focused on the histological characteristics of tumor cells, including aberrance in the expression of markers of malignant transformation, changes in cell morphology, senescence, and proliferation as well as the loss of barrier integrity due to invasion and microvascularization. However, increasing evidence has suggested that clinical outcomes can vary greatly among patients with the same histological stage (Galon et al., 2013, 2014). It has been well recognized that solid tumors consist of heterogeneous populations of neoplastic cells as well as recruited immune cells, fibroblasts, and endothelial cells, which are collectively known as the tumor micro-environment (TME) (Joyce and Pollard, 2009; McAllister and Weinberg, 2014). All these cellular compartments interact with each other and contribute to the development and progression of tumors.

As the predominant infiltrated immune cells in the TME, tumor-infiltrating lymphocytes (TILs) have been extensively studied for their prognostic values (Nagtegaal et al., 2011; Galon et al., 2013, 2014; Fridman et al., 2017). Since the infiltration of tumors by

Corresponding Author: Jintao Liu, Department of Gastroenterology, Shenzhen Baoan Traditional Chinese Medicine Hospital Group, Shenzhen, Guangdong, 518100, PR China. e-mail: jtliu118@163.com
www.hh.um.es. DOI: 10.14670/HH-18-824

T cells is interpreted as a sign of anti-tumor immunity, an abundance of TILs is often associated with a favorable clinical outcome across many cancer types (Galon et al., 2006). Studies in animal models have also revealed that progressively growing tumors contain weak or functionally exhausted TILs, such as PD-1+ and CD4+FOXP3+ T cells, while regressing tumors have highly reactive TILs, such as CD8+CD45RO+ T cells (Galon et al., 2013, 2014). Comprehensive multi-omic analyses have been performed to provide mechanistic insights into these associations between TILs and clinical outcomes (Bindea et al., 2013; Althobiti et al., 2018; Laumont et al., 2021). Besides contributing to anti-tumor immune responses, TILs can also serve as sensors to monitor TME as they are highly dynamic and heterogeneous both within and across tumors, which allows for rapid adaptions to alternations in the TME. However, the high degree of spatial heterogeneity of TILs poses a major challenge for the development of predictive or prognostic strategies that integrate the characteristics of TILs. Relatively little information has been available on the association between the TIL distribution pattern and the prognosis of GC. To address this issue, we respectively evaluated the relationship between TILs at different regions, including the tumor core (TC), the tumor stroma (TS), as well as the corresponding normal tissue adjacent to the tumor (AT), and clinicopathological features and progression-free survival (PFS), and a more effective prognostic variable may be identified, which would help guide therapeutic decisions.

Materials and methods

Patients

The ethics committee of the People's Hospital of Baoan Shenzhen (Date: March 9, 2021; NO: BYL20210336) approved this retrospective study and waived the requirement to obtain informed consent for participation. We enrolled 59 GC patients who underwent complete surgical resection at the People's Hospital of Baoan Shenzhen from 2017 to 2019. No GC patient received chemotherapy or radiotherapy treatment preoperatively. Their clinical and histopathological data were retrieved or reviewed by two experienced gastrointestinal pathologists (FLX and QWC) according to the 8th Edition of TNM staging in GC. The observation time in this study was the interval between diagnosis and last contact (recurrence, death, or last follow-up).

Immunohistochemistry

Paraffin-embedded biopsies with tumor and adjacent tissue were sectioned for immunohistochemistry. Firstly, the surgical specimens were assessed pathologically by hematoxylin and eosin staining; secondly, they were serially sectioned (5-μm thick) and deparaffinized, rehydrated, and pretreated in citrate buffer (0.01 M, pH 6) for antigen retrieval; and lastly, slides were incubated with primary antibodies (anti-CD3 antibody, ab5690; anti-CD8 antibody, ab4055, Abcam, Cambridge, UK) following blocking by 5% (v/v) human serum.

Cell Quantification

Immunohistochemically stained samples were digitalized using the 3D Histech Midi Scanner System and were viewed within the Panoramic Viewer Software (Histolab Products AB). Two experienced pathologists (SYS and FLX) double-blindly evaluated the amount of stained cells in five visual fields with the most abundant T-cell infiltration.

Table 1. Patient clinicopathological features and their correlations with PFS.

		PFS (95% CI)	*p*-value
Age			0.149
<50	20	30.0(20.6-39.4)	
>50	39	22.6(17.0-28.2)	
Sex			0.862
Male	35	25.5(18.6-32.4)	
Female	24	24.6(17.7-31.5)	
CEA (ng/ml)			0.418
Negative (<5)	48	26.1(20.5-31.6)	
Positive (>5)	11	21(10.6-31.4)	
CA19-9 (U/ml)			0.07
Negative (<37)	52	26.7(21.5-31.9)	
Positive (>37)	7	13.3(3.6-23.0)	
Tumor location			0.419
Proximal stomach	37	26.7(20.9-32.4)	
Distal stomach	22	22.6(13.4-31.8)	
Tumor size (cm)			0.297
<5	43	26.7(20.9-32.4)	
>5	16	21(11.6-30.4)	
Histological type			0.575
Tubular adenocarcinoma	36	25.7(19.8-31.6)	
Mucinous adenocarcinoma	6	30.8(4.7-57.0)	
Signet ring cell carcinoma	17	21.9(12.2-31.5)	
T stage			**<0.0001**
T1+T2	29	43.6(35.7-51.4)	
T3+T4	30	16.4(12.6-20.2)	
N stage			**<0.0001**
N0	23	37.3(28.2-46.4)	
N1+N2+N3	36	17.9(13.7-22.1)	
M stage			**0.005**
M0	52	27.6(22.5-32.7)	
M1	7	7.1(4.0-10.3)	
TNM Stage			**<0.0001**
I+II	27	38.7(31.6-45.9)	
III+ IV	32	14.4(10.8-418.0)	
Histological grade			0.146
Well-Moderate	11	32.5(17.8-47.1)	
Poor	48	23.5(18.4-28.5)	

Bold indicates statistical significance. PFS, progression-free survival; CI, confidence interval; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

Statistical Analysis

Pearson's χ^2 test or Fisher's exact (2-sided) test was used to compare categorical and continuous variables. PFS curves were assessed by the Kaplan–Meier analysis and the significant differences were determined by the log-rank test. Cox proportional hazards regression models were used to analyze the variables implicated in PFS. Analyses of the correlations between clinicopathological features and the recurrence of patients with early-stage diseases or responses to chemotherapy were performed using multivariate logistic regressions. A p-value <0.05 was considered statistically significant.

Results

Study Population

In total, 59 GC patients without preoperative treatment were enrolled in this study. Their clinicopathological characteristics are summarized in Table 1. Most patients had negative serum levels of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9, the most commonly used tumor markers in GC. Histologically, 42 patients (71.2%) were diagnosed with adenocarcinoma, and 30 (50.8%) presented with deep tumor invasion. Lymph node metastasis was observed in 36 patients (61.0%), and 7 patients (11.9%) developed distant metastasis. The median duration of PFS was 25.1 months (20.3-29.9 months, 95% confidence interval [CI]). Fifty-two patients (88.1%) experienced tumor progression before the end of the observation period. As shown in Table 1, lower tumor invasion depth (T stage, p<0.0001), absence of lymph node invasion (N stage, p<0.0001), and distant metastasis (T stage, p=0.005) as well as earlier TNM stage (p<0.0001) were significantly associated with improved PFS (Table 1).

TIL distribution pattern in GC

To explore the distribution pattern of TILs, stained cells were quantitatively estimated in three distinct regions for each patient: TC, TS, and AT. We found that both CD3+ and CD8+ TILs displayed similar distribution patterns. They were mainly located at the TC ($CD3+TILs^{TC}$, 690.8±231.6; $CD8+TILs^{TC}$, 470.7±164.4), and TILs were also present in the TS ($CD3+TILs^{TS}$, 355.8±143.7; $CD8+TILs^{TS}$, 223.0±85.3). Densities of TILs in tumor tissue were significantly higher than those in the AT ($CD3+TILs^{AT}$, 86.6±33.1; $CD8+TILs^{AT}$, 58.9±29.9). Representative pictures of TILs in three compartments are shown in Figure 1A-F, and statistics of TILs are shown in Figure 1G,H. A statistically significant association was observed between TIL density in TC and TS (p<0.001). The ratio of CD3+ to CD8+ TILs was also evaluated. The TC

Table 2. Correlations between TILs and clinicopathological features.

	CD3+				CD8+			
	TC		TS		TC		TS	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age								
<50 vs. >50	0.777(0.263-2.293)	0.531	1.426(0.483-4.211)	0.545	1.053(0.358-3.094)	0.103	1.941(0.649-5.808)	0.558
Sex								
Male vs. Female	1.663(0.582-4.747)	0.582	2.222(0.767-6.437)	0.249	1.663(0.582-4.747)	0.588	1.251(0.442-3.545)	0.747
CEA								
Negative vs. Positive	0.159(0.031-0.815)	**0.042**	0.268(0.125-1.871)	0.123	0.484(0.125-1.871)	0.314	0.159(0.031-0.815)	**0.045**
CA19-9								
Negative vs. Positive	0.533(0.115-2.471)	0.824	0.132(0.015-1.177)	0.179	0.132(0.015-1.177)	0.194	0.578(0.126-2.672)	0.841
Tumor size (cm)								
<5 vs. >5	0.522(0.161-1.692)	0.074	0.151(0.037-0.610)	**0.041**	0.240(0.067-0.866)	**0.045**	0.360(0.107-1.215)	0.100
Histological type								
Adenocarcinoma vs. others	1.125(0.364-3.476)	0.683	1.237(0.400-3.826)	0.890	1.125(0.364-3.476)	0.406	0.636(0.204-1.990)	0.597
T stage								
T1+T2 vs. T3+T4	0.308(0.097-0.977)	**0.012**	0.658(0.216-1.981)	0.314	0.238(0.072-0.791)	**0.003**	0.341(0.108-1.080)	0.169
N stage								
N0 vs. N1+N2+N3	0.298(0.099-0.900)	**0.039**	0.546(0.189-1.574)	0.363	0.469(0.157-1.401)	0.119	0.469(0.157-1.401)	0.179
TNM Stage								
I+II vs. III+ IV	0.191(0.063-0.583)	**0.0003**	0.402(0.140-1.153)	0.082	0.191(0.063-0.583)	**0.0006**	0.221(0.073-0.664)	**0.040**
Histological grade								
Well-Moderate vs. Poor	0.526(0.136-2.033)	0.286	0.903(0.244-3.346)	0.763	1.304(0.350-4.858)	0.783	1.304(0.350-4.858)	0.881

Bold indicates statistical significance. PFS, progression-free survival; CI, confidence interval; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

displayed a significantly lower CD3+/CD8+ ratio compared with TS (p=0.034, Fig. 1I), suggesting that the composition of infiltrated T cells was significantly different between the TC and TS.

The association between TIL densities and clinicopathological features

The cohort was divided into low- and high-density

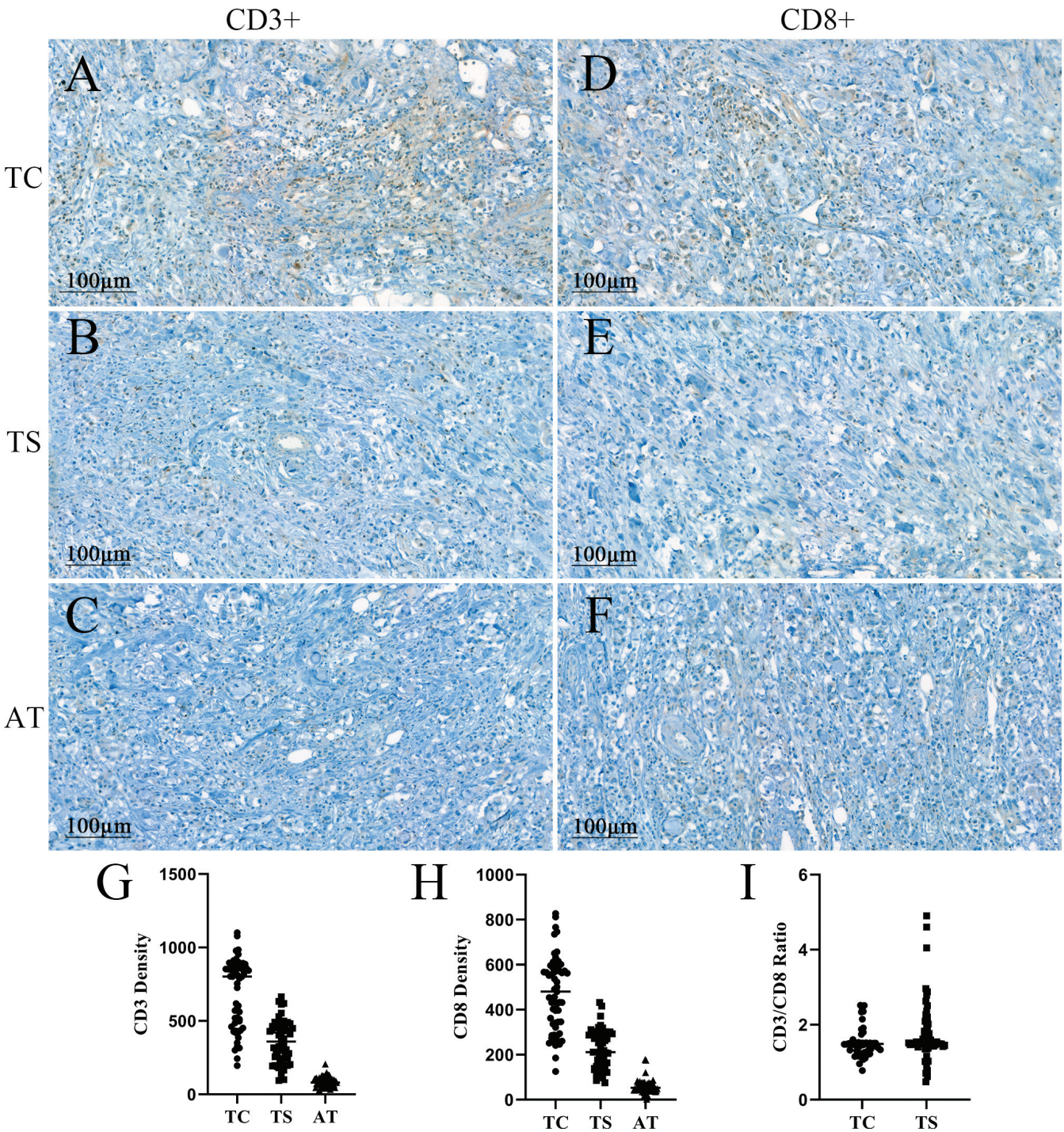

Fig. 1. The distribution pattern of tumor-infiltrating lymphocytes (TILs) in gastric cancer (GC). **A-F.** Representative immunohistochemistry staining for CD3 (left) and CD8 (right) at the tumor center (TC), the tumor stroma (TS), and adjacent tissue (AT). **G, H.** Densities of CD3+ and CD8+ TILs in three different regions. **I.** CD3+/CD8+ TILs ratio at TC and TS.

groups according to the median threshold, and Spearman's rank correlation was used to explore the correlation between TIL densities and clinicopathologic features. As no statistical difference in AT was observed within the studied patients, only intratumoral and peritumoral TILs were considered in the following analysis. As shown in Table 2, the associations of clinicopathological features with CD3+ and CD8+ TILs at the same region were not identical. For CD3+TILs, the density of CD3+ $TILs^{TC}$ was associated with the depth of tumor invasion (OR, 0.308; 95% CI, 0.097-0.977), incidence of lymph node metastasis (OR, 0.298; 95% CI, 0.099-0.900), and TNM stage (OR, 0.191; 95% CI, 0.063-0.583). Peritumoral CD3+ TILs were more frequently observed in patients with tumors <5 cm. For CD8+ TILs, intratumoral CD8+ TIL density was significantly higher in patients with smaller tumor size (OR, 0.240; 95% CI, 0.067-0.866), reduced depth of invasion (OR, 0.238; 95% CI, 0.072-0.791), and earlier TNM stage (OR, 0.191; 95% CI, 0.063-0.583). Peritumoral CD8+ TIL density was inversely correlated with TNM stage (OR, 0.221; 95% CI, 0.073-0.664). No significant association was observed between densities of TILs with age, sex, or level of CA19-9.

The association between TILs and GC patient outcomes

Table 3 shows the results of the univariate survival analysis of clinicopathological features. We found that the degree of tumor invasion (HR, 0.205; 95% CI, 0.117-0.357), the presence of lymph node metastasis (HR, 0.260; 95% CI, 0.175-0.531), and distant metastasis (HR, 0.167; 95% CI, 0.030-0.940) were the factors associated with GC patient survival. Kaplan-Meier analysis showed that high densities of intratumoral CD3+ and CD8+ TILs were implicated in favorable patient outcomes (Fig. 2).

A multivariate model was used to identify

Table 3. Univariate and multivariate analyses of factors associated with progression-free survival.

		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
T stage	T1+T2 vs. T3+T4	0.205(0.117-0.357)	**0.002**	0.131(0.043-0.359)	**0.0002**
N stage	N0 vs. N1+N2+N3	0.260(0.175-0.531)	**0.0001**	0.820(0.326-1.971)	0.664
CD3+ $TILs^{TC}$	Low vs. High	1.929(1.089-3.416)	**0.013**	3.618(1.254-10.182)	**0.016**
CD3+ $TILs^{TS}$	Low vs. High	1.389(0.789-2.445)	0.227		
CD8+ $TILs^{TC}$	Low vs. High	1.948(1.099-3.452)	**0.011**	0.539(0.197-1.519)	0.236
CD8+ $TILs^{TS}$	Low vs. High	1.439(0.822-2.519)	0.175		

Bold indicated a statistically significant. HR, hazard ratio; CI, confidence interval.

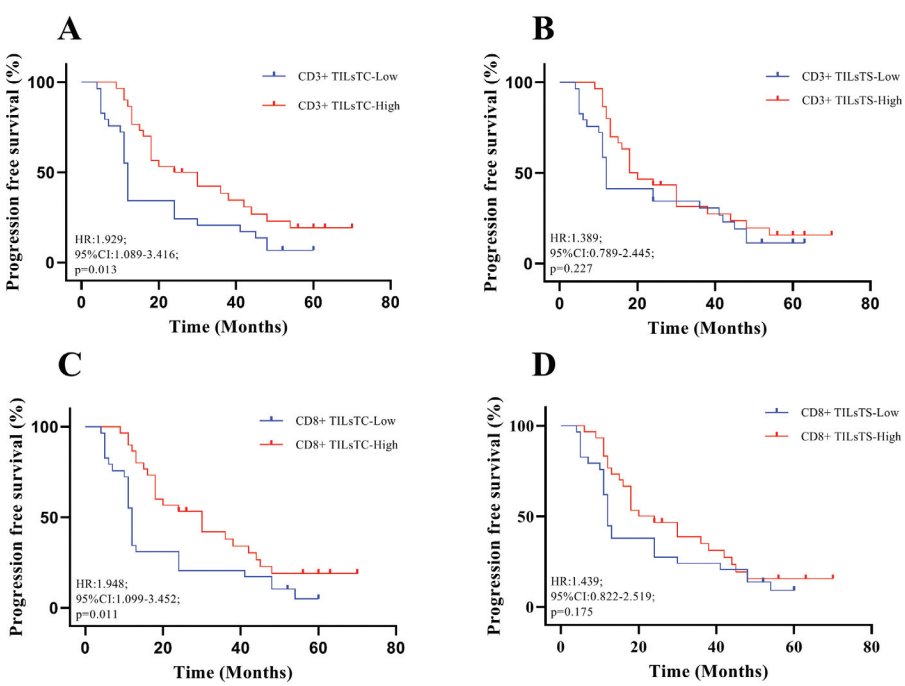

Fig. 2. Relationship between progression-free survival and the density of tumor-infiltrating lymphocytes (TILs). **A, B.** Kaplan-Meier curves illustrated the duration of progression-free survival according to the density of CD3+ TILs in the tumor center (TC) and tumor stroma (TS), respectively. **C, D.** Kaplan-Meier curves illustrated the duration of progression-free survival according to the density of CD8+ TILs in TC and TS, respectively.

Table 4. Univariate and multivariate analyses of factors associated with the effect of adjuvant chemotherapy.

		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
CD3+ $TILs^{TC}$	Low vs. High	6.960(2.374-20.400)	**0.0004**	11.942(1.307-94.162)	**0.017**
CD3+ $TILs^{TS}$	Low vs. High	2.712(1.046-7.032)	**0.040**	2.112(0.260-13.740)	0.952
CD8+ $TILs^{TC}$	Low vs. High	3.657(1.359-9.841)	**0.010**	1.784(0.329-7.290)	0.768
CD8+ $TILs^{TS}$	Low vs. High	0.940(0.436-2.028)	0.857		

Bold indicates statistical significance. HR, hazard ratio; CI, confidence interval.

independent prognostic factors, in which all features found to have significant prognostic value in the univariate analysis were included. Multivariate analysis revealed that T stage (HR, 0.131; 95% CI, 0.043-0.359; P=0.0002) and CD3+ $TILs^{TC}$ (HR, 3.618; 95% CI, 1.254-10.182; P=0.016) showed independent prognostic significance. These observations also confirmed that high densities of CD3+ $TILs^{TC}$ were associated with good patient outcomes.

The association between TILs and early-stage patient outcomes

In our study, 12 patients had early gastric cancer (EGC), which was defined as invasive carcinoma confined to mucosa and/or submucosa regardless of lymph node metastasis, and five patients developed disease recurrence. The presence of lymph node metastasis was found to be strongly associated with recurrence in EGC, while other clinicopathological features, including age, sex, serum CEA and CA19-9 levels, and histological grade, were not correlated with recurrence in EGC. We next studied whether using the densities of TILs in different regions could help stratify recurrence patients. However, we found that TIL density at different locations was not significantly associated with recurrence in EGC.

The association between TILs and the effect of adjuvant chemotherapy

Increasing evidence suggests that the host immune system makes a crucial contribution to the anti-tumor effects of adjuvant chemotherapy (ACT). Therefore, we evaluated the benefit of treatment according to the density of TILs in patients who received postoperative chemotherapy. Twenty-seven patients were treated with Fluorouracil (FU)-based chemotherapy. Although there were no significant differences in PSF according to the density of TILs, patients in high-density groups for CD3+ $TILs^{TC}$, CD3+ $TILs^{TS}$, and CD8+ $TILs^{TC}$ had a longer median duration of PSF than those in the corresponding low-density groups (CD3+ $TILs^{TC}$, 19.9±12.4 vs. 12.3±5.6, p=0.054; CD3+ $TILs^{TS}$, 19.4±12.6 vs. 12.8±5.8, p=0.099; CD8+ $TILs^{TC}$, 19.4±12.8 vs. 12.8±5.3, p=0.099). Cox regression analysis showed that GC patients with elevated densities of CD3+TILs at the TC had greater benefits with FU-based regimens (Table 4).

Discussion

Traditionally, cancer has been thought to be a cell-autonomous genetic disease, in which the malignant transformation of normal cells progressively occurs through a series of genetic and epigenetic alterations. Now, it has been well recognized that the evolution of cancer reflects complex interactions of tumor cells with their microenvironment (Joyce and Pollard, 2009; Pietras and Ostman, 2010; McAllister and Weinberg, 2014). Therefore, integrating the analysis of tumor-intrinsic and TME factors may improve the accuracy of patient stratification and prognosis (Nagtegaal et al., 2011; Galon et al., 2013, 2014; Fridman et al., 2017). Currently, infiltration of tumors by T cells is interpreted as a sign of anti-tumor immunity, and this feature is associated with a favorable clinical outcome across many cancer types. However, the prognostic significance of TILs in GC remains a subject of controversy, largely due to the marked spatial heterogeneity of TILs. To address this issue, a detailed immunohistochemical evaluation of TILs in three areas, including TC (designated as intratumoral compartments), TS (defined as peritumoral compartments), and AT (viewed as the normal control) was performed. We found that GC patients in the high-density TIL group were significantly associated with reduced tumor invasion depth, absence of lymph node metastasis, earlier TNM stage, improved PFS, and thus, lower risk of progression. These results agreed with previous reports, that an abundance of tumor tissue infiltration was linked to the gain of anti-tumor benefits and improvement in patient survival (Lee et al., 2008; Wakatsuki et al., 2013; Dai et al., 2016; Kang et al., 2016; Kim et al., 2017; Jiang et al., 2018; Uppal et al., 2020).

Regardless of the TNM stage of GC patients, TILs were detected in their tumor tissues, suggesting that TILs participated in all stages of tumor progression. Because TILs were mainly detected at the tumor center rather than the stroma, they suppressed tumor development through direct T cell-mediated cytotoxicity rather than the indirect action of secreted soluble

mediators, such as cytokines, chemokines, and other bioactive factors. In addition, we found that the ratio of CD3+/CD8+ TILs at the TC was significantly different from that at the TS. Therefore, these observations suggested that the composition of TILs at different regions of tumor tissue was highly heterogeneous, which might explain the variable impact of TILs on prognosis in GC. For example, Liu et al. (2015) pointed out that both intratumoral and peritumoral TILs were associated with superior overall survival independent of their locations. However, Uppal et al. found that high densities of TILs, including CD3+, CD4+, CR45RO+, and Treg subsets, along the invasive margin had a significant impact on better overall survival, whereas no intratumoral TIL subset correlated with prognosis (Uppal et al., 2020). In a retrospective pilot study of 479 GC patients receiving radical surgical resection, Wang et al. (2011) showed that intratumoral Tregs were associated with improved survival. In contrast, others demonstrated that Tregs played a role in immuno-suppression in tumor progression and led to a poorer prognosis (Liu et al., 2015). No clear explanation for these contradictory observations exists, although many hypotheses have been proposed, such as the highly intrinsic heterogeneity of TILs among patients, and differences in methods used to detect and count target cells.

Patients with EGC are best treated with surgical resection of the primary tumor, but 2.1- 12.4 % of patients still have disease recurrence (Wu et al., 2008; Yamamoto et al., 2008). Currently, the presence of lymph node metastasis is regarded as an independent risk factor of recurrence in EGC. In the present study, nearly all relapsed ECG patients had histologically detected lymph node metastasis. Our result also confirmed the correlation between lymph node metastasis and recurrence in EGC patients. Additionally, as defects in the immune response would contribute to tumor progression, considerable research attempts have been made to determine whether infiltrated immune cell signatures could help stratify early-stage patients who have a high risk of recurrence. Glaire et al. (2019), in a pooled analysis of colorectal cancer (CRC) patients with stage II disease, demonstrated that intratumoral CD8+ was a stronger predictor of CRC recurrence. Dieu-Nosjean et al. (2008) found that increased density of mature dendritic cells (DCs) was associated with a favorable clinical outcome for patients with early-stage non-small-cell lung cancer (NSCLC). They found that the number of tumor-infiltrating mature DCs was a reliable predictive indicator to identify patients with early-stage NSCLC who had a high risk of relapse. For GC, Kim et al. (2017) found that low TILs were significantly associated with lymph node metastasis in EGC. Therefore, low TILs were considered predictive of recurrence in EGC. However, we did not find any association between TILs and recurrence in EGC, possibly due to the limited sample size. Thus, further research in a large-scale cohort study is needed to more explicitly evaluate the impact of TILs on EGC patient outcomes.

Chemotherapy has been routinely applied in advanced-stage GC patients to overcome postoperative recurrence but the effectiveness of ACT is largely unpredictable. Accumulating evidence indicates that the efficacy of chemotherapy does not only involve direct cytostatic/cytotoxic effects but also relies on the (re)activation of tumor-targeting immune responses, which would lead to the recruitment and expansion of immune cells in the TME (Zitvogel et al., 2008). Therefore, the presence of TILs could be considered predictive of the effectiveness of ACT. In a large retrospective study of 1156 stage II colon cancer patients, Morris et al. (2008) found that patients with TILs gained more survival benefit from chemotherapy. For GC, by analyzing samples from patients undergoing radical resection, Zhang et al. (2023) demonstrated that a high density of cytotoxic CD8+ TILs at the TC was significantly associated with prolonged overall survival in patients treated with chemotherapy. In the present study, we found that the survival benefit of FU-based regimens in GC patients was significantly improved by the presence of a high density of CD3+ TILs at the TC. However, no significant association was observed between high intratumoral CD8+ TIL density and good prognosis, inconsistent with the observation of Zhang et al. (2023). Our results suggested that intratumoral CD3+ TILs rather than intratumoral CD8+ TILs, had an important impact on the effectiveness of ACT in GC patients.

In conclusion, we found that GC patients with a high density of intratumoral TILs exhibited improved clinical outcomes. Although TIL signatures failed to stratify EGC patients with a higher risk of recurrence, a high density of CD3+ TILs at the TC was identified as a predictive factor of chemotherapy efficacy, which may help in guiding therapeutic decisions.

Acknowledgements. This study was supported by the Shenzhen Foundation of Science and Technology (JCYJ20210324111013036 and JCYJ20190809160001751), and Funds for Scholar of Shenzhen Baoan People's Hospital (BAYYZDXK001).

A conflict of interest statement

The authors report no conflicts of interest in this work.

Data availability statement

Data are available from the corresponding author upon reasonable request.

References

Althobiti M., Aleskandarany M.A., Joseph C., Toss M., Mongan N., Diez-Rodriguez M., Nolan C.C., Ashankyty I., Ellis I.O., Green A.R. and Rakha E.A. (2018). Heterogeneity of tumour-infiltrating lymphocytes in breast cancer and its prognostic significance. Histopathology 73, 887-896.

Bindea G., Mlecnik B., Tosolini M., Kirilovsky A., Waldner M., Obenauf A.C., Angell H., Fredriksen T., Lafontaine L., Berger A., Bruneval P.,

Fridman W.H., Becker C., Pagès F., Speicher M.R., Trajanoski Z. and Galon J. (2013). Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 39, 782-795.

Dai C., Geng R., Wang C., Wong A., Qing M., Hu J., Sun Y., Lo A.W. and Li J. (2016). Concordance of immune checkpoints within tumor immune contexture and their prognostic significance in gastric cancer. Mol. Oncol. 10, 1551-1558.

Dieu-Nosjean M.C., Antoine M., Danel C., Heudes D., Wislez M., Poulot V., Rabbe N., Laurans L., Tartour E., de Chaisemartin L., Lebecque S., Fridman W.H. and Cadranel J. (2008). Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J. Clin. Oncol. 26, 4410-4417.

Eusebi L.H., Telese A., Marasco G., Bazzoli F. and Zagari R.M. (2020). Gastric cancer prevention strategies: A global perspective. J. Gastroenterol. Hepatol. 35, 1495-1502.

Fridman W.H., Zitvogel L., Sautès-Fridman C. and Kroemer G. (2017). The immune contexture in cancer prognosis and treatment. Nat. Rev. Clin. Oncol. 14, 717-734.

Galon J., Costes A., Sanchez-Cabo F., Kirilovsky A., Mlecnik B., Lagorce-Pagès C., Tosolini M., Camus M., Berger A., Wind P., Zinzindohoué F., Bruneval P., Cugnenc P.H., Trajanoski Z., Fridman W.H. and Pagès F. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313, 1960-1964.

Galon J., Angell H.K., Bedognetti D. and Marincola F.M. (2013). The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity 39, 11-26.

Galon J., Mlecnik B., Bindea G., Angell H.K., Berger A., Lagorce C., Lugli A., Zlobec I., Hartmann A., Bifulco C., Nagtegaal I.D., Palmqvist R., Masucci G.V., Botti G., Tatangelo F., Delrio P., Maio M., Laghi L., Grizzi F., Asslaber M., D'Arrigo C., Vidal-Vanaclocha F., Zavadova E., Chouchane L., Ohashi P.S., Hafezi-Bakhtiari S., Wouters B.G., Roehrl M., Nguyen L., Kawakami Y., Hazama S., Okuno K., Ogino S., Gibbs P., Waring P., Sato N., Torigoe T., Itoh K., Patel P.S., Shukla S.N., Wang Y., Kopetz S., Sinicrope F.A., Scripcariu V., Ascierto P.A., Marincola F.M., Fox B.A. and Pagès F. (2014). Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. J. Pathol. 232, 199-209.

Gambardella V. and Cervantes A. (2018). Precision medicine in the adjuvant treatment of gastric cancer. Lancet Oncol. 19, 583-584.

Glaire M.A., Domingo E., Sveen A., Bruun J., Nesbakken A., Nicholson G., Novelli M., Lawson K., Oukrif D., Kildal W., Danielsen H.E., Kerr R., Kerr D., Tomlinson I., Lothe R.A. and Church D.N. (2019). Tumour-infiltrating $CD8^+$ lymphocytes and colorectal cancer recurrence by tumour and nodal stage. Br. J. Cancer 121, 474-482.

Jiang Y., Zhang Q., Hu Y., Li T., Yu J., Zhao L., Ye G., Deng H., Mou T., Cai S., Zhou Z., Liu H., Chen G., Li G. and Qi X. (2018). ImmunoScore signature: A prognostic and predictive tool in gastric cancer. Ann. Surg. 267, 504-513.

Joyce J. A. and Pollard J.W. (2009). Microenvironmental regulation of metastasis. Nat. Rev. Cancer 9, 239-252.

Kang B.W., Seo A.N., Yoon S., Bae H.I., Jeon S.W., Kwon O.K., Chung H.Y., Yu W., Kang H. and Kim J.G. (2016). Prognostic value of tumor-infiltrating lymphocytes in Epstein-Barr virus-associated gastric cancer. Ann. Oncol. 27, 494-501.

Katai H., Ishikawa T., Akazawa K., Isobe Y., Miyashiro I., Oda I., Tsujitani S., Ono H., Tanabe S., Fukagawa T., Nunobe S., Kakeji Y. and Nashimoto A., Registration Committee of the Japanese Gastric Cancer Association (2018). Five-year survival analysis of surgically resected gastric cancer cases in Japan: a retrospective analysis of more than 100,000 patients from the nationwide registry of the Japanese Gastric Cancer Association (2001-2007). Gastric Cancer 21, 144-154.

Kim J.Y., Kim C.H., Lee Y., Lee J.H. and Chae Y.S. (2017). Tumour infiltrating lymphocytes are predictors of lymph node metastasis in early gastric cancers. Pathology 49, 589-595.

Laumont C.M., Wouters M.C.A., Smazynski J., Gierc N.S., Chavez E.A., Chong L.C., Thornton S., Milne K., Webb J.R., Steidl C. and Nelson B.H. (2021). Single-cell profiles and prognostic impact of tumor-infiltrating lymphocytes coexpressing CD39, CD103, and PD-1 in ovarian cancer. Clin. Cancer Res. 27, 4089-4100.

Lee H.E., Chae S.W., Lee Y.J., Kim M.A., Lee H.S., Lee B.L. and Kim W.H. (2008). Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer. Br. J. Cancer 99, 1704-1711.

Liu K., Yang K., Wu B., Chen H., Chen X., Chen X., Jiang L., Ye F., He D., Lu Z., Xue L., Zhang W., Li Q., Zhou Z., Mo X. and Hu J. (2015). Tumor-Infiltrating immune cells are associated with prognosis of gastric cancer. Medicine 94, e1631.

Machlowska J., Baj J., Sitarz M., Maciejewski R. and Sitarz R. (2020). Gastric cancer: Epidemiology, risk factors, classification, genomic characteristics and treatment strategies. Int. J. Mol. Sci. 21, 4012.

McAllister S.S. and Weinberg R.A. (2014). The tumour-induced systemic environment as a critical regulator of cancer progression and metastasis. Nat. Cell Biol. 16, 717-727.

Morris M., Platell C. and Iacopetta B. (2008). Tumor-infiltrating lymphocytes and perforation in colon cancer predict positive response to 5-fluorouracil chemotherapy. Clin. Cancer Res. 14, 1413-1417.

Nagtegaal I.D., Quirke P. and Schmoll, H.J. (2011). Has the new TNM classification for colorectal cancer improved care? Nat. Rev. Clin. Oncol. 9, 119-123.

Pietras K. and Ostman A. (2010). Hallmarks of cancer: interactions with the tumor stroma. Exp. Cell. Res. 316, 1324-1331.

Siegel R.L., Miller K.D., Fuchs H.E. and Jemal A. (2022). Cancer statistics, 2022. CA Cancer J. Clin. 72, 7-33.

Sumiyama K. (2017). Past and current trends in endoscopic diagnosis for early stage gastric cancer in Japan. Gastric Cancer 20, 20-27.

Uppal A., Dehal A., Chang S.C., Barrak D., Naeini Y., Jalas J.R. and Bilchik A.J. (2020). The immune microenvironment impacts survival in western patients with gastric adenocarcinoma. J. Gastrointest. Surg. 24, 28-38.

Van Cutsem E., Sagaert X., Topal B., Haustermans K. and Prenen H. (2016). Gastric cancer. Lancet 388, 2654-2664.

Wakatsuki K., Sho M., Yamato I., Takayama T., Matsumoto S., Tanaka T., Migita K., Ito M., Hotta K. and Nakajima Y. (2013). Clinical impact of tumor-infiltrating $CD45RO^+$ memory T cells on human gastric cancer. Oncol. Rep. 29, 1756-1762.

Wang B., Xu D., Yu X., Ding, T., Rao, H., Zhan Y., Zheng L. and Li L. (2011). Association of intra-tumoral infiltrating macrophages and regulatory T cells is an independent prognostic factor in gastric cancer after radical resection. Ann. Surg. Oncol. 18, 2585-2593.

Wu B., Wu D., Wang M. and Wang G. (2008). Recurrence in patients following curative resection of early gastric carcinoma. J. Surg. Oncol. 98, 411-414.

Yamamoto M., Yamanaka T., Baba H., Kakeji Y. and Maehara Y.

(2008). The postoperative recurrence and the occurrence of second primary carcinomas in patients with early gastric carcinoma. J. Surg. Oncol. 97, 231-235.

Zhang Y., Zhao Z.X., Gao J.P., Huang Y.K. and Huang H. (2023). Tumor-infiltrating $CD226^{+}CD8^{+}$ T cells are associated with postoperative prognosis and adjuvant chemotherapeutic benefits in gastric cancer patients. J. Cancer Res. Clin. Oncol. 149, 4381-4389.

Zitvogel L., Apetoh L., Ghiringhelli F. and Kroemer G. (2008). Immunological aspects of cancer chemotherapy. Nat. Rev. Immunol. 8, 59-73.

Accepted September 30, 2024