Original Article

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Pathology Section

Tumour-infiltrating Lymphocyte Score and its Association with Histopathological Parameters in Gastrointestinal Tumours: A Cross-sectional Study from a Tertiary Care Centre in Chengalpattu, Tamil Nadu, India

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ABSTRACT

Introduction: Gastrointestinal malignancies are biologically heterogeneous and show variable immune responses across anatomical sites. Tumour-Infiltrating Lymphocytes (TIL) reflects host immune response within the tumour microenvironment and can be assessed on routine haematoxylin and eosin-stained sections.

Aim: To evaluate stromal TIL score in primary gastrointestinal malignancies and assess its association with histopathological parameters.

Materials and Methods: This single-centre cross-sectional study included 45 primary gastrointestinal malignant tumours diagnosed between January 2024 and December 2025 at Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Chengalpattu, Tamil Nadu, India. Tumours from the stomach, colon, rectum, duodenum, and oesophagus/gastro-oesophageal junction were included. Histopathological parameters including tumour site, histological subtype, tumour grade, lymphovascular invasion, perineural invasion, lymph node status, pathological tumour stage, and TNM stage were recorded. Stromal TIL score was assessed as the percentage of stromal area occupied by mononuclear inflammatory cells. For dichotomised analysis, cases with stromal TIL score >50% were classified as high-TIL, whereas cases with stromal TIL score ≤50% were grouped as low-TIL.

Statistical Analysis: Data were analysed using SPSS software version 29.0. Fisher's-exact test was used for categorical variables, and Mann-Whitney U test was used for continuous non-parametric variables. A p-value <0.05 was considered statistically significant.

Results: Among 45 cases, 27 (60.00%) were High-TIL and 18 (40.00%) were Low-TIL. Low-TIL status showed significant association with poor differentiation, lymphovascular invasion, perineural invasion, nodal positivity, advanced pT stage, and advanced TNM stage. Positive lymph node count and exploratory composite adverse-feature score were significantly lower in the high-TIL group.

Conclusion: Low stromal TIL status was associated with adverse histopathological features. However, because stromal TIL distribution was uneven across tumour sites and histological subtypes, the findings should be interpreted as exploratory pooled associations rather than site-independent conclusions.

Keywords: Clinicopathological correlation, Haematoxylin and eosin, Lymphovascular invasion, Pathological staging, Perineural invasion, Stromal immune response

INTRODUCTION

Gastrointestinal tract tumours comprise a heterogeneous group of neoplasms arising across different anatomical sites, including the oesophagus/gastro-oesophageal junction, stomach, duodenum, colon, and rectum. The World Health Organisation classification of digestive system tumours provides the standard diagnostic framework for histopathological classification and tumour typing [1]. Recent updates to the WHO classification have further emphasised refined diagnostic criteria, standardised terminology, and integration of pathological, molecular, and clinically relevant features in digestive system tumours [2].

Tumour progression is influenced not only by neoplastic epithelial cells but also by the surrounding tumour microenvironment, including stromal and immune cell interactions, cytokine signalling, and immune checkpoint pathways. These interactions may influence tumour invasion, immune escape, metastatic potential, and response to therapy. The increasing use of immune checkpoint inhibitors in selected gastrointestinal malignancies has further highlighted the relevance of evaluating tumour immune interactions in diagnostic and research pathology [3].

The TILs represent a morphological reflection of the host immune response against tumour cells. They can be assessed on routine haematoxylin and eosin-stained sections, making stromal TILs evaluation a practical and low-cost histopathological parameter. The International Immuno-Oncology Biomarkers Working Group has proposed a standardised approach for stromal TILs assessment in solid tumours, including gastrointestinal tract carcinomas. In this method, stromal TILs are assessed as the percentage of stromal area occupied by mononuclear inflammatory cells, while tumour cell nests, necrosis, fibrosis, abscess formation, and granulocytic inflammation are excluded [4,5].

The relevance of TILs assessment has been most extensively studied in colorectal carcinoma. More recent studies have also explored the interaction between TILs, tumour budding, and immune checkpoint expression in colorectal carcinoma, further supporting the biological relevance of immune infiltration within the tumour microenvironment [6]. Routine histopathological parameters such as tumour site, histological subtype, tumour grade, lymphovascular invasion, perineural invasion, lymph node status, and pathological TNM stage remain central to gastrointestinal tumour reporting [1,7-9]. Correlating stromal TILs

status with these parameters may help determine whether host immune response patterns are associated with favourable or adverse pathological features.

Despite growing evidence regarding TILs in colorectal and gastric carcinomas, data evaluating stromal TILs scores across gastrointestinal tumours remain limited, particularly in regional institutional cohorts. Hence, the present study was undertaken with the primary objective of evaluating stromal TILs score in primary gastrointestinal malignancies and the secondary objective of assessing its association with histopathological parameters, including tumour site, histological subtype, tumour grade, lymphovascular invasion, perineural invasion, lymph node status, pathological tumour stage, and TNM stage.

MATERIALS AND METHODS

Study design and setting: This single-centre cross-sectional study was conducted in the Department of Pathology, Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Chengalpattu, Tamil Nadu, India, over a period of six months from March 2026 to August 2026. Archived cases diagnosed between January 2024 and December 2025 were retrieved and evaluated. The study was carried out after obtaining approval from the Institutional Ethics Committee. The IEC reference number was KIMS/PG/13/25.02.2026. The study involved no direct patient intervention.

Tumours Studied

The study included primary gastrointestinal malignancies from the stomach, colon, rectum, duodenum, and oesophagus/gastro-oesophageal junction. The histological subtypes studied included adenocarcinoma, NOS, mucinous adenocarcinoma, signet-ring cell carcinoma, poorly cohesive carcinoma, squamous cell carcinoma, adenosquamous carcinoma, and neuroendocrine carcinoma.

Inclusion Criteria: Reported cases of primary gastrointestinal malignancies with available formalin-fixed paraffin-embedded tissue blocks and corresponding haematoxylin and eosin-stained slides were included.

Exclusion Criteria: Cases treated with neoadjuvant therapy, metastatic tumours to the gastrointestinal tract, recurrent tumours, benign lesions, and cases with inadequate tissue or poor-quality slides were excluded.

The sample size was calculated using finite population correction, considering an available population of 50 cases, 95% confidence level, 5% margin of error, and expected proportion of 50%. The formula used was:

$$n = NZ^2p(1-p) / \{d^2(N-1) + Z^2p(1-p)\}$$

where, N=50, Z=1.96, p=0.50, and d=0.05. The calculated minimum sample size was 44.34, rounded to 45 cases. Cases were selected by purposive sampling [10].

Histopathological Assessment

All available haematoxylin and eosin-stained sections were reviewed. Histopathological parameters including tumour site, histological subtype, tumour size, tumour grade, lymphovascular invasion, perineural invasion, lymph node status, pathological tumour stage, and TNM stage were recorded. Tumour grading was assigned according to the World Health Organization classification of digestive system tumours, based on site-specific histomorphological differentiation criteria [1]. Pathological tumour stage and TNM stage were recorded according to routine gastrointestinal tumour reporting parameters and AJCC-based staging principles [9].

All slides were initially reviewed by the principal investigator under the supervision of a consultant pathologist. Stromal TILs scoring and histopathological parameters were assessed on haematoxylin and eosin-stained sections. The assessment was not independently blinded because the study involved retrospective review of archived diagnostic slides and corresponding clinicopathological records. Doubtful or discrepant histopathological findings were reviewed with the supervising consultant pathologist and resolved by consensus. Formal interobserver agreement analysis using Cohen's kappa or intraclass correlation coefficient was not performed.

Stromal TILs Assessment

Stromal TILs were assessed on haematoxylin and eosin-stained sections according to the recommendations proposed by the International TILs Working Group/International Immuno-Oncology Biomarkers Working Group [5,11]. Stromal TILs score was estimated as the percentage of stromal area occupied by mononuclear inflammatory cells, including lymphocytes and plasma cells, over the total stromal area within the invasive tumour border. Only the stromal compartment was evaluated. Tumour cell nests, areas outside the invasive tumour border, necrosis, ulceration, fibrosis, abscess formation, and neutrophilic/granulocytic infiltrates were excluded. The average stromal TILs density across the section was recorded, and assessment of only the highest lymphocyte concentrate area was avoided [5,11].

For descriptive assessment, stromal TILs scores were initially recorded in three categories: low TILs (0-10%), intermediate TILs (11-50%), and high TILs (>50%). The three-tier classification was used only for descriptive assessment of stromal TILs distribution. For dichotomised statistical analysis, 50% was used as the cut-off value for evaluation of TILs on haematoxylin and eosin-stained sections, according to the verification study of the recommendations proposed by the International TILs working group [12]. Accordingly, cases with stromal TILs score >50% were classified as high-TILs, whereas cases with stromal TILs score ≤50% were grouped as low-TILs for high-versus-low comparison. This dichotomised approach was selected to allow comparison between high-TILs and low-TILs groups in a small cohort and to avoid sparse subgroup counts in the low and intermediate categories.

An exploratory composite adverse-feature score was calculated by assigning one point each for the presence of poor differentiation, lymphovascular invasion, perineural invasion, nodal positivity, advanced pathological tumour stage (pT3-pT4), and advanced TNM stage (Stage III-IV). The total score ranged from 0 to 6. This score was used only as an exploratory summary measure and was not considered a validated prognostic scoring system.

STATISTICAL ANALYSIS

Data were entered in Microsoft Excel and analysed using SPSS software version 29. Categorical variables were expressed as frequency and percentage. Continuous variables were expressed as median with interquartile range for non-parametric analysis. Association between TILs status and categorical histopathological parameters was analysed using the two-sided Fisher's-exact test. Comparison of continuous non-parametric variables between high-TILs and low-TILs groups was performed using the Mann-Whitney U test. A p-value <0.05 was considered statistically significant. Odds ratios with 95% confidence intervals were calculated to estimate the strength of association, with the high-TILs group used as the reference category.

RESULTS

A total of 45 primary gastrointestinal malignancy cases were included in the study. The age of the patients ranged from 34 to 78 years, with a median age of 61 years. There were 23 males (51.11%) and 22 females (48.89%). The stomach was the most common tumour site, accounting for 15 cases (33.33%), followed by the colon and rectum with nine cases (20.00%) each. Adenocarcinoma, NOS was the most frequent histological subtype, seen in 28 cases (62.22%). Based on stromal TILs assessment, 27 cases (60.00%) were

classified as high-TILs, while 18 cases (40.00%) were classified as low-TILs for analysis. Baseline clinicopathological characteristics are summarised in [Table/Fig-1].

Parameters	Category	n (%)
Gender	Male	23 (51.11)
	Female	22 (48.89)
Site	Stomach	15 (33.33)
	Colon	9 (20.00)
	Rectum	9 (20.00)
	Duodenum	6 (13.33)
	Oesophagus/GEJ	6 (13.33)
Histology	Adenocarcinoma, NOS	28 (62.22)
	Mucinous adenocarcinoma	5 (11.11)
	Signet-ring cell carcinoma	3 (6.67)
	Squamous cell carcinoma	3 (6.67)
	Poorly cohesive carcinoma	2 (4.44)
	Adenosquamous carcinoma	2 (4.44)
	Neuroendocrine carcinoma	2 (4.44)

[Table/Fig-1]: Baseline clinicopathological characteristics of the gastrointestinal malignancy cohort.
TIL: Tumour-infiltrating lymphocytes; GEJ: Gastro-oesophageal junction; NOS: Not otherwise specified

Site-wise distribution of stromal TILs status is shown in [Table/Fig-2]. High-TILs status was seen in 9/15 stomach tumours, 9/9 colon tumours, and 9/9 rectal tumours. All duodenal tumours and oesophagus/GEJ tumours showed Low-TILs status in the present cohort. Due to small subgroup numbers and zero-cell categories, site-specific inferential analysis was not performed.

Tumour site	Total n	High-TIL n (%)	Low-TIL n (%)
Stomach	15	9 (60.00)	6 (40.00)
Colon	9	9 (100.00)	0 (0.00)
Rectum	9	9 (100.00)	0 (0.00)
Duodenum	6	0 (0.00)	6 (100.00)
Oesophagus/GEJ	6	0 (0.00)	6 (100.00)
Total	45	27 (60.00)	18 (40.00)

[Table/Fig-2]: Distribution of stromal TIL status according to gastrointestinal tumour site.
TIL: Tumour-infiltrating lymphocytes; GEJ: Gastro-oesophageal junction

Histology-wise distribution of stromal TILs status is shown in [Table/Fig-3]. High-TILs status was observed among adenocarcinoma, NOS, mucinous adenocarcinoma, and signet-ring cell carcinoma cases. All poorly cohesive carcinomas, squamous cell carcinomas, adenosquamous carcinomas, and neuroendocrine carcinomas showed low stromal TILs status in this cohort. Due to uneven histological subtype distribution and sparse subgroup counts, histology-specific inferential analysis was not performed.

Histological subtype	Total n	High-TIL n (%)	Low-TIL n (%)
Adenocarcinoma, NOS	28	20 (71.43)	8 (28.57)
Mucinous adenocarcinoma	5	5 (100.00)	0 (0.00)
Signet-ring cell carcinoma	3	2 (66.67)	1 (33.33)
Poorly cohesive carcinoma	2	0 (0.00)	2 (100.00)
Squamous cell carcinoma	3	0 (0.00)	3 (100.00)
Adenosquamous carcinoma	2	0 (0.00)	2 (100.00)
Neuroendocrine carcinoma	2	0 (0.00)	2 (100.00)
Total	45	27 (60.00)	18 (40.00)

[Table/Fig-3]: Distribution of stromal TIL status according to histological subtype.
TIL: Tumour-infiltrating lymphocytes; NOS: Not otherwise specified

Pathological tumour stage and TNM stage distribution stratified by gastrointestinal tumour site are presented in [Table/Fig-4]. Overall, pT1, pT2, pT3, and pT4 stages were observed in 13 (28.89%), 15 (33.33%), 8 (17.78%), and 9 (20.00%) cases, respectively. Stage I/II disease was observed in 29 (64.44%) cases, whereas Stage III/IV disease was observed in 16 (35.56%) cases.

The association between stromal TILs status and adverse histopathological parameters is summarised in [Table/Fig-5]. The frequencies of poor tumour differentiation, lymphovascular invasion, perineural invasion, nodal positivity, pT3–pT4 stage, and Stage III–IV disease were higher in the low-TILs group than in the high-TILs group, as shown in [Table/Fig-5].

Mann-Whitney U test was used for continuous non-parametric variables, as summarised in [Table/Fig-6]. Positive lymph node count was significantly lower in the High-TILs group than in the Low-TILs group (p=0.0052). The exploratory composite adverse-feature score was also significantly lower among High-TILs cases (p=0.0037). Age, tumour size, and number of lymph nodes examined did not show statistically significant differences between the two groups.

The distribution of adverse histopathological parameters according to stromal TILs status is illustrated in [Table/Fig-7].

Representative gross and microscopic findings are shown in [Table/Fig-8-10]. A gastrectomy specimen showing gastric adenocarcinoma with high stromal tumour-infiltrating lymphocytic response is illustrated in [Table/Fig-8]. A rectal adenocarcinoma specimen with low stromal tumour-infiltrating lymphocytic response is shown in [Table/Fig-9]. Microscopic evidence of lymphovascular invasion and lymph node metastatic deposits is demonstrated in [Table/Fig-10].

DISCUSSION

In the present study, stromal TILs status showed significant association with several adverse histopathological parameters in a pooled cohort of primary gastrointestinal malignancies. Low-TILs status was associated with higher frequencies of poor tumour differentiation, lymphovascular invasion, perineural invasion, nodal positivity, advanced pathological tumour stage, and advanced TNM stage. However, these findings should be interpreted as pooled cohort-level associations because the study included tumours from different gastrointestinal sites with uneven distribution of stromal TILs status across anatomical sites and histological subtypes.

The present cohort was heterogeneous with respect to tumour site and histological subtype. High-TILs cases were concentrated in stomach, colon, and rectal tumours, whereas all duodenal and oesophagus/GEJ tumours showed low stromal TILs status in this cohort. Similarly, poorly cohesive carcinoma, squamous cell carcinoma, adenosquamous carcinoma, and neuroendocrine carcinoma were represented only in the Low-TILs group. Therefore, the observed association between Low-TILs status and adverse histopathological features may partly reflect differences in tumour site and histological subtype. Confounding by tumour site and histology cannot be excluded. Hence, the findings should not be interpreted as site-independent conclusions applicable to all gastrointestinal malignancies.

In addition to uneven stromal TILs distribution, pTNM stage distribution also varied across gastrointestinal sites. Duodenal and oesophagus/GEJ tumours showed a higher proportion of Stage III/IV disease than stomach, colon, and rectal tumours in this cohort. Therefore, the association between Low-TILs status and adverse histopathological features may partly reflect underlying site-wise differences in stage distribution. This reinforces the need to interpret the findings as pooled exploratory associations rather than site-independent conclusions.

Tumour site	n	pT1 n (%)	pT2 n (%)	pT3 n (%)	pT4 n (%)	Stage I/II n (%)	Stage III/IV n (%)
Stomach	15	4 (26.67)	6 (40.00)	2 (13.33)	3 (20.00)	11 (73.33)	4 (26.67)
Colon	9	3 (33.33)	4 (44.44)	1 (11.11)	1 (11.11)	7 (77.78)	2 (22.22)

Tumour site	n	pT1 n (%)	pT2 n (%)	pT3 n (%)	pT4 n (%)	Stage I/II n (%)	Stage III/IV n (%)
Rectum	9	4 (44.44)	3 (33.33)	0 (0.00)	2 (22.22)	7 (77.78)	2 (22.22)
Duodenum	6	1 (16.67)	1 (16.67)	1 (16.67)	3 (50.00)	2 (33.33)	4 (66.67)
Oesophagus/GEJ	6	1 (16.67)	1 (16.67)	4 (66.67)	0 (0.00)	2 (33.33)	4 (66.67)
Total	45	13 (28.89)	15 (33.33)	8 (17.78)	9 (20.00)	29 (64.44)	16 (35.56)

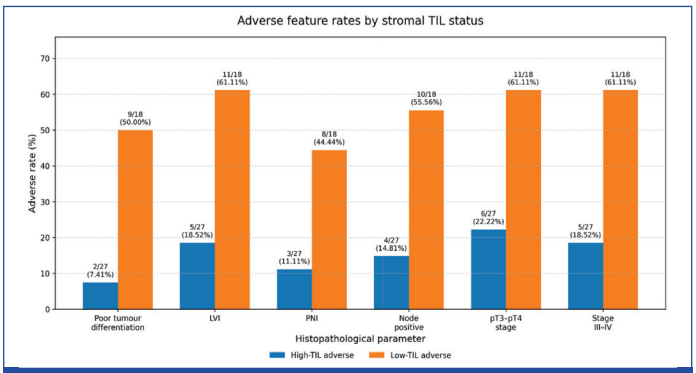
[Table/Fig-4]: Pathological tumour stage and TNM stage distribution stratified by gastrointestinal tumour site.
GEJ: Gastro-oesophageal junction; pT: Pathological tumour stage; TNM: Tumour-Node-Metastasis staging

Parameter	High-TIL adverse n (%)	Low-TIL adverse n (%)	OR for adverse feature in Low-TIL vs High-TIL (95% CI)	p-value
Poor tumour differentiation	2 (7.41)	9 (50.00)	12.50 (2.26-69.19)	0.0031
Lymphovascular invasion present	5 (18.52)	11 (61.11)	6.91 (1.78-26.85)	0.0050
Perineural invasion present	3 (11.11)	8 (44.44)	6.40 (1.40-29.21)	0.0157
Node positive	4 (14.81)	10 (55.56)	7.19 (1.75-29.48)	0.0075
pT3–pT4 stage	6 (22.22)	11 (61.11)	5.50 (1.48-20.42)	0.0127
Stage III–IV	5 (18.52)	11 (61.11)	6.91 (1.78-26.85)	0.0050

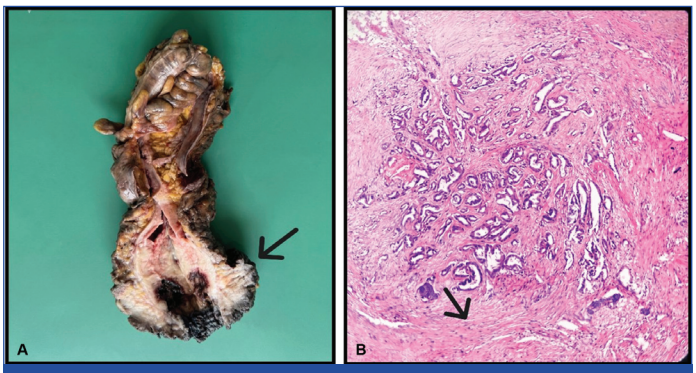
[Table/Fig-5]: Association of stromal TIL status with adverse histopathological parameters.
TIL: Tumour-infiltrating lymphocytes; OR: Odds ratio; CI: Confidence interval. OR represents odds of the adverse feature in the Low-TIL group compared with the High-TIL group. Fisher's-exact test was used; p<0.05 was considered statistically significant

Variable	High-TIL median (IQR)	Low-TIL median (IQR)	U statistic	p-value
Age (years)	61 (47.5–68.5)	61.5 (48.25–68.5)	246	0.9538
Tumour size (cm)	4.2 (3.1–4.75)	5.25 (3.2–5.78)	175.5	0.1204
Lymph nodes examined	19 (13.5-25.0)	20.5 (11.75-26.0)	227.5	0.7274
Positive lymph nodes	0 (0.0-0.0)	1 (0.0-3.75)	143.5	0.0052
Exploratory composite adverse-feature score	0 (0.0-0.0)	4.5 (0.0-6.0)	134	0.0037

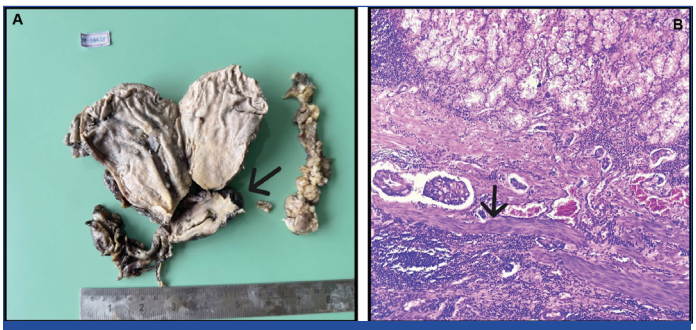
[Table/Fig-6]: Comparison of continuous variables between High-TIL and Low-TIL groups.
TIL: Tumour-infiltrating lymphocytes; IQR: Interquartile range; U statistic: Mann-Whitney U test statistic; p<0.05 was considered statistically significant.



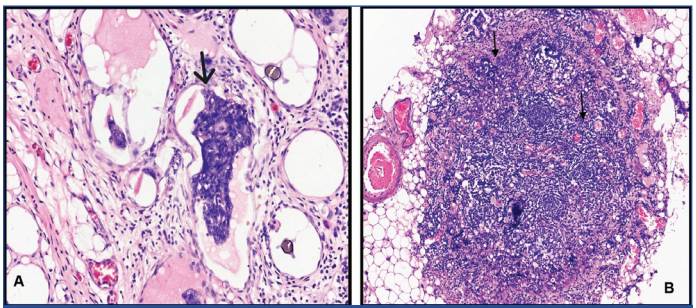
[Table/Fig-7]: Distribution of adverse histopathological parameters according to stromal TIL status.
Bar graph showing adverse-feature rates in High-TIL and Low-TIL groups. Data labels represent number of adverse cases/total cases with corresponding percentage; TIL: Tumour-infiltrating lymphocytes; LVI: Lymphovascular invasion; PNI: Perineural invasion.



[Table/Fig-9]: Abdominoperineal resection specimen showing rectal adenocarcinoma with low stromal Tumour-Infiltrating Lymphocytes (TIL).
a) Gross photograph showing an irregular ulcero-proliferative circumferential growth causing luminal narrowing; b) Photomicrograph showing infiltrating adenocarcinoma with low stromal tumour-infiltrating lymphocytic response. Arrow indicates sparse stromal lymphocytic infiltrate. Haematoxylin and eosin stain, 20× magnification



[Table/Fig-8]: Total gastrectomy specimen showing gastric adenocarcinoma with high stromal Tumour-Infiltrating Lymphocytes (TIL).
a) Gross photograph showing an ulcero-infiltrative grey-white tumour involving the gastric wall with wall thickening and firm cut surface; b) Photomicrograph showing infiltrating adenocarcinoma with high stromal tumour-infiltrating lymphocytic response. Arrows indicate stromal lymphocytic infiltrate. Haematoxylin and eosin stain, 20× magnification



[Table/Fig-10]: Lymphovascular invasion and lymph node metastasis in gastric adenocarcinoma.
a) Photomicrograph showing lymphovascular invasion. Arrow indicates tumour embolus within lymphovascular space. Haematoxylin and eosin stain, 40× magnification; b) Photomicrograph showing metastatic adenocarcinoma deposits in lymphnode. Haematoxylin and eosin stain, 20× magnification

The current findings are comparable with previous studies on colorectal carcinoma, where increased lymphocytic infiltration has been associated with favourable clinicopathological features. Fuchs TL et al., evaluated stromal TILs using the International TILs Working Group system in 1034 cases of colorectal carcinoma and reported that higher TILs scores were associated with lower tumour stage, lower histological grade, and better overall survival [5]. A systematic review and meta-analysis by Idos GE et al., also reported an

association between increased TILs density and improved survival outcomes in colorectal carcinoma [13].

Indian data have shown similar clinicopathological associations. Jose B et al., observed that low TILs density was significantly associated with poor tumour differentiation, lymphovascular invasion, nodal involvement, and advanced pathological stage [10]. Use of haematoxylin and eosin-stained sections for stromal TILs evaluation was described by Shibutani M et al., where a 50% cut-off was applied for classifying cases into High-TILs and Low-TILs groups [12]. Although the present study included tumours from multiple gastrointestinal sites, the direction of association was similar to earlier colorectal carcinoma observations [5,10,12,14].

Poor tumour differentiation was more frequent in the Low-TILs group. This cross-sectional observation indicates an association between reduced stromal lymphocytic infiltration and adverse tumour grade; however, no causal inference can be drawn from this study design. Similar associations between lymphocytic response and tumour differentiation have been reported in colorectal carcinoma cohorts [10,14]. Ko YS and Pyo JS also highlighted the clinicopathological significance of TILs in colorectal carcinoma, supporting their relevance as an immune-related histopathological parameter [15]. Since tumour differentiation remains a routine reporting variable, its association with stromal TILs status may reflect a relationship between host immune response patterns and tumour morphological features.

Lymphovascular invasion and perineural invasion were also more frequent in the Low-TILs group. These parameters indicate tumour involvement of vascular, lymphatic, and neural spaces and are generally regarded as adverse histopathological features. Previous studies have reported similar associations between reduced stromal or peritumoural lymphocytic response and adverse invasive features in colorectal carcinoma [10,16,17]. In the present cohort, lower stromal immune infiltration was associated with adverse invasive features such as lymphovascular invasion and perineural invasion. However, because the study was cross-sectional, it cannot establish whether reduced stromal TILs contribute to adverse tumour morphology or merely accompany it.

Low-TILs status was also associated with nodal positivity, advanced pT stage, and advanced TNM stage. These findings are relevant because nodal status and pathological stage remain central to the assessment of gastrointestinal malignancies. An association between higher stromal TILs scores and lower tumour stage was reported by Fuchs TL et al., while Jose B et al., found low TILs density to be associated with nodal involvement and advanced pathological stage [5,10]. Rozek LS et al., also showed that lymphocytic reaction was associated with stage and survival in colorectal carcinoma [18]. The present findings are therefore consistent with previous observations that stronger local immune response is often associated with less advanced pathological disease [5,10,15,18].

The biological basis for these findings may be related to the type, density, and spatial distribution of immune cells within the tumour microenvironment. Galon J et al., demonstrated that the type, density, and location of immune cells within colorectal tumours could provide information beyond conventional anatomical staging [19]. Pages F et al., further showed that cytotoxic and memory T-cell responses were associated with outcome in early-stage colorectal carcinoma [20]. These studies support the biological plausibility that immune cell density and spatial distribution are relevant to tumour behaviour; however, the present H&E-based cross-sectional study cannot demonstrate functional immune activity or tumour control. It is also important to recognise that gastrointestinal tumours arising at different anatomical sites may differ in tumour biology, histological subtype, and immune microenvironment [1,7,19,20]. In the present cohort, stage distribution also varied across tumour sites. Therefore, the pooled analysis in the present study should be interpreted cautiously, as stromal TILs distribution and its association with adverse histopathological parameters may not be uniform across all gastrointestinal tumour sites.

Several previous studies have evaluated TILs in relation to overall survival, disease-free survival, recurrence, and treatment response [5,13,18,20,21]. In contrast, the present study evaluated only histopathological parameters and did not include survival follow-up, recurrence data, or treatment-response outcomes. Therefore, prognostic implications cannot be directly inferred from the present findings. The observed associations with grade, lymphovascular invasion, perineural invasion, nodal status, pT stage, and TNM stage should be interpreted as histopathological associations rather than evidence of survival benefit.

In the present study, stromal TILs assessment was performed on haematoxylin and eosin-stained sections alone; therefore, the observed association reflects overall stromal mononuclear immune infiltration rather than a specific lymphocyte subset. The inflammatory response at the tumour invasive margin has also been investigated through other scoring systems. The Klintrup-Makinen inflammatory score and related approaches have shown that the local inflammatory reaction may carry clinicopathological and prognostic relevance in colorectal carcinoma [22]. Although the present study used percentage-based stromal TILs assessment rather than the Klintrup-Makinen score, both approaches emphasise the importance of host immune response within the tumour microenvironment.

A practical advantage of stromal TILs evaluation is that it can be performed on routine haematoxylin and eosin-stained sections without additional immunohistochemistry. This makes it a low-cost and accessible histopathological parameter, especially in resource-limited settings. The International Immuno-Oncology Biomarkers Working Group has proposed standardised recommendations for stromal TILs assessment, and subsequent studies have examined the applicability and reproducibility of this method in colorectal carcinoma [5,11,23]. In routine pathology, stromal TILs assessment may serve as an adjunct to established parameters such as tumour grade, lymphovascular invasion, perineural invasion, lymph node status, and TNM stage. It should complement, not replace, conventional histopathological reporting [24,25].

Future site-specific studies with larger and more balanced tumour representation are required to validate the role of stromal TILs assessment across different gastrointestinal malignancies. Incorporation of immune marker profiling, mismatch repair status, PD-1/PD-L1 expression, digital image analysis, and survival follow-up may help clarify the clinicopathological and clinical relevance of stromal TILs scoring.

Limitation(s)

The main limitation of the present study was the heterogeneous composition of the cohort. Tumours from different gastrointestinal sites were included, and these sites have distinct biological behaviour and immune microenvironments. Stromal TILs distribution was not balanced across anatomical sites or histological subtypes. Colon and rectal tumours were represented only in the High-TILs group, whereas duodenal and oesophagus/GEJ tumours were represented only in the Low-TILs group. All poorly cohesive carcinomas, squamous cell carcinomas, adenosquamous carcinomas, and neuroendocrine carcinomas also showed low stromal TILs status in this cohort. Therefore, confounding by tumour site and histological subtype cannot be excluded. Other limitations include small sample size, single-centre design, absence of survival follow-up, lack of immunohistochemical lymphocyte subset analysis, and absence of interobserver agreement analysis using Cohen's kappa or intraclass correlation coefficient. The exploratory composite adverse-feature score was not a validated prognostic score and assigned equal weight to adverse variables that may differ in biological and clinical importance. Site-specific inferential analysis and multivariable

adjustment were not attempted because of small subgroup numbers and sparse cells.

CONCLUSION(S)

In this pooled cohort of primary gastrointestinal malignancies, low stromal TILs status was significantly associated with adverse histopathological features, including poor tumour differentiation, lymphovascular invasion, perineural invasion, nodal positivity, advanced pathological tumour stage, and advanced TNM stage. These findings suggest that reduced stromal immune infiltration may accompany adverse tumour morphology. However, because the cohort included multiple gastrointestinal tumour sites and histological subtypes with uneven distribution of stromal TILs status, the observed associations should be interpreted cautiously and cannot be considered site-independent. Stromal TILs assessment on routine haematoxylin and eosin-stained sections may serve as a simple and cost-effective adjunctive histopathological parameter. Larger site-specific studies with survival follow-up and immune profiling are required to further define its clinicopathological and clinical significance.

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