APMIS JOURNAL OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY

APMIS 131: 19–25

DOI 10.1111/apm.13277

Tertiary lymphoid structures and gastric cancer prognosis

NIKO KEMI,[1] OLLI YLITALO,[1] JUHA P. VÄYRYNEN,[1,2] OLLI HELMINEN,[1,3] ANNA JUNTTILA,[3] JOHANNA MRENA,[3] JAN BÖHM[2] and JOONAS H. KAUPPILA[1,4]

[1]Cancer and Translational Medicine Research Unit, Medical Research Centre, University of Oulu and Oulu University Hospital, Oulu; [2]Department of Pathology, Central Finland Central Hospital, Jyväskylä; [3]Department of Surgery, Central Finland Central Hospital, Jyväskylä, Finland; and [4]Upper Gastrointestinal Surgery, Department of Molecular medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Kemi N, Ylitalo O, Väyrynen JP, Helminen O, Junttila A, Mrena J, Böhm J, Kauppila JH. Tertiary lymphoid structures and gastric cancer prognosis. APMIS. 2023; 131: 19–25.

Tertiary lymphoid structures (TLSs) are part of immune response against cancer. Their high density and high diameter have been shown to be associated with prognosis in different cancer types. The aim of this study was to examine the prognostic significance of TLS density and diameter in gastric cancer and reproducibility of their assessments. TLS densities and maximal TLS diameter were assessed from hematoxylin–eosin (HE) stained slides of 721 surgically treated gastric cancer patients from two hospitals in Finland. Mortality hazard ratios (HRs) for TLS densities and maximal TLS diameter were analyzed. TLS densities and maximal TLS diameter were assessed with moderate interobserver agreement (Cohen's kappa 0.50–0.62). Maximal TLS density was not associated with survival (adjusted HR 0.85, 95% CI 0.70–1.02) and neither was hotspot TLS density (adjusted HR 0.85, 95% CI 0.70–1.02). High maximal TLS diameter was associated with longer survival in overall study population (adjusted HR 0.74, 95% CI 0.61–0.89) and in diffuse type subgroup (adjusted HR 0.65, 95% CI 0.50–0.85). In conclusion, high maximal TLS diameter is associated with improved survival in gastric cancer and can be assessed from HE-stained slides. Its prognostic value might be limited to diffuse histological type.

Key words: Gastric cancer; lymphocyte; tertiary lymphoid structure; inflammation; prognosis.

Niko Kemi, Cancer and Translational Medicine Research Unit, Medical Research Center, University of Oulu and Oulu University Hospital, Aapistie 5, PO Box 5000, 90014, Oulu, Finland. e-mail: niko.kemi@oulu.fi

Niko Kemi, Olli Ylitalo equally contributed to this work.

INTRODUCTION

Gastric cancer presents with over 1,000,000 new cases and causes an estimated 783,000 deaths per year [1]. The tumor-node-metastasis (TNM) classification is the golden standard of gastric cancer staging, which includes invasion depth of the tumor, extent of lymph node metastasis, and distant metastases [2]. Complementary prognostic factors might be useful to better estimate cancer prognosis, as outcomes are heterogeneous even among patients within the same TNM-stage groups [3].

Tertiary lymphoid structures (TLSs) are ectopic lymphoid structures in non-lymphoid tissue sites that can emerge on sites of chronic inflammation including neoplasms [4]. They functionally resemble lymphoid tissue of lymph nodes that are essential for adaptive immunity, representing sites for lymphocyte activation and proliferation [4]. Cancer-related immune and inflammatory reactions may induce the formation of TLSs, contributing to anti-tumoral immunity [5].

TLSs have been shown to possess prognostic value in various cancers. A higher TLS density is associated with a better prognosis in colorectal cancer [6,7], pancreatic cancer [8], and lung cancer [9,10]. High diameter of TLSs associates with good prognosis in colorectal cancer [11]. TLS might have potential as a clinical prognostic marker in different cancer types [12]. A recent study from China

Received 29 May 2022. Accepted 10 October 2022

suggested association with high TLS density and improved prognosis in gastric cancer [13]. Also high maximal diameter of a TLS might have prognostic value [14]. These previous studies on TLSs in gastric cancer have been done in Eastern cohorts, differing in demographics and survival compared to the West. There is a lack of data on TLSs and gastric cancer in Western populations.

The aim of this study was to evaluate the prognostic value of average and hotspot TLS density and maximal TLS diameter assessed from hematoxylin–eosin (HE) stained slides in a two-center gastric cancer cohort, with subgroup analyses in intestinal and diffuse types.

MATERIALS AND METHODS

Study design

This study was a retrospective cohort study. The study cohort has been previously described [15,16]. Totally, 583 patients from Oulu University Hospital between 1983 and 2016 who underwent gastrectomy for gastric adenocarcinoma and available diagnostic HE slides were included in the study. In addition to this, 138 patients from Central Finland Central Hospital treated in years 1997–2018 with available HE slides were also included. The study was approved by Oulu University Hospital Ethics Committee and the need for informed consent was waived by the Finnish National Authority for Medicolegal Affairs (VALVIRA).

Data collection

The patients were identified from the archives of pathology departments at Oulu University Hospital and Central Finland Central Hospital. Clinical data for each patient were obtained from patient records and pathology reports. The tumor stages were evaluated according to The American Joint Committee on Cancer's 8th edition of TNM classification [2]. The follow-up data were retrieved from the Causes of Death Registry at Statistics Finland. Immutable national personal numbers that are assigned to each resident in the country were used. The follow-up data were complete and available until the end of 2019 from Oulu University Hospital and until the end of August 2019 from Central Finland Central Hospital.

The diagnostic HE-stained slides for each patient used primarily for clinical decision making were obtained from hospital archives. Representative slide with the deepest invasion was selected for each patient and digitalized with Aperio AT2 (Leica Biosystems, Wetzlar, Germany).

Assessment of TLSs

QuPath was used for analysis of scanned slides [17]. TLSs were defined as ectopic lymphoid structures in non-lymphoid tissue sites. The smallest acceptable diameter for a TLS was 200 μm. Assessments were done by N.K. and O.Y, blinded to clinical and follow-up data. Mean values of the two assessors were used for further analysis for all TLS properties that were studied.

Average density of TLSs was assessed as described by Väyrynen *et al.* [7] in colorectal cancer. All qualified TLSs were counted from the slide and the number of TLSs was divided by the length of the invasive edge of the tumor to obtain average TLS density. Patients were divided into high and low average TLS density groups by the median value of density among all patients.

Hotspot density of TLSs was assessed by counting the highest number of individual TLSs in a single field of vision at 4× objective magnification (40× total magnification) in the slide. The area of the field of vision was approximately 9.0 mm^2. If necessary, multiple fields of vision were analyzed and the one with the most TLSs was considered decisive. Patients were divided into high and low hotspot TLS density groups by the median value of density among all patients.

The maximal diameter of the TLSs was assessed similarly to Omura *et al.* [14] by measuring the diameter of the largest TLS in the slide. Several TLSs were measured if necessary, and the one with largest diameter was considered decisive. Patients were divided into high and low maximal TLS diameter groups by the median value of maximal diameter among all patients.

Outcomes

The primary outcome of the study was 5-year overall survival, which was defined as death for any cause during the time between the date of surgery and death of the patient during 5 years or at the end of 5-year follow-up. The secondary outcome of the study was overall survival which was defined as death for any cause during the time between the date of surgery and death of the patient or the end of follow-up.

Statistical analysis

To assess the interobserver agreement Cohen's kappa was used. Kaplan–Meier method was used to compare survival between groups. Cox regression was used to compare survival between groups. For Cox regression, there were an unadjusted crude and an adjusted model, which was used in the multivariate analysis. In the adjusted model, potential confounding variables were year of surgery (<2000 or ≥2000), age at diagnosis (continuous variable), sex (male or female), administration of perioperative chemotherapy (yes or no), tumor stage (stage 0–II or stage III–IV), Laurén classification (intestinal, diffuse), radical resection (R0 or R1/2), and center (Oulu University Hospital or Central Finland Central Hospital). Patients were divided into intestinal and diffuse type subgroups by Laurén's classification for subgroup analyses, which were performed for both subgroups separately. In the intestinal-type subgroup, histological grade (I–II or III) was used as an additional confounder in the multivariate model. A sensitivity analysis that excluded patients with R1/2 resection was performed. The point estimates were similar to the main analysis and therefore only the results of the main analysis are presented. IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY) was used for all statistical analyses.

RESULTS

Patients

There were 721 surgically treated gastric adenocarcinoma patients in the study. The median age for the patients was 69 years (range from 27 to 90 years), with 425 (58.9%) of them being men and 296 (41.1%) women. Of the patients, 559 (77.5%) had histologically confirmed R0 resection, while 162 (22.5%) had R1/2 resection including patients treated with palliative intent. The median follow-up time was 28 months (range = 0–432 months) and there was no loss to follow-up. During five-year follow-up, primary outcome occurred in 482 (66.9%) patients. Assessments of average TLS density, hotspot TLS density, and maximal TLS diameter were successfully performed for all 721 patients.

Average TLS density

The median TLS density was 0.71 TLS/mm, and after dichotomization, 360 (49.9%) patients had high average TLS density (Fig. 1). Interobserver agreement for average TLS density measured by Kappa coefficient was 0.62. High average TLS density was associated with younger age at diagnosis and diffuse type histology (Table S1).

In low average TLS density group, 5-year survival was 28.6% and in high average TLS density group 35.6% (Fig. S1). Patients with high average TLS density had longer five-year survival in univariate, but not in multivariate analysis (hazard ratio [HR] 0.85, 95% CI 0.70–1.02), compared to low average TLS density (Table S2), the results being similar for secondary outcome overall survival. In subgroup analyses, high average TLS density was not associated with improved five-year survival in multivariable analyses of either intestinal (HR 0.84, 95% CI 0.64–1.10) or diffuse type subgroups (HR 0.84, 95% CI 0.65–1.10).

Hotspot TLS density

The median hotspot TLS density was 4, and after dichotomization, 342 (47.4%) of the patients had high hotspot TLS density (Fig. 1). The Kappa coefficient for hotspot TLS density was 0.623. High hotspot TLS density was associated with younger age at diagnosis (Table S3).

Five-year survival was 28.9% in low hotspot TLS density group and 35.7% in high hotspot TLS density group (Fig. S2). High hotspot TLS density was associated with longer five-year survival in univariate, but not in multivariate analysis (HR 0.85, 95% CI 0.70–1.02), compared to low hotspot TLS density (Table S4). The results were similar for secondary outcome overall survival. In subgroup analyses, high hotspot TLS density was not associated with improved five-year survival in multivariable analyses of either intestinal (HR 0.86, 95% CI

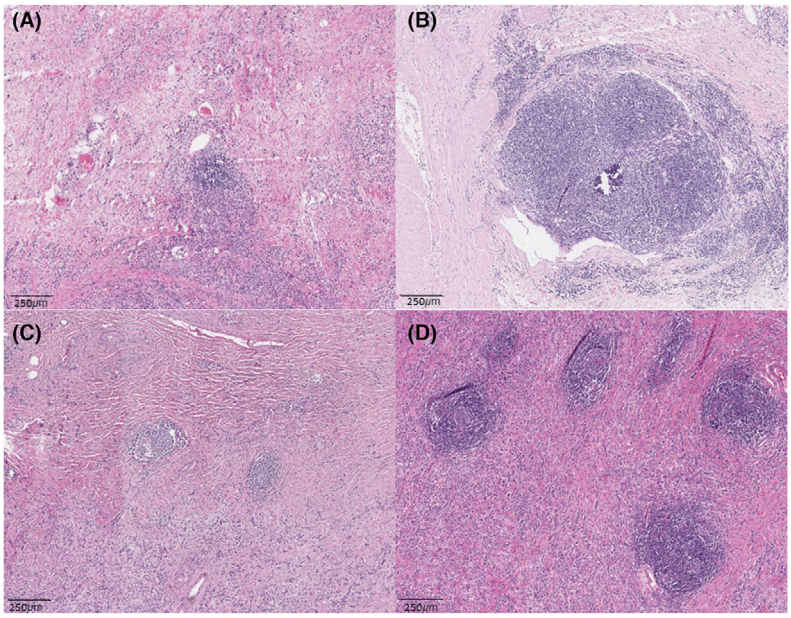

Fig. 1. Examples of low maximal TLS diameter (A), high maximal TLS diameter (B), low hotspot TLS density (C), and high TLS density (D) in gastric cancer at 4×objective magnification.

0.66–1.12) or diffuse type subgroups (HR 0.85, 95% CI 0.67–1.09).

Maximal TLS diameter and survival

The median maximal TLS diameter was 673.5 μm. After dichotomization, 359 (49.8%) of the patients had low maximal TLS diameter (Fig. 1). Kappa coefficient of maximal TLS diameter was 0.501. High maximal TLS diameter was associated with younger age at diagnosis, radical resection, and surgery at Oulu University Hospital (Table 1).

The five-year survival was 26.4% in low maximal TLS diameter group and 37.8% in high maximal TLS diameter group (Fig. 2). High maximal TLS diameter was associated with longer five-year survival in both univariate (HR 0.71, 95% CI 0.60–0.85) and multivariate (HR 0.74, 95% CI 0.61–0.89) analyses compared to low maximal TLS diameter (Table 2). Similarly, high maximal TLS diameter was associated with longer overall survival in univariate and multivariate analysis (HR 0.72, 95% CI 0.61–0.85) compared to low maximal TLS diameter.

In subgroup analysis of intestinal type histology, maximal TLS diameter was not associated with five-year survival (adjusted HR 0.87, 95% CI 0.67–1.13) or overall survival (adjusted HR 0.88, 95% CI 0.70–1.11), compared to low maximal TLS diameter (Table 2). In contrast, in diffuse type subgroup high maximal TLS diameter was associated with improved five-year survival (adjusted HR 0.65, 95% CI 0.50–0.85) and improved overall survival (adjusted HR 0.63, 95% CI 0.49–0.80), compared to low maximal TLS diameter.

Table 1. Associations between maximal TLS diameter and clinicopathological variables in 721 surgically treated gastric cancer patients

	Low maximal TLS diameter (N = 362)	High maximal TLS diameter (N = 359)	p-value
Year of surgery			0.10
≥2000	203 (56.1%)	179 (49.9%)	
<2000	159 (43.9%)	180 (50.1%)	
Mean age at diagnosis	68.0	65.9	**0.018**
Sex			0.29
Male	206 (56.9%)	219 (61.0%)	
Female	156 (43.1%)	140 (39.0%)	
Center			**<0.001**
Oulu	270 (74.6%)	313 (87.2%)	
Central Finland	92 (25.4%)	46 (12.8%)	
Perioperative chemotherapy			0.29
Yes	20 (5.5%)	13 (3.6%)	
No	342 (94.5%)	346 (96.4%)	
Tumor stage			0.59
1 or 2	223 (61.6%)	229 (63.8%)	
3 or 4	139 (38.4%)	130 (36.2%)	
Lauren class			0.31
Intestinal	179 (49.4%)	170 (47.4%)	
Diffuse	175 (48.3%)	174 (48.5%)	
Mixed	8 (2.2%)	15 (4.2%)	
Histological grade in intestinal type			0.58
I or II	118 (65.9%)	107 (62.9%)	
III	61 (34.1%)	63 (37.1%)	
Radicality of resection			**<0.001**
R0	261 (72.1%)	298 (83.0%)	
R1 or R2	101 (27.9%)	61 (17.0%)	

p-values under 0.05 are in bold.

DISCUSSION

In this retrospective cohort study, high diameter of the largest TLS was independently associated with favorable prognosis in a Western population of gastric cancer. In subgroup analysis, this association was only seen in diffuse type adenocarcinoma. The average and hotspot density of TLSs were not independently associated with prognosis in this study. Assessments of both TLS densities and maximal TLS diameter had moderate interobserver agreement.

This study was the largest study assessing maximal TLS diameter and the second largest assessing TLS density in gastric cancer. Furthermore, this is the first large study on TLS in the Western world. The main strength of the study was a representative study population of two patient cohorts from separate institutions with minimal selection bias and complete follow-up data. There are also some limitations that might limit applicability of the results. The study period between 1983 and 2018 was long and included development of treatments. Confounding effect of treatment changes was taken into account by adjusting the multivariate model with treatment year. To avoid selection bias, palliative resections were included in the cohort, which also explains low observed overall survival rates in the whole cohort. Margin positive resection status was included in multivariable model to reduce any confounding arising from margin positivity. A sensitivity analysis including only patients with radical, curative resection showed similar point estimates than the main analysis. The study cohort also included neoadjuvant treated patients, which was taken into account in the multivariable model. Small number of patients treated with neoadjuvant therapy limits the applicability of results to neoadjuvant-treated patients. The study was based

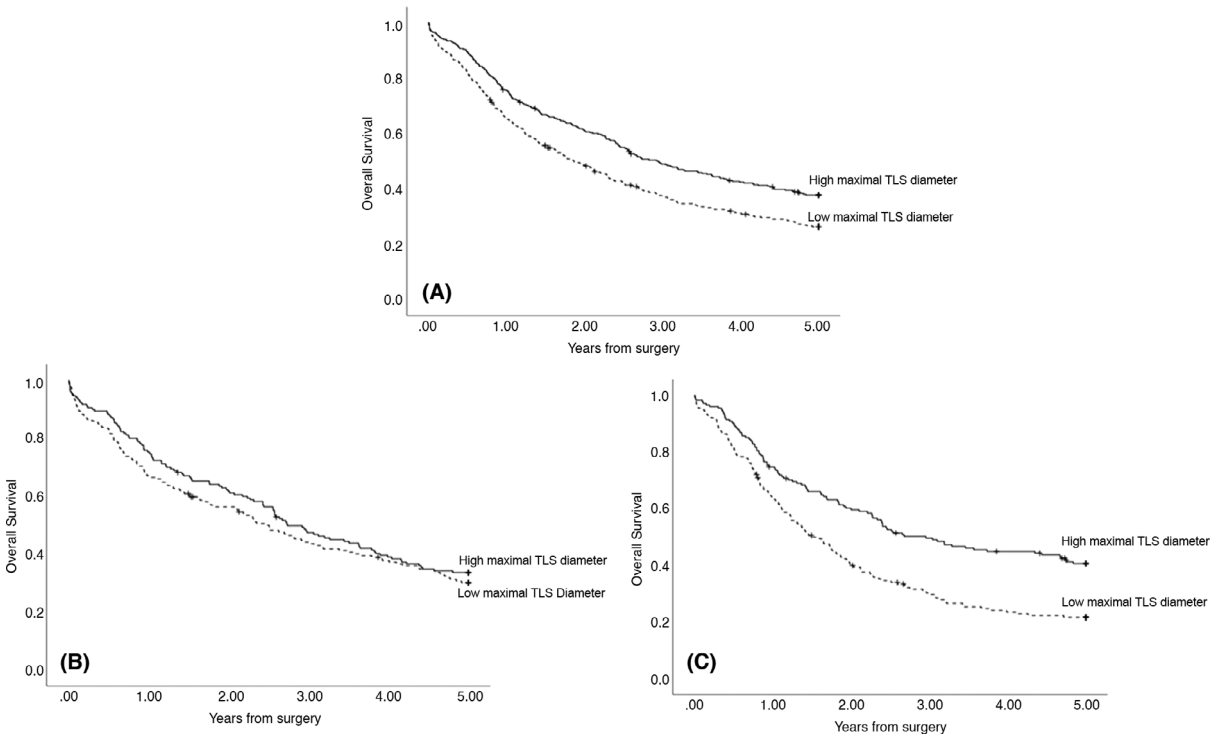

Fig. 2. Five-year overall survival stratified by maximal TLS diameter in the whole study cohort of 721 gastric adenocarcinoma patients (A), in intestinal type subgroup (B), and in diffuse type subgroup (C).

only on HE-stained slides, which might reduce its comparability with studies utilizing other methods like immunohistochemistry.

High density of TLSs was associated with good prognosis in a recent large Chinese study of 914 gastric cancer patients (adjusted HR 0.79, 95% CI 0.67–0.94) [13]. The study was based on estimating cumulative area of TLSs from HE-stained slides. In few small immunohistochemistry-based studies, high number of TLSs has also been associated with good prognosis in gastric cancer [18,19]. In our study, neither average nor hotspot density was independently associated with prognosis. However, the point estimates of both average and hotspot TLS densities were similar to the aforementioned study, and prognostic relevance of TLS density cannot be ruled out.

A recent Japanese study (n = 170) assessed the TLSs in stage II/III gastric cancer patients and suggested that an improvement in the patient's nutritional status might be associated with an increase in these peritumoral TLSs, which again could contribute to a better prognosis [14]. In that study, the outcome measure was cancer-specific survival instead of overall survival, which could be considered problematic due to the majority of gastric cancer patients dying of gastric cancer and potentially resulting in misclassification. Subgroup analysis according to histology was not provided in that study. In the present study, high maximal TLS diameter was associated with longer five-year survival but the effect was limited to diffuse type in subgroup analysis.

TLSs have been proposed to reflect the antitumor immune response [5,20]. Inside TLSs, dendritic cells introduce antigens to CD4+ T cells leading to T cell activation, B cell maturation, and antibody production [20]. This may explain the better prognosis of patients with high maximal diameter of TLSs. TLS size might also associate with maturity of TLSs, which seems to associate with good prognosis. Higher TLS diameter might also be associated with TLS maturation, which seems to have prognostic significance at least in lung squamous cell carcinoma and colorectal cancer [21,22].

The present study shows directions for future research and has also some potential clinical implications. The prognostic value of TLS density and maximal TLS diameter should be further studied in large retrospective studies to fully understand their prognostic significance in gastric cancer. Subgroup analyses of different histological types should be included to better understand differences between diffuse and intestinal types. The finding the TLS

Table 2. Maximal TLS diameter and survival in the study cohort of 721 gastric cancer patients

Survival model	Number of patients	Low diameter HR (95% CI)	High diameter HR (95% CI)
Five-year survival			
All patients (crude)	721	1.00 (Reference)	0.71 (0.60–0.85)
All patients (adjusted)[a]	721	1.00 (Reference)	0.74 (0.61–0.89)
Subgroup analysis			
Intestinal type (crude)	349	1.00 (Reference)	0.88 (0.68–1.14)
Intestinal type (adjusted)[b]	349	1.00 (Reference)	0.87 (0.67–1.13)
Diffuse type (crude)	349	1.00 (Reference)	0.59 (0.45–0.76)
Diffuse type (adjusted)[c]	349	1.00 (Reference)	0.65 (0.50–0.85)
Overall survival			
All patients (crude)	721	1.00 (Reference)	0.70 (0.59–0.82)
All patients (adjusted)[a]	721	1.00 (Reference)	0.72 (0.61–0.85)
Subgroup analysis			
Intestinal type (crude)	349	1.00 (Reference)	0.86 (0.69–1.08)
Intestinal type (adjusted)[b]	349	1.00 (Reference)	0.88 (0.70–1.11)
Diffuse type (crude)	349	1.00 (Reference)	0.59 (0.46–0.75)
Diffuse type (adjusted)[c]	349	1.00 (Reference)	0.63 (0.49–0.80)

[a]Adjusted for year of diagnosis, center, age, sex, tumor stage, Lauren classification, perioperative chemotherapy, and radical resection.
[b]Adjusted for year of diagnosis, center, age, sex, tumor stage, tumor grade, perioperative chemotherapy, and radical resection.
[c]Adjusted for year of diagnosis, center, age, sex, tumor stage, perioperative chemotherapy, and radical resection.

diameter is prognostic in diffuse gastric cancer could be particularly relevant for clinical practice, as several other histological properties, such as tumor budding or tumor-stroma ratio, might be difficult or impossible to assess and could function poorly in the uncohesive growth pattern of diffuse cancer. Use of artificial intelligence models could also be studied to further improve TLS assessment. TLSs might also be potential targets for immunotherapy in the future [20,23].

In conclusion, high maximal TLS diameter is associated with improved survival in gastric cancer, but its effect might be limited to diffuse histological type. Maximal TLS diameter can be assessed from HE-stained slides with moderate interobserver agreement. It cannot be ruled out that TLS density might also be associated with prognosis in gastric cancer.

The authors thank Mrs. Riitta Vuento for important technical assistance. The study benefited from samples/data from Northern Finland Biobank Borealis and Central Finland Biobank.

FUNDING

This study was supported by grants from Vatsatautien tutkimussäätiö (NK), The Finnish Medical Foundation (NK), Sigrid Jusélius Foundation (JHK), Finnish Cancer Foundation (JHK), Orion Research Foundation (JHK), Thelma Mäkikyrö Foundation (JHK), Mary and Georg C. Ehrnroot Foundation (JHK, OH), and Instrumentarium Science Foundation (OH).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
2. Amin MB, Edge S, Greene F, Byrd DR, Brookland RK, Washington MK, et al. AJCC cancer staging manual. 8th ed. New York: Springer; 2017.
3. Sano T, Coit DG, Kim HH, Roviello F, Kassab P, Wittekind C, et al. Proposal of a new stage grouping of gastric cancer for TNM classification: international gastric cancer association staging project. Gastric Cancer. 2017;20:217–25.
4. Neyt K, Perros F, GeurtsvanKessel CH, Hammad H, Lambrecht BN. Tertiary lymphoid organs in infection and autoimmunity. Trends Immunol. 2012;33:297–305.
5. Hiraoka N, Ino Y, Yamazaki-Itoh R. Tertiary lymphoid organs in cancer tissues. Front Immunol. 2016;7:244.
6. Rozek LS, Schmit SL, Greenson JK, Tomsho LP, Rennert HS, Rennert G, et al. Tumor-infiltrating lymphocytes, Crohn's-like lymphoid reaction, and survival

from colorectal cancer. J Natl Cancer Inst. 2016;108: djw027.
7. Väyrynen JP, Sajanti SA, Klintrup K, Mäkelä J, Herzig KH, Karttunen TJ, et al. Characteristics and significance of colorectal cancer associated lymphoid reaction. Int J Cancer. 2014;134:2126–35.
8. Hiraoka N, Ino Y, Yamazaki-Itoh R, Kanai Y, Kosuge T, Shimada K. Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. Br J Cancer. 2015;112:1782–90.
9. Germain C, Gnjatic S, Tamzalit F, Knockaert S, Remark R, Goc J, et al. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. Am J Respir Crit Care Med. 2014;189:832–44.
10. Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol. 2008;26:4410–7.
11. Ueno H, Hashiguchi Y, Shimazaki H, Shinto E, Kajiwara Y, Nakanishi K, et al. Objective criteria for crohn-like lymphoid reaction in colorectal cancer. Am J Clin Pathol. 2013;139:434–41.
12. Munoz-Erazo L, Rhodes JL, Marion VC, Kemp RA. Tertiary lymphoid structures in cancer – considerations for patient prognosis. Cell Mol Immunol. 2020;17:570–5.
13. He W, Zhang D, Liu H, Chen T, Xie J, Peng L, et al. The high level of tertiary lymphoid structure is correlated with superior survival in patients with advanced gastric cancer. Front Oncol. 2020;10:980.
14. Omura Y, Toiyama Y, Okugawa Y, Yamamoto A, Yin C, Kusunoki K, et al. Crohn's-like lymphoid reaction is associated with oncological prognosis and nutritional status in patients with pathological stage II/III gastric cancer. Ann Surg Oncol. 2020;27:259–67.
15. Kemi N, Eskuri M, Herva A, Leppänen J, Huhta H, Helminen O, et al. Tumour-stroma ratio and prognosis in gastric adenocarcinoma. Br J Cancer. 2018;119:435–9.
16. Kemi N, Hiltunen N, Väyrynen JP, Pohjanen VM, Helminen O, Junttila A, et al. Immune cell infiltrate and prognosis in gastric cancer. Cancers. 2020;12:3604.
17. Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, et al. QuPath: open source software for digital pathology image analysis. Sci Rep. 2017;7:16878.
18. Hennequin A, Derangere V, Boidot R, Apetoh L, Vincent J, Orry D, et al. Tumor infiltration by Tbet+ effector T cells and CD20+ B cells is associated with survival in gastric cancer patients. Onco Targets Ther. 2016;5:e1054598.
19. Yamakoshi Y, Tanaka H, Sakimura C, Deguchi S, Mori T, Tamura T, et al. Immunological potential of tertiary lymphoid structures surrounding the primary tumor in gastric cancer. Int J Oncol. 2020;57:171–82.
20. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer. 2019;19:307–25.
21. Siliņa K, Soltermann A, Attar FM, Casanova R, Uckeley ZM, Thut H, et al. Germinal centers determine the prognostic relevance of tertiary lymphoid structures and are impaired by corticosteroids in lung squamous cell carcinoma. Cancer Res. 2018;78:1308–20.
22. Posch F, Silina K, Leibl S, Mündlein A, Moch H, Siebenhüner A, et al. Maturation of tertiary lymphoid structures and recurrence of stage II and III colorectal cancer. Onco Targets Ther. 2018;7:e1378844.
23. Kang W, Feng Z, Luo J, He Z, Liu J, Wu J, et al. Tertiary lymphoid structures in cancer: the double-edged sword role in antitumor immunity and potential therapeutic induction strategies. Front Immunol. 2021;12:689270.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Five-year overall survival stratified by average TLS density in the study cohort of 721 gastric adenocarcinoma patients.
Figure S2 Five-year overall survival stratified by hotspot TLS density in the study cohort of 721 gastric adenocarcinoma patients.
Table S1 Associations between average TLS density and clinicopathological variables in 721 surgically treated gastric cancer patients.
Table S2 Average TLS density and survival in the study cohort of 721 gastric cancer patients.
Table S3 Associations between hotspot TLS density and clinicopathological variables in 721 surgically treated gastric cancer patients.
Table S4 Hotspot TLS density and survival in the study cohort of 721 gastric cancer patients.