Retrospective Cohort Study

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Association of systemic inflammatory markers and tertiary lymphoid structure with pathological complete response in gastric cancer patients receiving preoperative treatment: a retrospective cohort study

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Background: Assessment of systemic and local immune responses is crucial in determining the efficacy of cancer interventions. The identification of specific factors that correlate with pathological complete response (pCR) is essential for optimizing treatment decisions.

Methods: In this retrospective study, a total of 521 patients diagnosed with gastric adenocarcinoma who underwent curative gastrectomy following preoperative treatment were reviewed. Of these patients, 463 did not achieve pCR (non-pCR) and 58 achieved pCR. Clinicopathological factors were evaluated to identify predictors for pCR using a logistic regression model. Additionally, a smaller cohort ($n = 76$) was derived using propensity score matching to investigate local immune response, specifically the features of tertiary lymphoid structure (TLS) using H&E staining, immunohistochemistry, and multiplex immunofluorescence.

Results: The multivariate regression analysis demonstrated a significant association between low systemic inflammatory status and pCR, as evidenced by reduced levels of the combined systemic immune-inflammation index (SII) and neutrophil-to-lymphocyte ratio (NLR) (SII + NLR) (odds ratio: 3.33, 95% CI: 1.79–6.17, $P < 0.001$). In the smaller cohort analysis, distinct TLS characteristics were correlated with the presence of pCR. Specifically, a higher density of TLS and a lower proportion of PD1+ cells and CD8+ cells within TLS in the tumor bed were strongly associated with pCR.

Conclusion: Both systemic and local immune profile were associated with pCR. A low level of SII + NLR served as an independent predictor of pCR, while distinct TLS features were associated with the presence of pCR. Focusing on the immune profile was crucial for optimal management of gastric cancer patients receiving preoperative treatment.

Keywords: gastric cancer, pathological complete response, preoperative treatment, systemic inflammatory marker, tertiary lymphoid structure

Introduction

Preoperative treatments, including neoadjuvant and conversion therapy, are commonly employed in gastric cancer to downstage tumors, enhance R0 resection rates, and increase the likelihood of curative surgery. Pathological complete response (pCR), which represents the complete eradication of gastric cancer cells by preoperative treatments, holds promise as the surrogate marker for long-term survival[1–3]. pCR is a distinct phenomenon from the broader category of patients with partial pathological/clinical treatment response. This has drawn the attention of breast cancer and colorectal cancer surgeons, as such patients may be suitable candidates for de-escalation of surgical treatment in the current landscape of multidisciplinary medicine[4,5]. However, advancing this concept requires the identification of reliable predictors for pCR. Unfortunately, such discoveries have not been extensively carried out in the context of gastric cancer.

Inflammation is a hallmark of cancer development and progression[6]. Given that cancer is a systemic disease, the systemic inflammatory response has been linked to treatment

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response in a variety of cancers[7]. The systemic immune-inflammation index (SII) and neutrophil-to-lymphocyte ratio (NLR) are widely-used biomarkers for evaluating preoperative partial treatment response in gastric cancer. However, the association between the combined SII and NLR with pCR has not been assessed[8–12].

Chronic exposure to inflammatory signals can lead to the emergence of tertiary lymphoid structures (TLSs) in the local tumor microenvironment, which indicates regions of active immune reactions against cancer[13,14]. Distinct features of TLS and their predictive role in pCR rates have been described in lung cancer[15,16], breast cancer[17,18], and colorectal cancer[19,20], but their relationship with pCR in gastric cancer is not yet clear.

In this study, we aimed to investigate the association of the immune profile with pCR in patients with gastric cancer, taking into account both the systemic immune status and the TLS in the local immune microenvironment. Specifically, we explored the correlation between the combined systemic immune biomarkers (SII + NLR), TLS density, the components of TLS and pCR. Our research yielded significant findings regarding the relationship between the immune profile and pCR. These insights can guide clinicians in making informed decisions about the optimal treatment strategies for patients with gastric cancer.

HIGHLIGHTS

- The preoperative treatment response in patients with gastric cancer was influenced by both systemic and local immune responses.
- A lower level of combined systemic immune-inflammation index and neutrophil-to-lymphocyte ratio was predictive of pathological complete response (pCR) as systemic immune factors.
- Unique tertiary lymphoid structure features were associated with the presence of pCR as a local immune factors.
- Incorporating immunotherapy into a multimodal preoperative treatment regimen induced a higher rate of pCR and impacted both systemic and local immune responses.

Methods

Study design

This was a retrospective single-center study. We adhered to the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS, Supplemental Digital Content 1, http://links.lww.com/JS9/B54) 2021 guidelines to ensure the quality of our reporting[21]. Ethical approval for this study was provided by the ethical committee of our center on 15 July 2023.

Patients

The study reviewed records of patients with gastric adenocarcinoma who received preoperative chemotherapy, with or without radiotherapy and immunotherapy, followed by curative gastrectomy (R0 resection) during the period spanning from June 2010 to May 2022. Exclusion criteria were as follow: other histology subtype including squamous cell carcinoma, neuroendocrine tumors, and stromal tumors; concurrent primary malignant disease of other organs; R1/R2 resection; unclear preoperative treatment regimen. Ultimately, a total of 521 patients were included in the study, of which 463 were non-pCR patients and 58 patients received pCR.

Blood parameters

Venous blood test data after the completion of preoperative therapy and prior to surgery were collected. SII and NLR were calculated using the formulas (neutrophils × platelets)/lymphocytes and neutrophils/lymphocytes, respectively.

Pathological complete response

pCR was defined as the absence of any remaining tumor component in both the primary tumor and the dissected regional lymph node[22].

Pathological analysis

Paraffin-embedded surgical specimens were cut into serial sections with a thickness of 4 μm. All sections were taken from either peripheral tumor tissue or tissue showing regression in patients who achieved pCR.

Immunohistochemistry (IHC) staining and multiplex immunofluorescence (mIF) staining were performed to detect the presence of antigens CD19 (90176S, Cell Signaling Technology), CD8 (D263403, Sangon Biotech), PD1 (ab137132, abcam) and FOXP3 (ab215206, abcam). Standard IHC procedures were followed, including dewaxing and rehydration of tissue sections, antigen retrieval, and blockade of endogenous peroxidase for IHC or crosslinking for mIF. Sections were then blocked and incubated with primary antibodies overnight at 4°C for IHC or incubated with primary antibodies at room temperature for 1 h for mIF. After washing, horseradish peroxidase conjugated secondary antibody (Servicebio) incubation was performed. For IHC, 3,3'-diaminobenzidine solution (DAB) (DAKO) colorization and hematoxylin counterstaining were performed. For mIF, fluorescent staining amplification solution (Absin) was used.

Quantitative analysis of hematoxylin and eosin (H&E) staining and IHC/mIF staining was performed on digitally scanned whole slide images (WSI) using Qupath software (v0.4.3).

TLS were identified based on their morphology on H&E staining slides. Only mature TLS (maturation classification ii and iii[23]) were evaluated in this study. TLS were categorized into two group according to their location: those in the tumor bed and those in adjacent normal tissue. The tumor bed was defined as the area that included the residual tumor, the tumor stroma, and the regression bed. TLS density was calculated as the number of TLS per cm^2 of the whole slide area. If a single WSI contained more than five TLS, five were randomly selected for component analysis. If there were fewer than five TLS, all were included in the analysis. The proportion of positive cells within the TLS was determined using the cell detection function in Qupath.

Statistical analysis

The nonparametric data were summarized as median and interquartile range, and categorial data were presented as absolute numbers and percentages. The optimal cut-off values of SII and NLR were determined by the receiver operating characteristic curve, and patients were categorized into high and low groups accordingly. Patients were further stratified based on the combination of SII and NLR into high SII + NLR (either or both

variables were high) and low SII + NLR (both variables were low). Differences were evaluated by the Mann–Whitney *U*-test for nonparametric numeric data, and Pearson's χ^2test or Fisher's exact test for categorical data. Overall survival (OS) was defined as the time interval from initial diagnosis (pathology-confirmed) to death of any cause or to the last follow-up. The Kaplan–Meier method was used to analyze OS, and differences were compared using log-rank tests.

Univariate and multivariate analyses were performed using a logistic regression model. Variables that showed statistical significance in the univariate analysis were included in the multivariate analysis. The odds ratio (OR) was reported with a 95% CI.

To evaluate the association between TLS and pCR, a smaller cohort (38 pairs) was extracted using propensity score matching. A 1:1 ratio PSM method was employed, utilizing optimal matching, exact matching, and no replacement. Optimal matching was used to match tumor site, cT stage, cN stage, signet-ring cell carcinoma, and SII + NLR grade, while exact matching was used to match unresectable reason and combination with immunotherapy. The TLS parameters were compared using either a paired nonparametric Wilcoxon test or an unpaired nonparametric Mann–Whitney test. For the analysis of TLS components, the average TLS parameters were first calculated for each WSI slide, followed by subsequent comparison tests.

All statistical tests were two-sided and *P*-values < 0.05 were considered statistically significant.

Statistical analysis was performed using SPSS software (SPSS Statistics, version 26) and R (4.1.2).

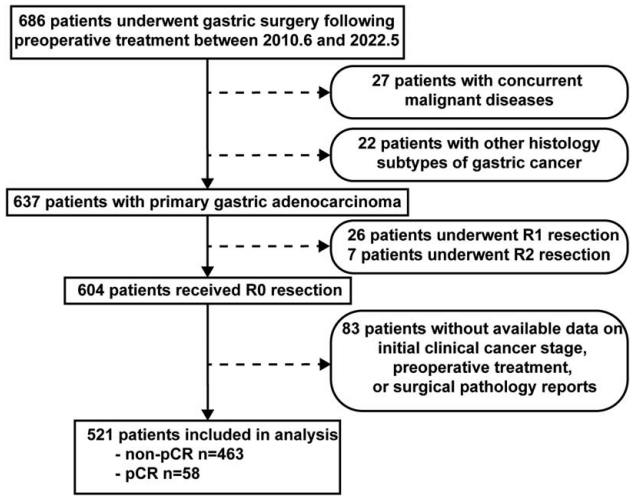

Figure 1. Study flowchart of patient inclusion and exclusion criteria for the analysis. A total of 686 patients underwent gastric surgery following preoperative treatment between June 2010 and May 2022. Of these, 27 patients with concurrent malignant diseases and 22 patients with other histology subtypes of gastric cancer were excluded. Additionally, 26 patients who underwent R1 resection and 7 patients who underwent R2 resection were excluded. Of the remaining 604 patients who received R0 resection, 83 patients did not have available data on initial clinical cancer stage, preoperative treatment, or surgical pathology reports were also excluded. A total of 521 patients were included in the final analysis. Of these patients, 463 did not achieve pathological complete response (non-pCR) and 58 achieved pCR.

Results

Baseline characteristics

The study flowchart was presented in Figure 1. A total of 521 patients who underwent R0 gastrectomy following preoperative treatment between June 2010 and May 2022 were included for analysis (Table 1).

Based on the receiver operating characteristic curve analysis, the cut-off points for SII and NLR were 215.66 ($\times 10^9$/l) and 1.36, respectively. Patients were stratified into SII high (70.2%) and low (29.8%) groups, as well as NLR high (70.6%) and low (29.4%) groups. Furthermore, by combining SII and NLR, patients were classified into SII + NLR grade high (79.1%) and low (20.9%) groups (Table 1).

Among the 521 patients, 58 (11.1%) patients achieved pCR after preoperative treatment. The non-pCR patients had a median OS time of 56 months, while the median OS for pCR patients was not reached. The pCR patients demonstrated a significantly better OS (hazard ratio: 2.968, 95% CI: 1.89–4.66, $P = 0.002$) (Fig. 2).

Pretreatment patient and tumor characteristics and pCR

In the univariate analysis (Table 2), prognostic factors including tumor location and signet-ring cell subtype were significantly associated with pCR. Specifically, patients with upper 1/3 gastric cancer (OR: 2.57, 95% CI: 1.15–5.75, $P = 0.021$) and lower 1/3 gastric cancer (OR: 2.50, 95% CI: 1.13–5.50, $P = 0.023$) had a higher rate of pCR than those with middle 1/3 gastric cancer. Additionally, patients with nonsignet-ring cell type had a higher rate of pCR compared to those with signet-ring cell type (OR: 2.94, 95% CI: 1.30–6.64, $P = 0.01$), and this remained to be an independent predictor of pCR in multivariate analysis (OR: 2.63, 95% CI: 1.13–6.14, $P = 0.025$) (Table 3).

Post-treatment peripheral blood biomarkers and pCR

In the analysis of post-treatment peripheral blood biomarkers and their association with pCR, univariate analysis revealed that neutrophil count (OR: 0.74, 95% CI: 0.58–0.96, $P = 0.023$), platelet count (OR: 0.99, 95% CI: 0.99–1.00, $P = 0.016$), and lymphocyte count (OR: 0.49, 95% CI: 0.27–0.88, $P = 0.017$) were significantly associated with pCR rate (Table 2). To gain a more comprehensive understanding of these three parameters, the SII and NLR indexes were subsequently calculated. Patients with lower SII (OR: 2.28, 95% CI: 1.31–3.97, $P = 0.004$) and NLR (OR: 1.98, 95% CI: 1.14–3.47, $P = 0.016$) exhibited higher rates of pCR (Table 2). Notably, the predictive value of the SII and NLR was more pronounced when the two indexes were combined. A lower SII + NLR grade demonstrated a significant predictive role in achieving pCR in both univariate (OR: 2.88, 95% CI: 1.62–5.12, $P < 0.001$) (Table 2) and multivariate analysis (OR: 3.33, 95% CI: 1.79–6.17, $P < 0.001$) (Table 3).

Tertiary lymphoid structure and pCR

A subset of 38 pairs of patients was selected from the original cohort using the PSM method. The baseline characteristics between the two groups in the smaller cohort, which had exhibited significant differences in the larger cohort, were balanced (SDC, Table 1, Supplemental Digital Content 2, http://links.lww.com/JS9/B55).

Table 1

Clinicopathological factors of included patients.

	N = 521
Pretreatment patient and tumor characteristics	
Sex (%)	
Male	396 (76.0)
Female	125 (24.0)
Age of diagnosis [median (IQR), yr)	62.0 [54.0, 68.0]
Tumor site (%)	
Upper 1/3	169 (32.4)
Middle 1/3	156 (29.9)
Lower 1/3	196 (37.6)
cT stage (%)	
cT3	90 (17.3)
cT4	388 (74.5)
Unclear	43 (8.3)
cN stage (%)	
N0	9 (1.7)
N+	502 (96.4)
Unclear	10 (1.9)
cM stage (%)	
M0	415 (79.7)
M1	106 (20.3)
Unresectable reason (%)	
Locally unresectable	30 (22.7)
Retroperitoneal or distant region lymph nodes	27 (20.5)
Positive peritoneal lavage cytology	8 (6.1)
Peritoneal cavity metastasis	34 (25.8)
Distant organ metastasis	33 (25.0)
Signet-ring cell carcinoma (%)	
No	381 (73.1)
Yes	140 (26.9)
Lauren classification (%)	
Intestinal	217 (41.7)
Diffuse	128 (24.6)
Mixed	146 (28.0)
Unclear	30 (5.8)
Stage of differentiation (%)	
Poorly differentiated	300 (57.6)
Well differentiated	211 (40.5)
Unclear	10 (1.9)
Preoperative treatment	
Treatment type	
Neoadjuvant	391 (75.0)
Conversion	130 (25.0)
Number of chemotherapy agent (%)	
2	323 (62.0)
≥3	198 (38)
Combination with immunotherapy	
No	469 (90.0)
Yes	52 (10.0)
Post-treatment peripheral markers	
Hemoglobin [median (IQR), g/l]	118.0 [108.0, 130.0]
Neutrophil count [median (IQR), $\times 10^9$/l]	2.6 [2.0, 3.4]
Platelet count [median (IQR), $\times 10^9$/l]	162.0 [126.0, 210.0]
Lymphocyte count [median (IQR), $\times 10^9$/l]	1.4 [1.1, 1.8]
Monocyte count [median (IQR), $\times 10^9$/l]	0.5 [0.4, 0.6]
LDH [median (IQR), U/l]	185.0 [163.0, 209.5]
ALB [median (IQR), g/l]	40.0 [37.0, 43.0]
SII (%)	
High	366 (70.2)
Low	155 (29.8)
NLR (%)	
High	368 (70.6)
Low	153 (29.4)
SII + NLR (%)	
High	412 (79.1)
Low	109 (20.9)
Pathological response	
pCR (%)	
No	463 (88.9)
Yes	58 (11.1)
Becker-TRG (%)	
1a	63 (12.1)
1b	51 (9.8)
2	168 (32.2)
3	239 (45.9)

ALB, albumin; IQR, interquartile range; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; pCR, pathological complete response; SII, systemic immune-inflammation index; TRG, tumor regression grade; yr, year.

The density of tumor bed TLS was significantly higher in pCR patients compared to non-pCR patients ($P = 0.009$) (Fig. 3A). Notably, this difference was observed specifically in tumor bed TLS and not in normal tissue TLS. Moreover, pCR patients had a higher density of tumor bed TLS compared to that of normal tissue TLS ($P < 0.001$), while no such difference was observed in non-pCR patients. Further analysis of the TLS components (Fig. 3B) revealed a decreased proportion of PD1+ cells within TLS in both tumor bed ($P < 0.001$) and normal tissue ($P = 0.011$) in pCR patients, while a decreased proportion of CD8+ cells within TLS was only detected in tumor bed ($P = 0.038$). No significant difference was observed between pCR and non-pCR patients in the proportion of FOXP3+ or CD19+ cells. Additionally, a consistent trend was observed in the proportion of PD1+ and FOXP3+ cells in both non-pCR ($P = 0.018$,

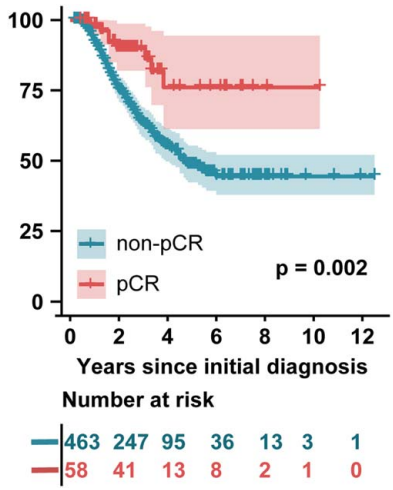

Figure 2. Kaplan–Meier curves of overall survival in patients with or without pathological complete response. A total of 521 patients were included in the analysis, of which 58 patients belonged to the pCR group and 463 patients belonged to the non-pCR group. The median survival of the pCR group was not reached, while it was 56 months for the non-pCR group. The overall survival (OS) of the pCR group was superior to the non-pCR group, with a log-rank test *P*-value of 0.002.

Table 2

Univariate analysis: factors associated with pathological complete response.

	non-pCR	pCR			95% CI		
	N = 463	*N* = 58	*P*[a]	OR	lower	upper	*P*[a]
Pretreatment patient and tumor characteristics							
Sex (%)							
Male	349 (75.4)	47 (81.0)	0.416	1.40	0.70	2.78	0.343
Female	114 (24.6)	11 (19.0)		1.00			
Age of diagnosis [median (IQR), yr]	62.0 [53.5, 68.0]	63.5 [56.0, 68.8]	0.231	1.02	0.99	1.05	0.174
Tumor site (%)							
Upper 1/3	146 (31.5)	23 (39.7)	**0.031**	2.57	1.15	5.75	**0.021**
Middle 1/3	147 (31.7)	9 (15.5)		1.00			
Lower 1/3	170 (36.7)	26 (44.8)		2.50	1.13	5.50	**0.023**
cT stage (%)							
cT3	79 (17.1)	11 (19.0)	0.71	1.15	0.57	2.33	0.704
cT4	346 (74.7)	42 (72.4)		1.00			
Unclear[b]	38 (8.2)	5 (8.6)					
cN stage (%)							
N0	8 (1.7)	1 (1.7)	1	1.00	0.12	8.11	0.997
N +	446 (96.3)	56 (96.6)		1.00			
Unclear[b]	9 (1.9)	1 (1.7)					
cM stage (%)							
M0	374 (80.8)	41 (70.7)	0.083	1.00			
M1	89 (19.2)	17 (29.3)		1.74	0.95	3.21	0.075
Unresectable cancer (%)							
Locally unresectable	27 (23.9)	3 (15.8)	0.249	1.00			
Retroperitoneal or distant region lymph nodes	21 (18.6)	6 (31.6)		2.57	0.58	11.51	0.217
Positive peritoneal lavage cytology	7 (6.2)	1 (5.3)		1.29	0.12	14.33	0.838
Peritoneal cavity metastasis	32 (28.3)	2 (10.5)		0.56	0.09	3.62	0.545
Distant organ metastasis	26 (23.0)	7 (36.8)		2.42	0.57	10.39	0.233
Signet-ring cell carcinoma (%)							
No	330 (71.3)	51 (87.9)	**0.007**	2.94	1.30	6.64	**0.010**
Yes	133 (28.7)	7 (12.1)		1.00			
Lauren classification (%)							
Intestinal	193 (41.7)	24 (41.4)	0.286	1.58	0.64	3.87	0.322
Diffuse	116 (25.1)	12 (20.7)		1.89	0.85	4.20	0.116
Mixed	137 (29.6)	9 (15.5)		1.00			
Unclear[b]	17 (3.7)	13 (22.4)					
Stage of differentiation (%)							
Poorly differentiated	267 (57.7)	33 (56.9)	0.771	1.12	0.63	1.99	0.705
Well differentiated	190 (41.0)	21 (36.2)		1.00			
Unclear[b]	6 (1.3)	4 (6.9)					
Preoperative treatment							
Treatment type							
Neoadjuvant	351 (75.8)	40 (69.0)	0.262	1.00			
Conversion	112 (24.2)	18 (31.0)		1.41	0.78	2.56	0.258
Number of chemotherapy agent (%)							
2	284 (61.3)	39 (67.2)	0.473	1.29	0.73	2.31	0.384
≥ 3	179 (38.7)	19 (32.8)		1.00			
Combination with immunotherapy							
No	426 (92.0)	43 (74.1)	< **0.001**	1.00			
Yes	37 (8.0)	15 (25.9)		4.02	2.04	7.90	< **0.001**
Post-treatment peripheral markers							
Hemoglobin [median (IQR), g/l]	118.0 [108.0, 130.0]	118.0 [112.0, 130.8]	0.781	1.00	0.99	1.02	0.7
Neutrophil count [median (IQR), ×10^9/l]	2.7 [2.0, 3.5]	2.2 [1.6, 2.8]	**0.002**	0.74	0.58	0.96	**0.023**
Platelet count [median (IQR), ×10^9/l]	164.0 [126.5, 217.5]	143.0 [124.2, 171.8]	**0.023**	0.99	0.99	1.00	**0.016**
Lymphocyte count [median (IQR), ×10^9/l]	1.4 [1.2, 1.8]	1.3 [1.0, 1.6]	**0.008**	0.49	0.27	0.88	**0.017**
Monocyte count (median [IQR], ×10^9/L)	0.5 [0.4, 0.6]	0.5 [0.4, 0.6]	0.36	0.49	0.11	2.22	0.356
LDH [median (IQR), U/l]	185.0 [163.2, 209.0]	185.0 [161.0, 210.0]	0.584	1.01	1.00	1.01	0.183
ALB [median (IQR), g/l]	40.0 [37.0, 43.0]	40.0 [38.0, 43.0]	0.443	1.03	0.96	1.10	0.435
SII (%)							
High	335 (72.4)	31 (53.4)	**0.006**	1.00			
Low	128 (27.6)	27 (46.6)		2.28	1.31	3.97	**0.004**
NLR (%)							
High	335 (72.4)	33 (56.9)	**0.021**	1.00			

Table 2

(*Continued*)

	non-pCR	pCR			95% CI		
	N = 463	*N* = 58	*P*[a]	OR	lower	upper	*P*[a]
Low	128 (27.6)	25 (43.1)		1.98	1.14	3.47	**0.016**
SII + NLR (%)							
High	377 (81.4)	35 (60.3)	< **0.001**	1.00			
Low	86 (18.6)	23 (39.7)		2.88	1.62	5.12	< **0.001**

[a]Significant *P*-values are indicated in bold.
[b]Unclear and mixed are not included in analysis.
ALB, albumin; IQR, interquartile range; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; pCR, pathological complete response; SII, systemic immune-inflammation index; yr, year.

$P = 0.003$) and non-pCR ($P = 0.035$, $P = 0.004$) groups, which were found to be higher in normal tissue TLS compared to tumor bed TLS. The representative IHC staining figures of TLS were displayed in Figure 3C.

To corroborate the results from IHC analysis, the mIF technique was employed on a subset of the IHC cohort (nine pairs) (Fig. 4). The results consistently demonstrated reduced proportions of PD1 + and CD8 + cells within TLS in the tumor bed of pCR patients compared to non-pCR patients ($P = 0.012$, $P = 0.008$) (Fig. 4B–C).

Preoperative treatment and pCR

As shown in Table 3, incorporating immunotherapy into preoperative treatments was independently associated with a higher likelihood of achieving pCR (OR: 4.97, 95% CI: 2.38–10.38, $P < 0.001$).

To investigate the potential effect of immunotherapy on systemic and local immune response, subgroup analyses were performed (SDC Table 2, Supplemental Digital Content 3, http://links.lww.com/JS9/B56, SDC Fig. 1, Supplemental Digital Content 4, http://links.lww.com/JS9/B57 and SDC Figure 2, Supplemental Digital Content 5, http://links.lww.com/JS9/B58). In terms of systemic immunity, the incorporation of immunotherapy was associated to higher level of SII, NLR and combined SII + NLR in pCR patients ($P = 0.033$, $P = 0.008$, $P = 0.030$). However, no similar effect was observed in the non-pCR group (SDC Table 2, Supplemental Digital Content 3, http://links.lww.com/JS9/B56, SDC Fig. 1, Supplemental Digital Content 4, http://links.lww.com/JS9/B57). Regarding local immunity, immunotherapy was linked to a lower density of TLS in normal tissue (SDC, Fig. 2A, Supplemental Digital Content 5, http://links.lww.com/JS9/B58). Immunotherapy also demonstrated distinct effects on the composition of tumor bed TLS. Specifically, the addition of immunotherapy led to a higher proportion of PD1 + cells in pCR patients ($P = 0.001$) (SDC, Fig. 2B, Supplemental Digital Content 5, http://links.lww.com/JS9/B58), a lower proportion of CD8 + cells in non-pCR patients ($P = 0.024$) (SDC, Fig. 2C, Supplemental Digital Content 5, http://links.lww.com/JS9/B58), and a higher proportion of FOXP3 + cells in the total cohort ($P = 0.021$) (SDC, Fig. 2D, Supplemental Digital Content 5, http://links.lww.com/JS9/B58). The proportion of CD19 + cells remained comparable (SDC, Fig. 2D, Supplemental Digital Content 5, http://links.lww.com/JS9/B58). Immunotherapy did not result in notable changes in the composition of normal tissue TLS.

Table 3

Multivariate analysis: factors associated with pathological complete response.

		95% CI		
	OR	lower	upper	*P*[a]
Pretreatment characteristics				
Tumor site				
Upper 1/3	1.95	0.85	4.50	0.116
Middle 1/3	1.00			
Lower 1/3	2.21	0.97	5.03	0.058
Signet-ring cell carcinoma (%)				
No	2.63	1.13	6.14	**0.025**
Yes	1.00			
Preoperative treatment				
Combination with immunotherapy				
No	1.00			
Yes	4.97	2.38	10.38	< **0.001**
Post-treatment systemic inflammatory marker				
SII + NLR (%)				
High	1.00			
Low	3.33	1.79	6.17	< **0.001**

[a]Significant *P*-values are indicated in bold.
NLR, neutrophil-to-lymphocyte ratio; SII, systemic immune-inflammation index.

Discussion

Cancer is a complicated disease, and systemic and local immune responses dictate the fate of cancer patients[24–26]. In this retrospective study, we aimed to investigate the correlation between systemic inflammatory markers (SII + NLR) and TLS in the local TME with pCR in patients with gastric cancer.

The correlation between systemic immune response and treatment response is significant, as a patient's overall immune system activity plays a crucial role in determining the effectiveness of therapeutic interventions[27,28]. Peripheral blood markers serve as convenient and low-cost indicators of systemic immune status. Post-treatment low levels of SII and NLR have been found to be associated with treatment response in various malignancies[29–33], though different cut-off values were applied for lack of standards. Here we report that a low level of SII + NLR following preoperative treatment serves as an independent predictor of pCR (Table 3). Neutrophilia is known to promote angiogenesis and mediate of immunosuppression[34], while the levels of circulating lymphocytes have been linked to tumor-infiltrating lymphocytes and the antitumor T-cell responses[35]. Additionally, platelets are

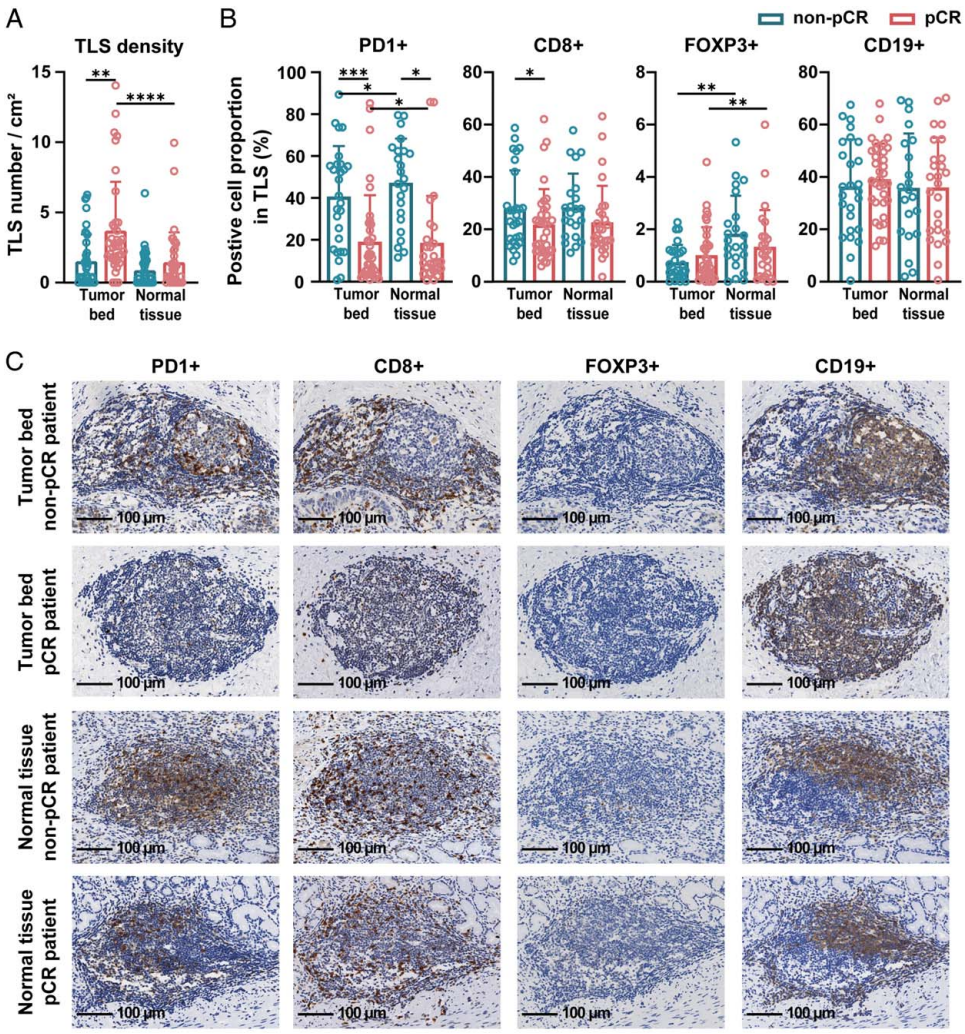

Figure 3. Characteristics of tertiary lymphoid structures detected by immunohistochemistry. (A) The density of tertiary lymphoid structure (TLS) was quantified as the number of TLS per square centimeter of tissue area on the whole slide, and a comparison was made between matched patients who achieved pCR and those who did not. (B) The proportion of cells positive for PD1, CD8, FOXP3, and CD19 within TLS was quantified using immunohistochemistry (IHC) and compared between matched pCR and non-pCR patients. Each empty circle represented the average proportion for an individual patient, calculated from the whole slide image. (C) Representative IHC images depicting PD1, CD8, FOXP3, and CD19-positive cells within TLS in a matched pair of patients were presented. Statistical significance levels were denoted by asterisks. *, **, ***, and **** represent *P*-values less than 0.05, 0.01, 0.001, and 0.0001, respectively.

recognized for shielding cancer cells from immune surveillance[36]. SII and NLR, derived from these blood parameters, can reflect the balance between protumoral inflammation and antitumor immune response. We propose that low levels of post-treatment systemic inflammatory markers represent a less inflammatory systemic status, which indicates the effective tumor clearance and an increased likelihood of achieving pCR.

pCR patients had a unique local immune profile, reflected by unique TLS features (Fig. 3). TLS can histologically and functionally resemble secondary lymphoid organs, and destroy tumor cells[37,38]. Higher density of TLS is correlated with a favorable prognosis[39] and treatment response[40–42]. Not only the presence but also the localization of TLS is related to its prognostic and predictive potential[43]. Consistent with previous discoveries[18,44], we found that the density of tumor bed TLS was correlated with a higher pCR rate, while the density of nontumoral TLS was comparable between pCR and non-pCR patients (Fig. 3A). The interaction of immune cells within TLS can strongly impact tumor response to treatment, thus analysis on TLS composition is gaining increased attention[16,45]. In our analysis, higher proportion of PD1 + and CD8 + cells within tumor bed TLS were detected in non-pCR patients compared to pCR patients(Fig. 3B–C and Fig. 4B–C), which is consistent with the diverse roles of PD1 in cancer. Expression of PD1 on T cells is a marker of tumor antigen-mediated activation, which remains high under sustained tumor antigen exposure, while it decreases after clearance of activating antigens[46,47]. Moreover, PD1 plays a notorious role in maintaining T cell exhaustion[47], leading to unsatisfactory tumor control. In pCR patients, tumor antigens were cleared, and thus, TLS of pCR patients exhibited lower levels of PD1 + cells and CD8 + T cells. The significantly higher PD1 + cell infiltration in TLS in non-pCR patients also reflected the immune-suppressive status in the microenvironment, likely contributing to the less optimal treatment response.

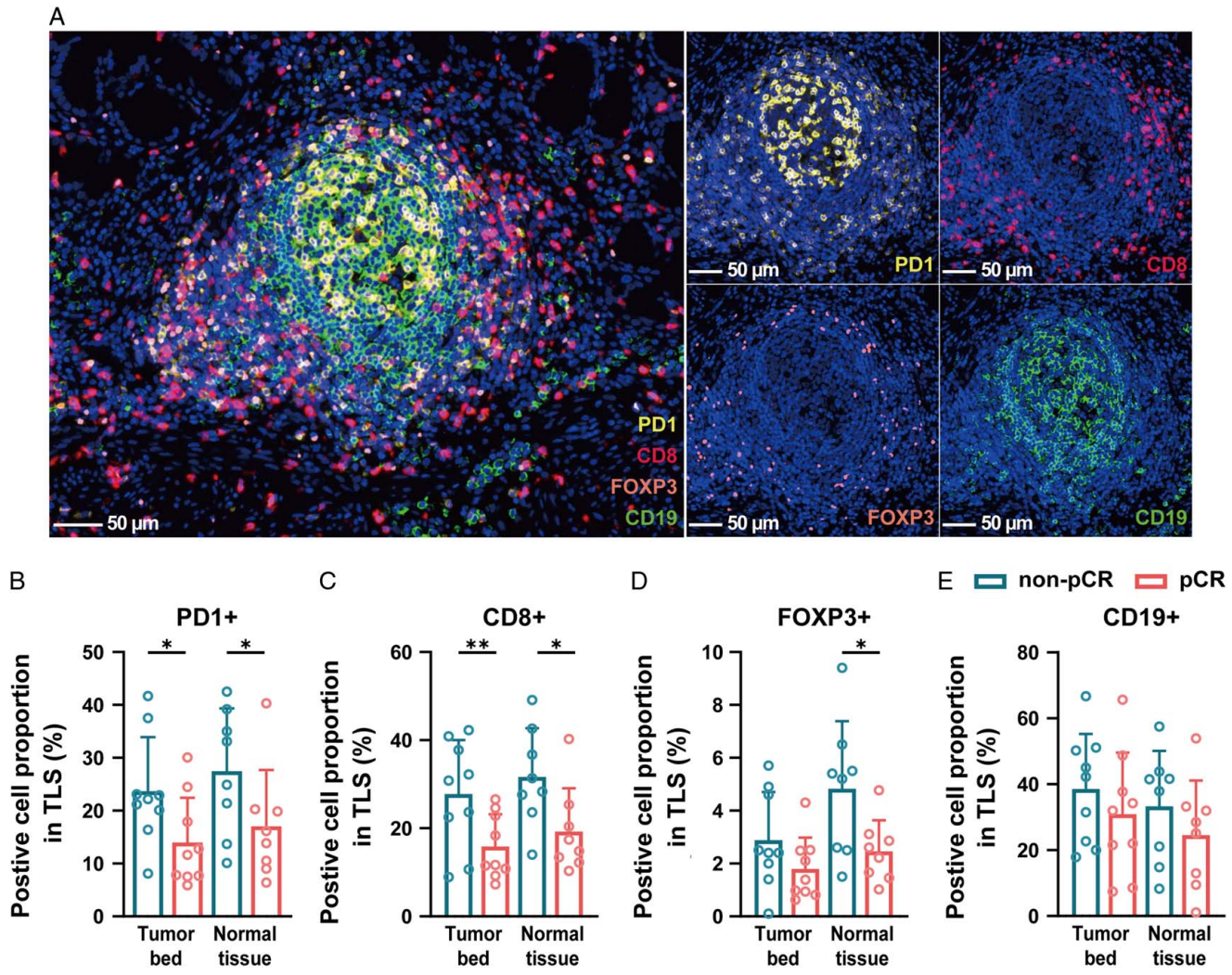

Figure 4. Characteristics of tertiary lymphoid structures detected by immunofluorescence. (A) The presented case depicted a TLS visualized through multicolor immunofluorescence (mIF) staining. The left Images showed the expression of individual markers. (B-E) The proportion of PD1 + , CD8 + , FOXP3 + and CD19 + within TLS in the tumor bed and in the peripheral normal tissue was quantified and compared. Each empty circle represented the average proportion for an individual patient, calculated from the whole slide image. Statistical significance levels were denoted by asterisks. * and ** represented *P*-values less than 0.05 and 0.01, respectively.

Through the integration of systemic and local immune status findings, we have developed an immune profile that is associated with the presence of pCR. This approach is rooted in the cancer-immunity cycle concept[24], which asserts that cancer is a disease in which systemic and local immunity are interconnected, and thus the clearance of tumor can be anticipated by assessing systemic and local immune status. With a more comprehensive understanding of immunity and cancer, immunotherapy has shifted the paradigm of cancer treatment by evoking an immune response or restoring tumor-induced immune deficiency, providing a distinct benefit to cancer patients compared to conventional chemotherapy and radiotherapy[48–52]. The potential impact of immunotherapy combined with multimodal preoperative treatment on achieving pCR in gastric cancer is quite intriguing, as evidenced by several phase II clinical trials[53–55] reporting pCR rates exceeding 30%. Our own data also suggest that combining immunotherapy is associated with an increased pCR rate (Table 3). Through a preliminary analysis of the impact of immunotherapy on both systemic and local immunity characteristics, our findings suggest that the induction of pCR by immunotherapy may differ from that of conventional chemotherapy and radiotherapy (SDC Table 2, Supplemental Digital Content 3, http://links.lww.com/JS9/B56, SDC Fig. 1, Supplemental Digital Content 4, http://links.lww.com/JS9/B57, and SDC Figure 2, Supplemental Digital Content 5, http://links.lww.com/JS9/B58). Previous studies have demonstrated the influence of immunotherapy on systemic immunity[56,57], as well as its ability to modulate the formation and function of TLS in various cancer types[16,40–42,58,59]. However, these studies have primarily focused on the effects of immunotherapy as monotherapy or on the broader population of patients with partial response. There is a dearth of research on its impact as part of preoperative multimodal treatment or on the unique pCR population in gastric cancer. Our findings suggest that further investigation is needed to

gain a more comprehensive understanding of the effects of immunotherapy on immune response and treatment efficacy.

There are several limitations in our study that need to be addressed. Firstly, the utilization of a retrospective design and the reliance on data from a single-center's experience necessitate the need for further validation. Additionally, there may have been bias in the clinical setting, particularly with regards to immunotherapy combined treatment modalities. Patients with dMMR status, high PD-L1 scores, and high tumor mutation burden are more likely to be recommended for immunotherapy and more likely to benefit from it[60], which could have a higher pCR rate. Although we acknowledge this bias, insufficient clinical data on molecular pathological diagnosis prevented us from addressing this issue in our study. Moreover, the data on systemic immune status (SII and NLR) and the local immune profile (TLS) were collected post-treatment, which restricts the possibility of analyzing dynamic changes in these variables and drawing robust conclusions about the causal relationship between immune profile and pCR rate. The implementation of further prospective study design and animal model holds importance in the generation of robust evidence and the elucidation of underlying mechanisms.

Despite the limitations of our study, our findings shed light on the role of immunity in achieving pCR. By reviewing patients over a 12-year period and including a relatively large number of pCR cases, we were able to focus on this unique patient group instead of merging them with partial response patients who still had viable tumors. The immune-related factors we discovered could be derived from routine clinical management, such as peripheral blood tests and gastric endoscopies. This less invasive approach to collecting evidence for pCR prior to surgery could be the first step in reconsidering the paradigm of current gastric cancer surgery in selective patients. This is particularly relevant in the context of the foreseeable increasing pCR rate in gastric cancer in the future. A similar route has already been taken in colorectal cancer and breast cancer[4,5].

Conclusion

In conclusion, this retrospective study revealed an immune profile that correlated with pCR. Specifically, a low level of systemic inflammation, as indicated by a reduced level of SII + NLR, was an independent predictor to pCR. In local TME, an increased TLS density and reduced proportions of PD1 + cells and CD8 + cells within TLS in the tumor bed were associated with the presence of pCR. These findings underscore the significance of considering systemic and local immune profiles when managing gastric cancer patients receiving preoperative treatment.

Ethical approval

Ethical approval for this study (B2023-229) was provided by the Ethical Committee of Zhongshan Hospital, Fudan University on 15 July 2023.

Consent

Written informed consent was obtained from the patient for publication of this study and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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Author contribution

Y.W.: investigation, writing – original draft, writing – review and editing; J.Z.: formal analysis; Z.W.: writing – original draft, writing – review and editing; D.L.: data curation; C.T.: resources; B.Y.: resources; Y.S.: funding acquisition; H.L.: supervision; X.W.: conceptualization and supervision.

Conflicts of interest disclosure

The authors declare that they have no financial conflicts of interest with regard to the content of this report.

Research registration unique identifying number (UIN)

Research Registry UIN: researchregistry9169. Link: https://www.researchregistry.com/registernow#home/registrationdetails/64928022b96d020028e53ade/.

Guarantor

The guarantor for this study is Yingying Wu and Junjie Zhao. They accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

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