Tumor Lymphocytic Infiltration as a Prognostic Factor in Gastric Carcinoma

Abdalla AZ[1], Ibrahim HA[1], Hussien MT[2], Abdelrehim SS[1]

[1] Medical Oncology Department, South Egypt Cancer Institute, Assiut University.
[2] Oncologic Pathology Department, South Egypt Cancer Institute, Assiut University.

Abstract:

Background: Gastric cancer has a relatively high prevalence and is one of the most common causes of cancer-related death worldwide. Despite many improvements in diagnosis and treatment, the prognosis for gastric cancer remains poor especially in the advanced stages. Several studies suggested that tumor lymphocytic infiltration (TLI) has a prognostic role in gastric carcinoma and may direct patient selection for immunotherapy. Our study aims to evaluate TLI in gastric carcinoma and its impact on survival.

Material and Method: This was a cohort retrospective study involved 73 gastric carcinoma patients at South Egypt Cancer Institute, in period from the beginning of 2016 to the end of 2020 to evaluate the relation between TLI and clinicopathological features, and its impact on survival outcomes in gastric carcinoma patients.

Results: Patients with high grade tumor, advanced stage, lymph node positive patients' group and the presence of patient distant metastasis (M1) in the presence of low TLI density were significantly associated with poor OS(p=0.033), (p=0.014), (p=0.001) and (p=0.006), respectively. A borderline significance impact on OS was noted in patients who responded to treatment in the presence of high TLI (p=0.067).

Conclusion: High TLI density has favorable outcome regarding OS in gastric carcinoma. These results may give us some valuable prognostic factors for medical management of gastric carcinoma.

Keyword: gastric carcinoma, tumor lymphocytic infiltration, prognostic factor, outcome.

Received: 23 August 2023
Accepted: 14 September 2023

Authors Information:

Ashraf Zeidan Abdalla
Medical Oncology Department, South Egypt Cancer Institute, Assiut University
email: profzedan@yahoo.com

Hasnaa Abdelghany Ibrahim
Medical Oncology Department, South Egypt Cancer Institute, Assiut University
email: drhasnaa1111@gmail.com

Marwa T. Hussien
Oncologic Pathology Department, South Egypt Cancer Institute, Assiut University
email: Marwat.hussien@aun.edu.eg

Sanaa Saber Abdelrehim
Medical Oncology Department, South Egypt Cancer Institute, Assiut University
email: sanaasaber26@yahoo.com

Corresponding Author:
Marwa T. Hussien
Oncologic Pathology Department, South Egypt Cancer Institute, Assiut University
email: Marwat.hussien@aun.edu.eg

Introduction:

Gastric cancer is the fifth most common cancer and the third leading cause of cancer-related deaths in the world[1]. Meta –analysis shows that depth of tumor invasion and presence or absence of lymph node metastasis are the most important prognostic factors in gastric cancer [2]. Histochemical and molecular biological techniques have made it possible to identify many different prognostic factors. The immune cell infiltrate which is an essential component of the tumor microenvironment, have a profound effect on tumor development and clinical outcomes, including tumor prognosis [3]. Tumors can be classified into 2 groups, T-cell-inflamed tumors and non- inflamed tumors, this is depending on the degree of immune cell infiltrations, [4].

Tumor lymphocytic infiltration (TLI) defined as the different infiltrating mononuclear inflammatory cells that directly in contact with the tumor cells (e.g., T cells, B cells, natural killer cells and macrophages) that contribute to either pro- or anti-tumor activities[5].

TLI act as major determinants of the host immune response to tumor cells[6], and the degree of TLI is thought to be associated with controlling the growth, progression, and metastasis of cancer[7]. In ovarian cancer, breast cancer, and colorectal cancer, TLI are essential for inhibiting cancer progression and have implications for the success of immunotherapy, it is predictive for response to neoadjuvant therapy and adjuvant chemotherapy for breast cancer patients[8].

In gastric cancer, studies revealed strong correlations between clinical outcomes and immune cells[9]. Many meta- analysis have shown that high

densities of TLI are associated with a favorable prognosis in gastric cancer[10].

The high TLI density was significantly correlated with small tumor size well-differentiation histological grade negative LN metastasis, negative nerve invasion, negative tumor thrombus, early stage and with favorable OS. This suggests that the adaptive immunity mediated by T lymphocytes acts as an active antitumor response by eradicating cancer cells and avoiding tumor growth[11].

Immune checkpoint inhibitors have attracted significant attention in recent years, with therapies targeting immune receptors such as PD-1 and CTLA-4 being capable of limiting T cell activity by modulating various signaling pathways[12], resulting in positive outcomes in clinical trials across various solid malignancies, including gastric carcinomas [13].

This research was conducted to evaluate the role of tumor lymphocytic infiltration as a prognostic factor in gastric carcinoma by using H&E stain, because it is a cheap and easy method and it gives satisfactory information about the prognosis of the disease, It is possible to predict which patients will respond to immunotherapy.

Patients and Methods:

Study design and patients' methods

This was a retrospective cohort study of 73 patients of gastric carcinoma at South Egypt Cancer Institute (SECI), in the period from 2016 to the end of 2020. Pathologically confirmed gastric adenocarcinoma NOS subtypes, age ≥ 18 years, both genders included and who with qualified and sufficient medical record. Patients who had no available H&E slides and/or formalin fixed paraffin embedded tissue blocks for evaluation of the TLI density excluded Methods:

This study included all patients of gastric adenocarcinoma NOS at South Egypt Cancer Institute from the beginning of 2016 to the end of 2020. All medical records of the patients were assessed for clinicopathological data including age, sex, site, size, tumor invasion, lymph node metastasis, lympho-vascular invasion perineural invasion, distant metastasis, Pathological diagnosis was revised and confirmed by the pathologist, TLI both stromal and intratumoral evaluated separately, by visual assessment of standard haematoxylin and eosin stained tissue sections. Stromal TLI: can be defined as a tumor stromal area containing infiltrating mononuclear inflammatory cells, while, intratumoral TLI: are intraepithelial lymphocytes or mononuclear cells within tumor cells, The response to chemotherapy was done by response evaluation criteria in solid tumors (Recist) criteria. We calculate the PFS which is the time from start of treatment to the first documentation of objective tumor progression or death or missed follow up. we also calculated the OS which is the time from the diagnosis to time of death due to any cause or last follow up.

Evaluation of TLI density

For TLI density evaluation, we used a modified the TLI scoring system [14]. At first, TLI intensity and percentage were estimated separately in the center (CT), and invasive margin of the tumor (IM). The IM was defined as the junctional area between the tumor invading edge area and the host stroma. The CT TLI is corresponding to intratumoral TLI, while IM TLI is corresponding to stromal TLI [15].

The intensity of TLI score ranged from 0-3 which corresponds to absent, mild, moderate, and dense TLI, respectively. The percentage of CT or IM region infiltrated by TLI was assessed. Finally, TLI assessment was done using five scoring system which illustrated in table 1[14] .

Table 1: Modified scoring system for assessment of TLI density in gastric carcinoma

TLI scoring system	Definition
Type 1 score	The intensity of TLI in the CT
Type 2 score	The intensity of TLI in the IM
Type 3 score TLI-CT region score	The multiplication of type 1 score by the % of the CT region infiltrated by TLI
Type 4 score TLI-IM region score	The multiplication of type 2 score by the % of the IM area infiltrated by TLI
Type 5 score TLI-total score	The sum of type 3 score and type 4 score

Statistical methods:

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 22. Quantitative data were statistically described in terms of mean ± SD and median (range) when not normally distributed. Qualitative data were statistically described in terms of frequencies (number of cases) and relative frequencies (percentages) when appropriate. Comparison of quantitative variables was done using Mann Whitney U test, and Kruskal Wallis test for comparing mean of more than quantitative variables because the data were not normally distributed. Kaplan-Meier's method with log rank test was used for overall and progression free survival analysis. P-value is always 2 tailed set significant at 0.05 level.

Results:

Clinicopathologic characteristics of patients

The present study was a five year retrospective observational study to evaluate the role of tumor lymphocytic infiltration as a prognostic factor in gastric carcinoma patients admitted at South Egypt Cancer Institute in the period from 2016-2020. The study included 73 gastric cancer cases.

The mean age of the studied cases was 51.51 ± 12.35 years, ranged from 21 to 78 years, 31 cases

(42.5%) were less than 50 years old, while 42 (57.5%) were ≥ 50 years old. Out of 73 studied cases; 39 (53.4%) were males and 34 (46.6%) were females with male: female ratio of 1.1:1.

More than half 43(58.9%) of the studied cases were suffered from adenocarcinoma, not otherwise specified (NOS) tubular variant, while 30 (41.15) diagnosed with other pathological variant which 18 (24.7%) diagnosed as signet ring carcinoma, mucinous adenocarcinoma was present in 7(9.6%), papillary adenocarcinoma was 1 (1.4%) and poorly cohesive adenocarcinoma was noticed in 4(5.5%) of cases.

Seven cases (9.6%) were tumor grade 1, 20 (27.4%) with tumor grade 2, and 46 (63.0%) were tumor grade 3 tumor.

Table 2: Clinico-pathological features of the studied patients (number (N)=73)

Variable name	N	(%)
Age (years)		
• Mean ± SD	51.51 ± 12.35	
• Median (range)	50 (21 – 78)	
• <50	31	(42.5)
• ≥ 50	42	(57.5)
Gender		
• Male	39	(53.4)
• Female	34	(46.6)
Male:Female ratio	1.1:1	
Tumor variant		
• Adenocarcinoma NOS, tubular	43	(58.9)
• Other variant	30	(41.1)
Grade		
• Grade 1	7	(9.6)
• Grade 2	20	(27.4)
• Grade 3	46	(63.0)
TNM staging system		
T		
• Early stage (T1+T2)	8	(11.0)
• Advanced stage(T3+T4)	65	(89.0)
N		
• N0	9	(12.3)
• N1	12	(16.4)
• N2	22	(30.1)
• N3	30	(41.1)
Distant metastasis		
• M0	30	(41.1)
• M1	43	(58.9)
Tumor Size		
• < 5cm	12	(16.4)
• ≥ 5cm	61	(83.6)
Tumor site		
• Gastro-esophageal junction	9	(12.3)
• Cardia	11	(15.1)
• Fundus	7	(9.6)
• Body	18	(24.7)
• Pylorus	28	(38.4)
Lympho-vascular invasion	67	(91.8)
Peri-neural invasion	53	(72.6)

Variables are presented as mean ± standard deviation, median (range) or frequency (%)

Regarding the TNM staging among the studied cases; according to T – staging, 8 cases (11.0%) with early stage (T1-T2), while 65 cases (89.0%) with advanced stage. According to N – staging, nine cases (12.3%) were free nodal metastasis, 12 cases (16.4%) with N1, 22 (30.1%) with N2, and 30 (41.1%) with N3 nodal metastasis. More than half of the studied cases (58.9%) suffered from distant metastasis. 83.6% have tumor size ≥ 5cm.

Regarding to tumor site; gastric pylorus was the commonest site documented in 28 cases (38.4%), followed by gastric body in 18 cases (24.7%), then cardia in 11 (15.1%), while gastro-esophageal junction, and fundus were documented in 9 (12.3%) and 7 (9.6%) of studied cases respectively. Most of studied cases 67 (91.8%) have lympho-vascular invasion, and 53 (72.6%) have peri-neural invasion.

Association of TLI and clinic-pathologic characteristic of the patients:

By studying the association between TLI (type 1, 2, 3, 4, and 5 scores) and clinic-pathologic variables of the studied patients, we observed that the median of all TLI scores were significantly higher among patients with positive nodal metastasis (P=0.001, 0.032, 0.002, 0.007, and 0.005) for TLI type 1, 2, 3, 4, and 5 scores respectively.

According to tumor site we observed that; TLI type 1 & type 5 scores were significantly higher in patients with pyloric sphincter tumor (P=0.008, and 0.034) respectively. While TLI type 3 score was significantly higher in patients with fundus or pyloric sphincter tumor (P=0.002) compared to other tumor site.

Also TLI type 1, 3, 4 and 5 scores were significantly higher in patients suffered from LVI compared to patients who didn't developed LVI (P=0.046, 0.047, 0.036 and 0.048) respectively, but PNI show no significant association with TLI scores.

Other clinic-pathologic variables of the studied patients namely (tumor variant, tumor grade, T-stage, M-stage, tumor size, and PNI) show no significant association with TLI scores (P>0.05, for all), Table 3.

Association of TLI and response of treatment:

By studying the association between TLI (type 1, 2, 3, 4, and 5 scores) and treatment received by the studied patients and its response, we observed that the median TLI type 1, 3, 4 and 5 scores were significantly higher among patients with stationary disease course (P=0.050, 0.016, 0.002,

0.009, and 0.008) for TLI type 1, 3, 4, and 5 scores respectively compared to patients with regressive or progressive disease course, as shown in Table 4.

Regarding to overall survival:

At 36 months of follow-up, tumor grade, T-stage, M-stage & site, and TLI type 3 score were shown to affect the overall survival of the studied patients (Table 5). Survival was 100% in G1, 43.3% in G2, and 25.6% in G3 (P= 0.013). Survival was 100% in early stage (T1+T2), and 32.9% in advanced stage (T3+T4), (P= 0.027). Survival was 77.2% in M0, and 18.1% M1

(P<0.001). Survival was highest 81.8% in patients with gastric cardia tumor, and lowest 0.0% in patients with gastric fundus tumor (P=0.026). TLI type 3 score at 36 months of follow-up, overall survival was 43.6% for those with intense lymphocytic infiltration compared to 33.5% for those with low TLI type 3 score (P= 0.021) (Figure 4).

Regarding to progression free survival:

At 36 months of follow-up; tumor variant, tumor grade, M-stage, and tumor site were shown to affect the progression free survival among the studied patients (Table 5). Progression free was 44.3% in patients with gastric adenocarcinoma, NOS, tubular subtype and 3.3% in patients with other subtypes (P= 0.002). Progression free was 100% in G1, 30.0% in G2, and 11.2% in G3 (P= 0.001). Progression free was 41.9% in M0, and 15.4% M1 (P=0.042). Progression free was highest 81.8% in patients with gastric cardia tumor, and lowest 0.0% in patients with gastric fundus tumor (P<0.001) (Figure 5).

Survival outcome of TLI density regarding clinicopathological variables:

In patients with high grade tumor and advanced stage, low TLI density was associated with short overall survival OS (p=0.033) and (p=0.014), respectively. In lymph node positive patients' group, low TLI density was associated with short overall survival OS (p=0.001).

Patients with small sized tumor < 5 cm with high TLI density, have better overall survival OS, in comparison with large sized tumor ≥5cm showed short OS (p=0.005). The presence of patient distant metastasis (M1) in the low TLI density was significantly associated with poor OS. (p=0.006). Positive LVI and PNI in high TLI density have a significant good impact on OS (p=0.010) and (p=0.030), respectively.

Regarding therapy response, a borderline significance impact on OS was noted in patients who responded to treatment in the presence of high TLI (p=0.067).

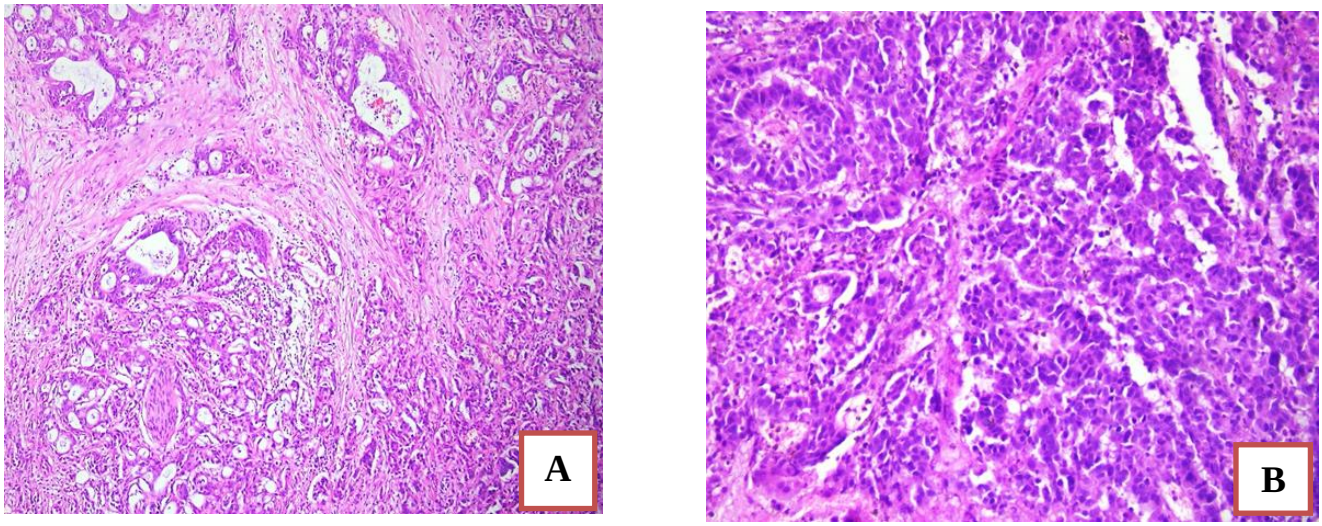


Figure 1: TLI in gastric adenocarcinoma, NOS tubular subtype (A) Moderately differentiated adenocarcinoma with tumor TLI score 1 and stromal TLI score 2 with perineural invasion (arrow) (x20). (B) Poorly differentiated adenocarcinoma with tumor TLI score 1 and stromal TLI score 2 (x40) H&E stain.

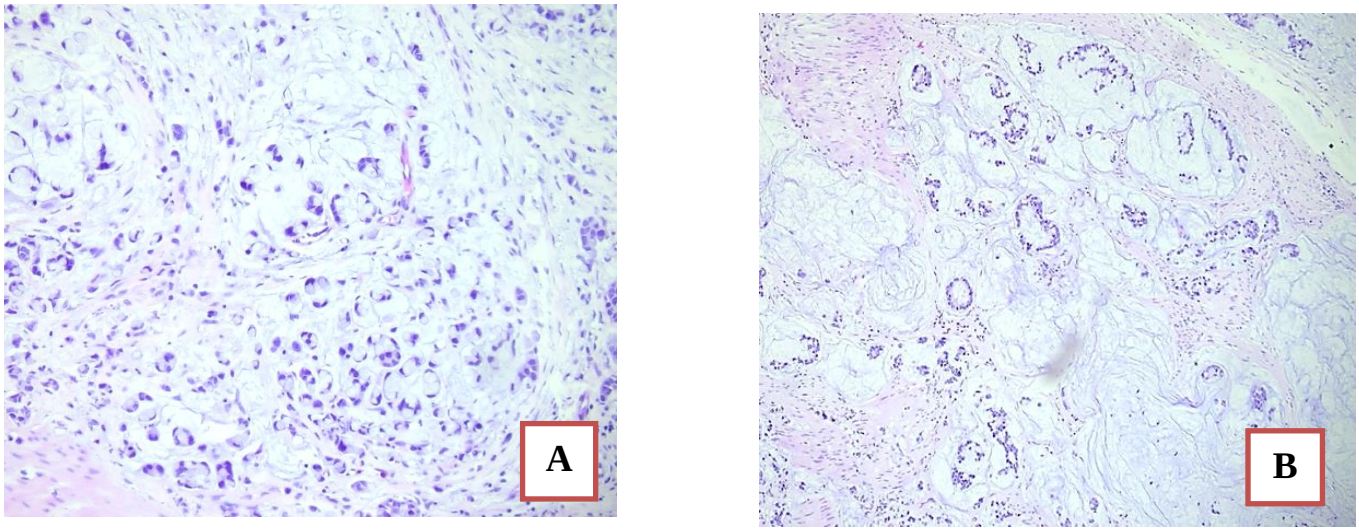


Figure 2: TLI in gastric mucinous and signet ring carcinoma (A) Mucinous carcinoma with tumor TLI score 1 and stromal TLI score 2 (x20). (B) Signet ring carcinoma with tumor TLI score 1 and stromal TLI score 2 (x40) H&E stain

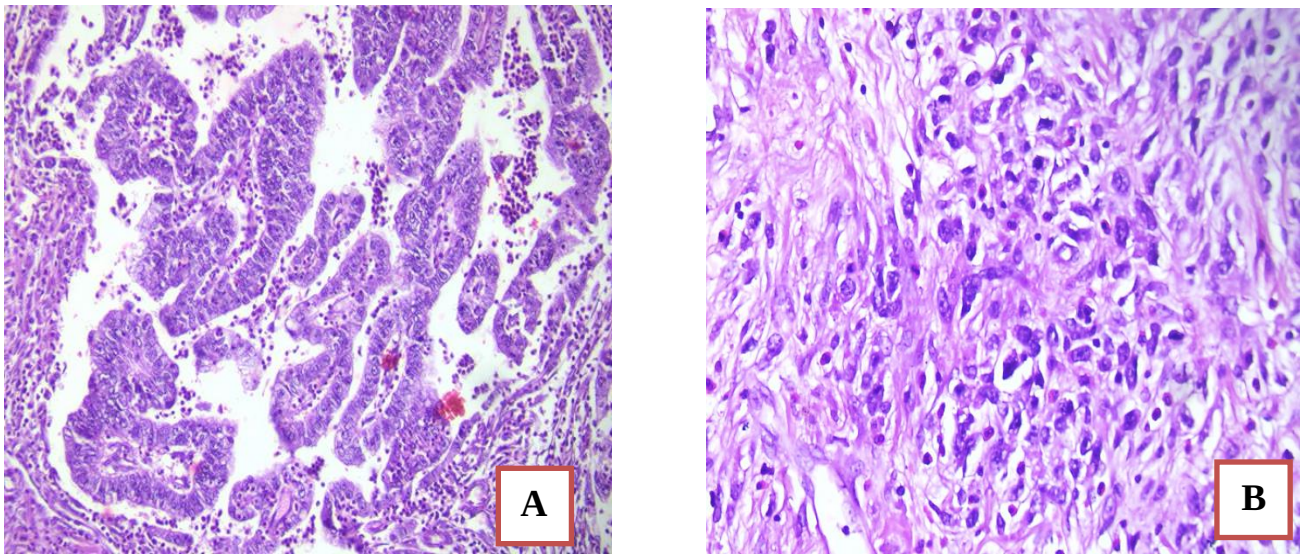
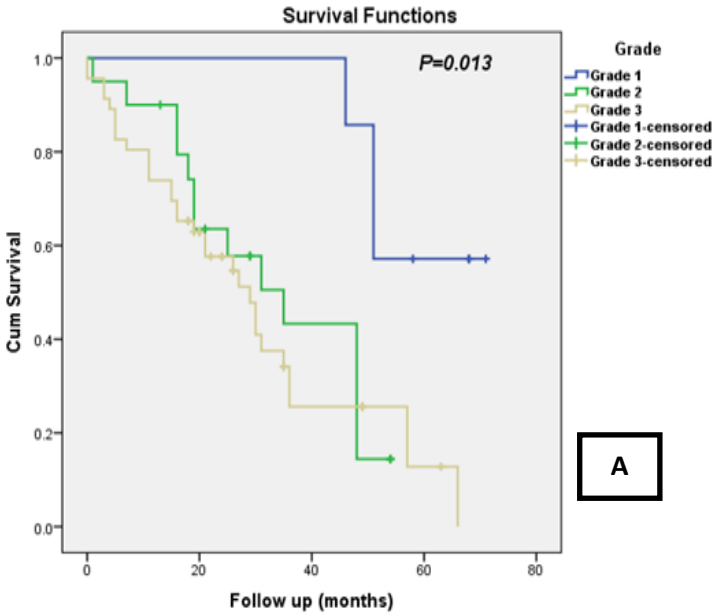
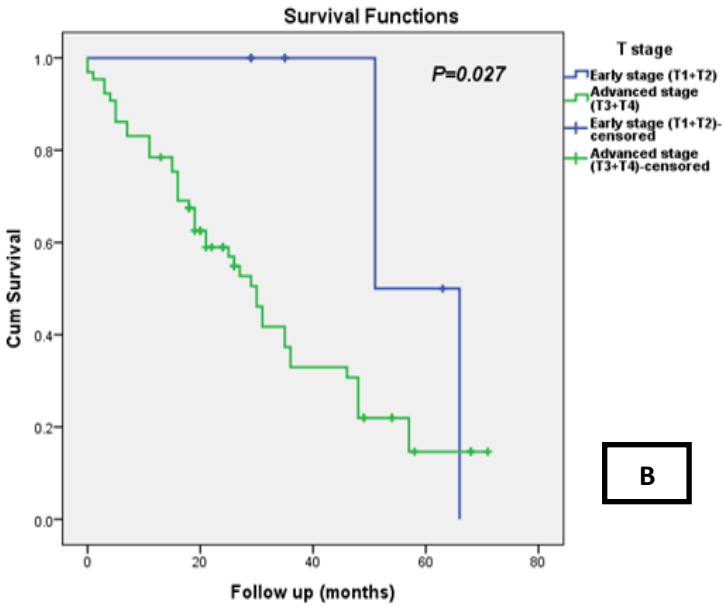
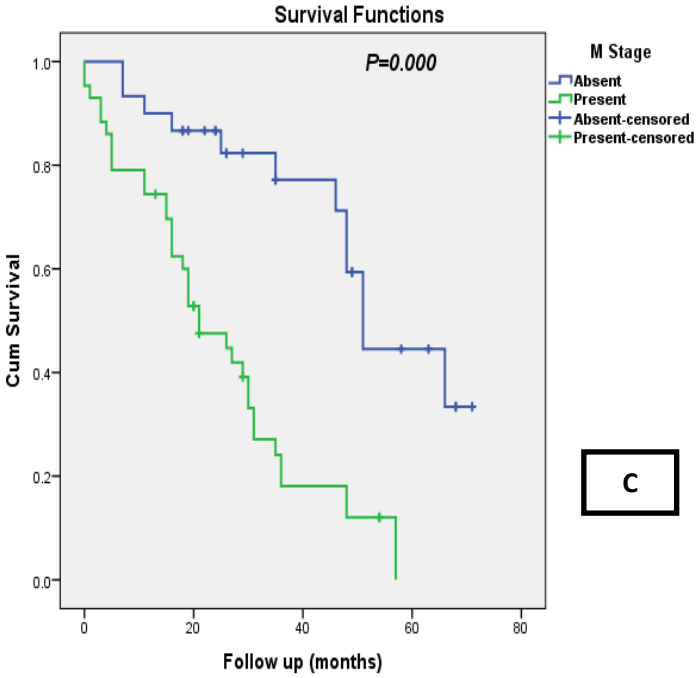


Figure 3: TLI in gastric papillary adenocarcinoma and poorly cohesive carcinoma
(A) Papillary adenocarcinoma with tumor TLI score 1 and stromal TLI score 2 (x20). (B) Poorly cohesive carcinoma with tumor TLI score 1 and stromal TLI score 2 (x40) H&E stain.







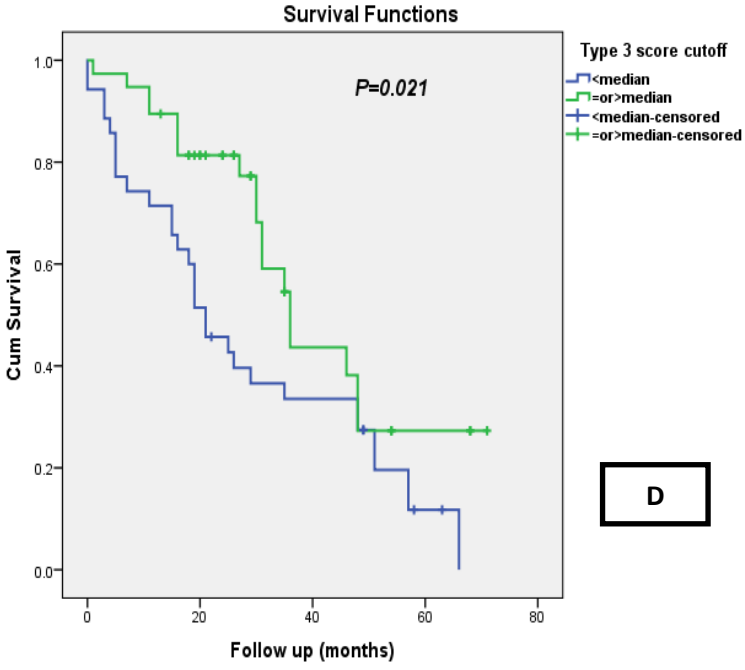


Figure 4: Overall survival of clinicopathological variables and TILs in gastric cancer cases
(A)Grade 3 gastric cancer is significantly associated with poor OS. (B) Advanced stage gastric cancer is significantly associated with shorter OS. (C) The presence of distance metastasis is significantly associated with poor OS. (D) Tumor lymphocytic infiltration (TLI) high is associated with decreased OS.

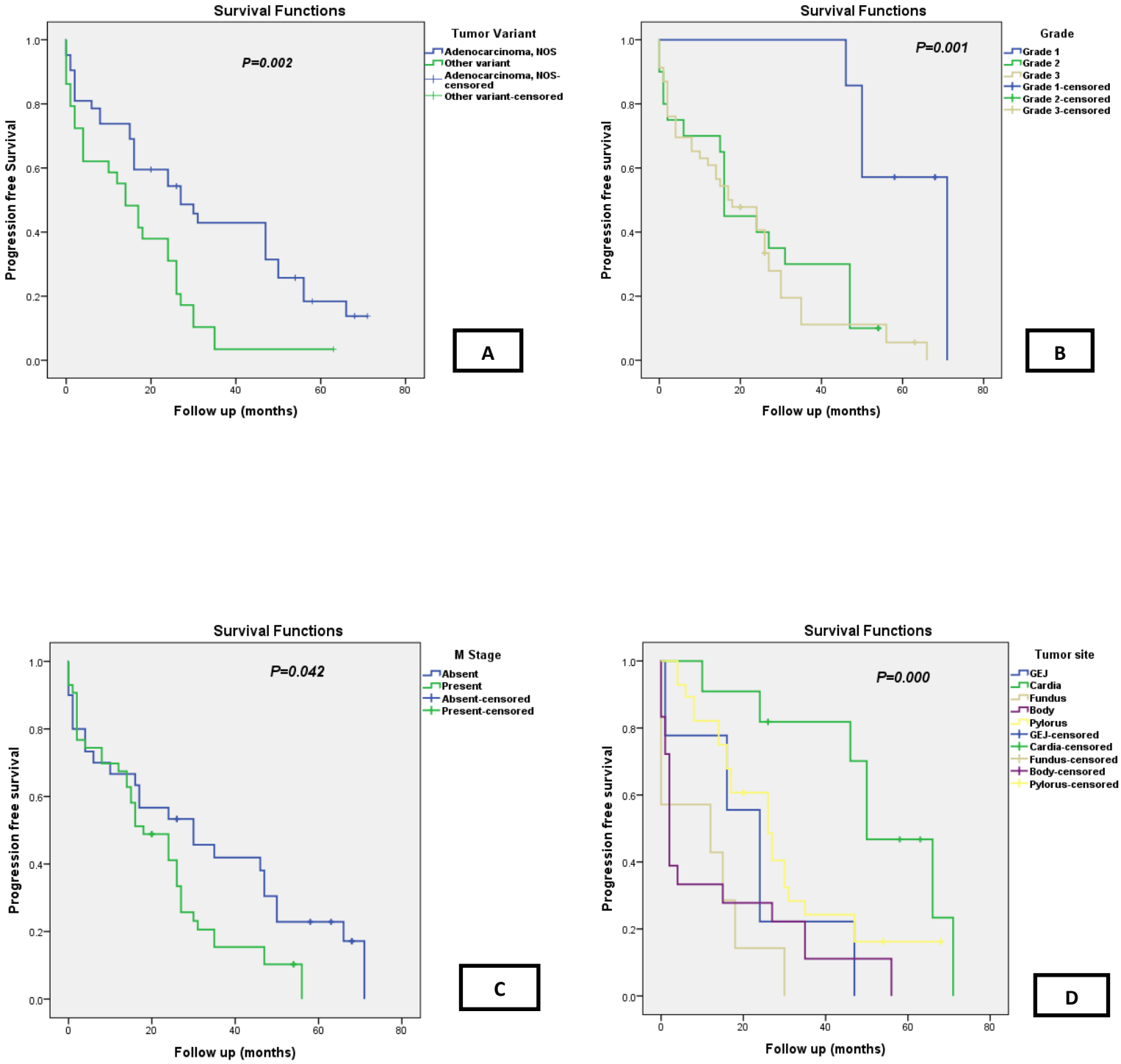


Figure 5: Progression free survival curves of clinicopathological variables cancer studied cases (A) Adenocarcinoma NOS histologic variant is significantly associated with prolonged PFS. (B) Grade 3 gastric cancer is significantly associated with shorter PFS. (C) The presence of distance metastasis is significantly associated with poor PFS. (D) Cardia tumor site is associated with better PFS.

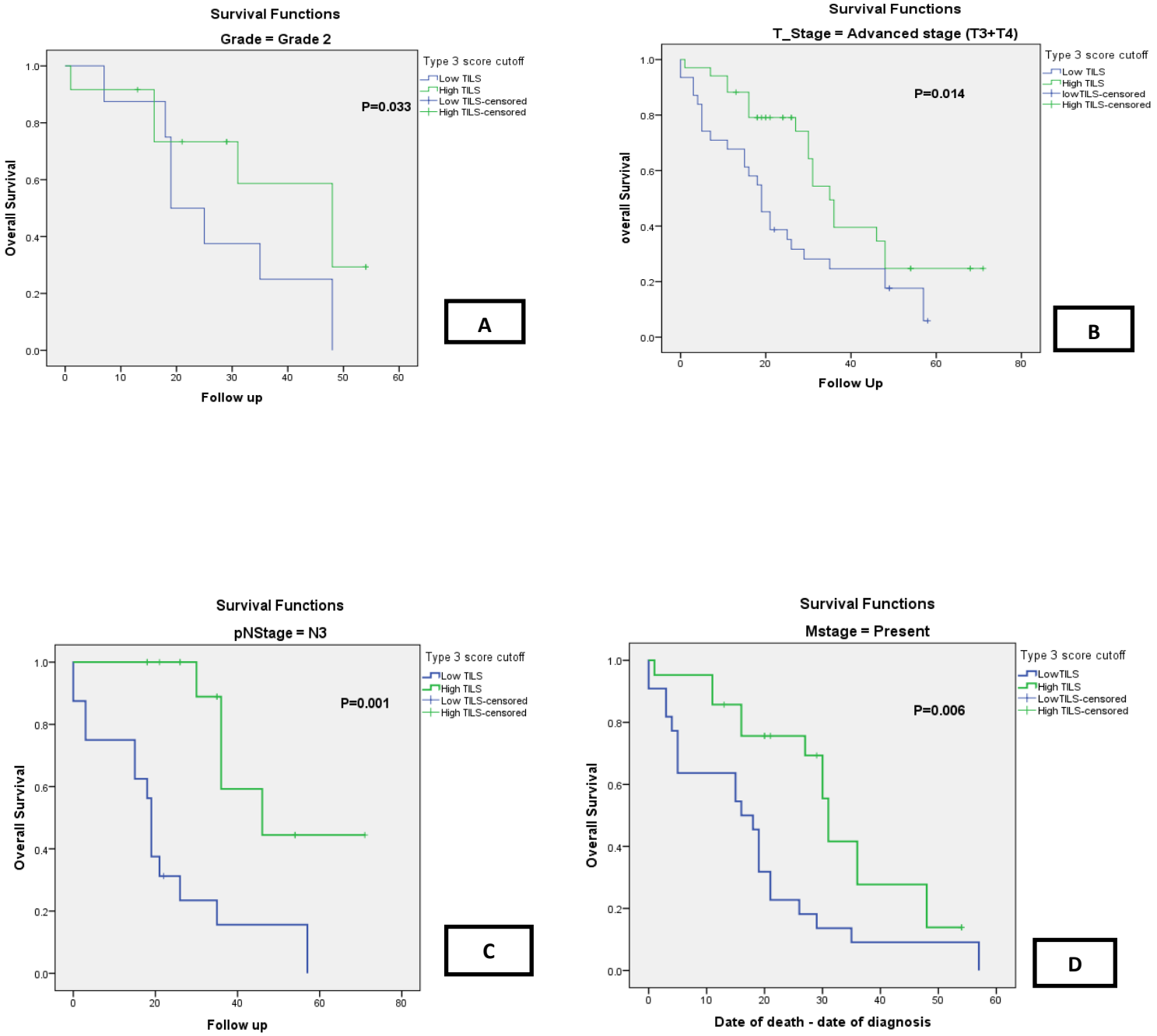


Figure 6: Survival outcome of TLI density regarding clinicopathological variables and TILs in gastric carcinoma. (A) patients with high grade tumor, low TLI density was associated with short overall survival OS. (B) advanced stage in the presence of low TLI density was associated with short overall survival OS. (C) In lymph node positive patients' group, low TLI density was associated with short OS. (D) The presence of patient distant metastasis (M1) in the low TLI density was significantly associated with poor OS.

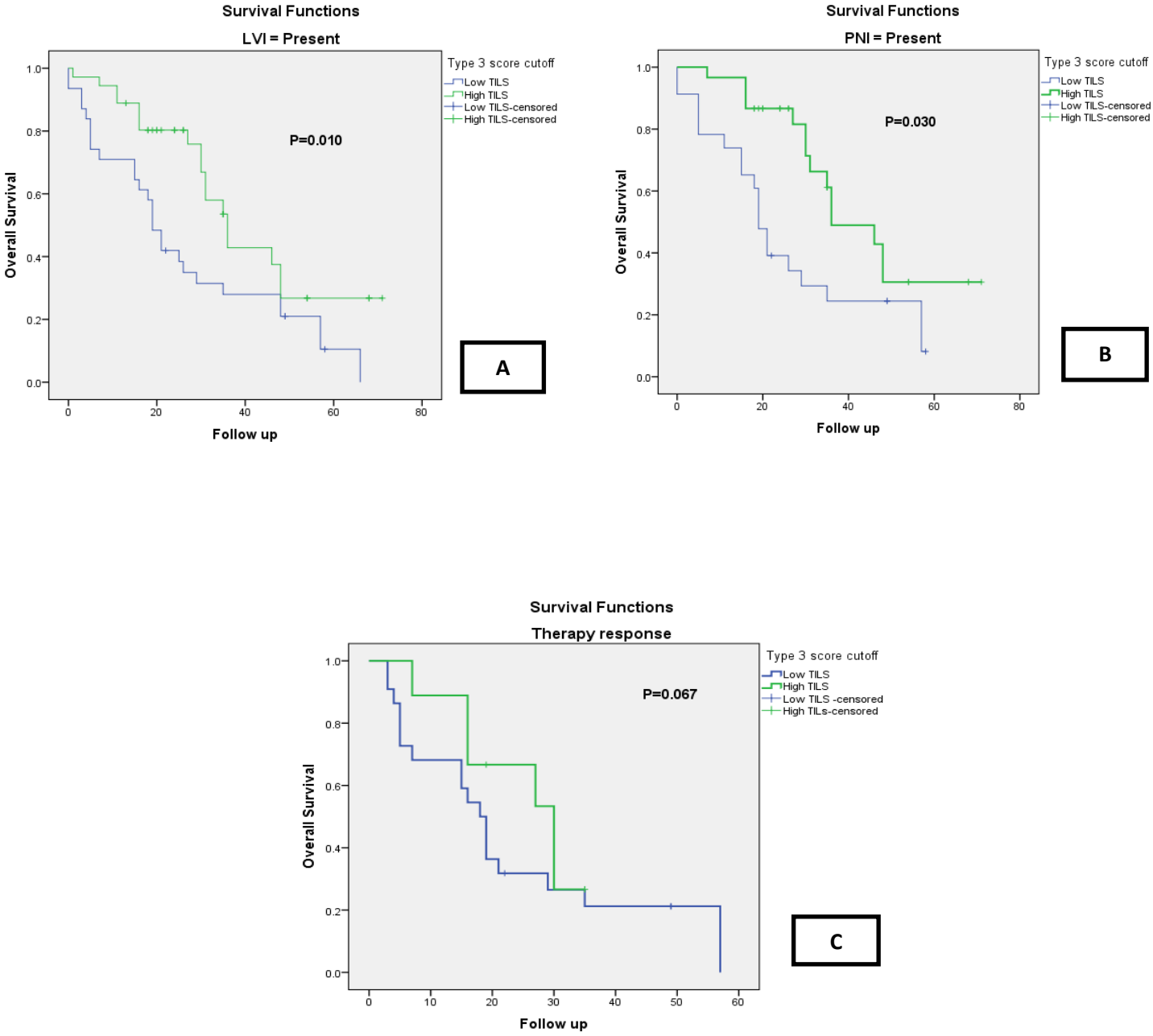


Figure 7: Survival outcome of TLI density regarding clinicopathological variables
(A) Positive LVI and (B) PNI in high TLI density have a significant good impact on OS. (C) A borderline significance impact on OS was noted in patients who responded to treatment in the presence of high TLI (p=0.067).

Table 3: Association of TLI and clinic-pathologic characteristic of the patients(n=73)

Variable name	Type 1 score Mean ± SD Median (range)	P value	Type 2 score Mean ± SD Median (range)	P value	Type 3 score Mean ± SD Median (range)	P value	Type 4 score Mean ± SD Median (range)	P value	Type 5 score Mean ± SD Median (range)	P value
Tumor variant		0.885		0.339		0.933		0.684		0.585
Adenocarcinoma NOS	2.02 ± 0.64 2 (1 – 3)		2.42 ± 0.66 3 (1 – 3)		105.35 ± 65.55 100 (20 – 270)		146.05 ± 59.61 140 (30 – 270)		247.44 ± 118.02 220 (50 – 540)	
Other variant	2.00 ± 0.69 2 (1 – 3)		2.30 ± 0.60 2 (1 – 3)		106.33 ± 68.30 100 (20 – 210)		151.33 ± 64.74 140 (30 – 240)		257.67 ± 124.28 260 (50 – 450)	
Grade		0.650		0.714		0.973		0.803		0.788
Grade 1	1.86 ± 0.90 2 (1 – 3)		2.29 ± 0.95 3 (1 – 3)		117.14 ± 109.50 100 (20 – 270)		151.43 ± 92.63 160 (50 – 270)		268.57 ± 200.29 260 (80 – 540)	
Grade 2	1.95 ± 0.60 2 (1 – 3)		2.45 ± 0.69 3 (1 – 3)		102.00 ± 60.14 100 (20 – 210)		139.50 ± 58.62 130 (30 – 240)		231.50 ± 104.54 215 (50 – 420)	
Grade 3	2.07 ± 0.65 2 (1 – 3)		2.35 ± 0.57 2 (1 – 3)		105.65 ± 62.17 80 (20 – 210)		151.52 ± 58.19 140 (30 – 240)		257.83 ± 113.00 255 (50 – 450)	
T		0.233		0.504		0.379		0.423		0.512
Early stage	1.75 ± 0.89 1.5 (1 – 3)		2.13 ± 0.99 2.5 (1 – 3)		91.25 ± 79.90 65 (20 – 210)		128.75 ± 76.24 150 (30 – 210)		220.00 ± 151.28 215 (50 – 420)	
Advanced stage	2.05 ± 0.62 2 (1 – 3)		2.40 ± 0.58 2 (1 – 3)		107.54 ± 64.86 100 (20 – 270)		150.62 ± 59.58 140 (30 – 270)		255.54 ± 116.30 250 (50 – 540)	
N		**0.001***		**0.032***		**0.002***		**0.007***		**0.005***
Negative	1.33 ± 0.50 1 (1 – 2)		1.89 ± 0.78 2 (1 – 3)		46.67 ± 32.40 30 (20 – 100)		96.67 ± 54.54 80 (30 - 180)		143.33 ± 83.52 120 (50 – 280)	
Positive	2.11 ± 0.62 2 (1 – 3)		2.44 ± 0.59 2 (1 – 3)		114.06 ± 65.65 100 (20 – 270)		155.47 ± 59.12 150 (30 – 270)		266.88 ± 116.74 260 (50 – 540)	
Tumor Size		0.379		0.927		0.753		0.636		0.923
< 5cm	2.17 ± 0.58 2 (1 – 3)		2.42 ± 0.51 2 (2 – 3)		108.33 ± 57.02 100 (30 – 210)		155.83 ± 60.07 145 (60 – 24)		255.83 ± 103.35 205 (110 – 390)	
≥ 5cm	1.98 ± 0.67 2 (1 – 3)		2.36 ± 0.66 2 (1 – 3)		105.25 ± 68.30 100 (20 – 270)		146.72 ± 62.01 140 (30 – 270)		250.82 ± 123.63 250 (50 – 540)	
M		0.051		0.653		0.484		0.261		0.306
Absent	1.83 ± 0.75 2 (1 – 3)		2.30 ± 0.75 2 (1 – 3)		102.33 ± 77.18 100 (20 – 270)		137.67 ± 69.51 140 (30 – 270)		236.67 ± 143.17 250 (50 – 540)	
Present	2.14 ± 0.56 2 (1 – 3)		2.42 ± 0.54 2 (1 – 3)		108.14 ± 58.24 80 (20 – 210)		155.58 ± 54.65 140 (60 – 240)		262.09 ± 101.06 240 (100 – 450)	
Tumor site		**0.008***		0.467		**0.002***		0.124		**0.034***
GEJ	1.67 ± 0.50 2 (1 – 2)		2.44 ± 0.88 3 (1 – 3)		61.11 ± 31.80 60 (20 – 100)		128.89 ± 64.51 150 (30 – 180)		190.00 ± 95.13 210 (50 – 280)	
Cardia	1.55 ± 0.52 2 (1 – 2)		2.09 ± 0.83 2 (1 – 3)		60.91 ± 35.62 60 (20 – 100)		111.82 ± 52.69 120 (30 – 180)		172.73 ± 84.51 150 (50 – 260)	

Variable name	Type 1 score Mean ± SD Median (range)	P value	Type 2 score Mean ± SD Median (range)	P value	Type 3 score Mean ± SD Median (range)	P value	Type 4 score Mean ± SD Median (range)	P value	Type 5 score Mean ± SD Median (range)	P value
Fundus	1.86 ± 0.69 2 (1 – 3)		2.29 ± 0.49 2 (2 – 3)		105.71 ± 70.20 140 (20 – 210)		141.43 ± 62.83 140 (80 – 240)		247.14 ± 130.35 280 (100 – 450)	
Body	2.11 ± 0.32 2 (2 – 3)		2.33 ± 0.49 2 (2 – 3)		100.00 ± 49.35 80 (60 – 210)		146.67 ± 58.41 130 (60 – 240)		248.33 ± 100.72 200 (120 – 420)	
Pylorus	2.29 ± 0.76 2 (1 – 3)		2.50 ± 0.58 3 (1 – 3)		141.43 ± 73.97 120 (30 – 270)		171.43 ± 59.36 195 (8 – 270)		305.71 ± 126.45 330 (110 – 540)	
LVI		**0.046***		0.098		**0.047***		**0.036***		**0.048***
Absent	1.50 ± 0.55 1.5 (1 – 2)		1.83 ± 0.98 1.5 (1 – 3)		56.67 ± 36.15 45 (20 – 100)		95.00 ± 67.75 65 (30 – 180)		151.67 ± 103.62 110 (50 – 280)	
Present	2.06 ± 0.65 2 (1 – 3)		2.42 ± 0.58 2 (1 – 3)		110.15 ± 66.69 100 (20 – 270)		152.99 ± 59.01 140 (30 – 270)		260.60 ± 117.79 250 (50 – 540)	
PNI		0.088		0.062		0.101		0.244		0.138
Absent	1.80 ± 0.70 2 (1 – 3)		2.10 ± 0.79 2 (1 – 3)		85.50 ± 59.60 80 (20 – 210)		132.00 ± 70.46 130 (30 – 240)		217.50 ± 126.19 200 (50 – 420)	
Present	2.09 ± 0.63 2 (1 – 3)		2.47 ± 0.54 2 (1 – 3)		113.40 ± 67.51 100 (20 – 270)		154.34 ± 57.13 150 (60 – 270)		264.53 ± 116.03 260 (100 – 540)	

Quantitative data are presented as mean ± SD and median (range), Comparison of quantitative variables was done using Mann Whitney U test, and Kruskal Wallis test . * Significance defined by $p < 0.05$.

Table 4: Association of TLI and response of treatment (n=73)

Variable name	Type 1 score Mean ± SD Median (range)	P value	Type 2 score Mean ± SD Median (range)	P value	Type 3 score Mean ± SD Median (range)	P value	Type 4 score Mean ± SD Median (range)	P value	Type 5 score Mean ± SD Median (range)	P value
Response to treatment										
Regressive	2.00 ± 0.69 2 (1 – 3)	**0.050***	2.34 ± 0.73 2 (1 – 3)	0.067	110.86 ± 71.31 100 (20 – 270)	**0.016***	138.57 ± 67.53 140 (30 – 270)	**0.009***	249.43 ± 132.40 260 (50 – 540)	**0.008***
Stationary	2.57 ± 0.53 3 (2 – 3)		2.86 ± 0.38 3 (2 – 3)		162.86 ± 46.45 180 (20 – 210)		214.29 ± 20.70 210 (180 – 240)		377.14 ± 54.38 390 (260 – 420)	
Progression	1.90 ± 0.60 2 (1 – 3)		2.29 ± 0.53 2 (1 – 3)		87.10 ± 56.40 80 (20 – 210)		144.19 ± 51.43 140 (80 – 240)		225.81 ± 98.31 200 (100 – 420)	

Quantitative data are presented as mean ± SD and median (range), Comparison of quantitative variables was done using Kruskal Wallis test.
* Significance defined by $p < 0.05$.

Table 5: Overall survival and progression free survival according to clinic-pathological details of the studied gastric cancer cases (n=73)

	OS (3 years)		PFS (3 years)	
	Estimate ± SE	**P value**	**Estimate ± SE**	**P value**
Age (years)		0.797		0.207
• < 50	25.7 ± 10.5%		9.2 ± 6.1%	
• ≥ 50	47.8 ± 8.0%		35.7 ± 7.4%	
Gender		0.054		0.222
• Male	53.6 ± 8.9%		34.2 ± 7.8%	
• Female	25.1 ± 8.8%		17.0 ± 6.8%	
Tumor variant		0.235		**0.002***
• Adenocarcinoma NOS	51.9 ± 8.2%		44.3 ± 7.9%	
• Other variant	20.5 ± 9.6%		3.3 ± 3.3%	
Grade		**0.013***		**0.001***
• Grade 1	100.0 ± 13.2%		100.0 ± 13.2%	
• Grade 2	43.3 ± 12.3%		30.0 ± 10.2%	
• Grade 3	25.6 ± 8.0%		11.2 ± 5.1%	
T – stage		**0.027***		0.401
• Early stage (T1+T2)	100.0 ± 25.0%		50.0 ± 17.7%	
• Advanced stage (T3+T4)	32.9 ± 6.6%		23.1 ± 5.5%	
N – stage		0.094		0.146
• Negative	77.8 ± 13.9%		55.6 ± 16.6%	
• Positive	35.0 ± 6.8%		21.8 ± 5.4%	
M – stage		**0.000***		**0.042***
• No metastasis	77.2 ± 8.4%		41.9 ± 9.2%	
• Metastasis	18.1 ± 6.5%		15.4 ± 5.7%	
Tumor site		**0.026***		**0.000***
• GEJ	44.4 ± 16.6%		22.2 ± 13.9%	
• Cardia	81.8 ± 11.6%		81.8 ± 11.6%	
• Fundus$	0.0 ± 0.0%		0.0 ± 0.0%	
• Body	21.9 ± 2.4%		11.1 ± 7.4%	
• Pylorus	42.0 ± 9.9%		24.3 ± 8.5%	
LVI		0.236		0.609
• Present	36.8 ± 6.7%		24.0 ± 5.5%	
• Absent	83.3 ± 15.2%		50.0 ± 20.4%	
PNI		0.383		0.392
• Present	38.5 ± 7.6%		24.6 ± 6.3%	
• Absent	46.0 ± 12.1%		30.0 ± 10.2%	
TLI type 1 score		0.140		0.140
• < median	65.5 ± 12.6%		46.7 ± 12.9%	
• ≥ median	31.7 ± 7.4%		20.7 ± 5.6%	
TLI type 2 score		0.378		0.093
• < median	83.3 ± 15.2%		83.3 ± 15.2%	
• ≥ median	35.6 ± 6.8%		20.8 ± 5.2%	
TLI type 3 score		**0.021***		0.554
• < median	33.5 ± 8.1%		25.7 ± 7.4%	
• ≥ median	43.6 ± 10.5%		26.7 ± 7.8%	
TLI type 4 score		0.819		0.136
• < median	51.3 ± 9.4%		41.4 ± 9.1%	
• ≥ median	26.0 ± 8.9%		14.8 ± 5.9%	
TLI type 5 score		0.151		0.761
• < median	38.4 ± 8.2%		30.6 ± 7.7%	
• ≥ median	36.0 ± 10.7%		20.9 ± 7.3%	

Kaplan-Meier's method with log rank test was used to calculate overall survival analysis* Significance defined by $p < 0.05$. $ Not reach follow up of three years.

Discussion:

One persistent debate is whether tumor immune response has a prognostic role in gastric carcinoma or not. This study was conducted to investigate the role of tumor lymphocytic infiltration (TLIs), as mirror of host response to tumor, as a prognostic factor in gastric carcinoma [16]. This done by evaluation TLIs density in H&E stain slides, because it is a cheap and easy method and it gave satisfactory information about the prognosis of the disease, it is possible to predict which patients will respond to immunotherapy.

To achieve our goal, we studied all patients treated from gastric adenocarcinoma in South Egypt Cancer Institute (SECI) in the period from (2016-2020) to evaluate the role of tumor lymphocytic infiltration as a prognostic factor in gastric carcinoma.

The mean age of our patients at time of diagnosis was 51.5 years, which in agreement with Gaballah et al with mean age of their studied cases was 52 years old [17] and Ibrahim et al study in which the mean age of patients was 54 years old [18].

In our study, there was no obvious sex predilection, with male to female ratio is 1.1 : 1. This result agrees with study done by Magdy et al that demonstrate male to female ratio was 1:1 [19]. This contrasts with GLOBOCAN 2018 estimates incidence rates for gastric cancer were two-fold to three-fold higher for men than women[20], this is may be due to differences in habits and behavior.

More than half of the studied cases were suffered from adenocarcinoma, NOS, while the adenocarcinoma variant account for 41.15 %. This finding matched to study done by Zeeneldin et al which noted that adenocarcinoma, NOS was the commonest histological subtype [21] and this is compatible with the universal most common pathological type.

The current study showed that grade 3 tumors (63.0%) were more prevalent than grade 2 and grade 1 (27.4% and 9.2%, respectively). This finding was in agreement with the studies done by Akl et al and Darwish et al [22,23], where the commonest tumor grade (according to Broder's classification) was grade 3, which may be explained by the fact that gastric carcinoma is an aggressive tumor.

Concerning the pathologic stage of the tumor, advanced stage (T3-T4) was the most prevalent stage in our study which was agree with finding found in an Egyptian study done by Darwish et al [23]. Because gastric carcinoma in its early stage is mostly asymptomatic, and in our country, it has not yet been applied the screening program for gastric carcinoma so the most prevalent stage is advanced stage, when the patients have symptoms.

About 58% of the studied cases were suffered from distant metastasis. That similar to study done by Darwish et al, at which 59.7% of their patients presented with metastasis [23]. This may be explained by most cases of gastric carcinoma are discovered in the advanced stages.

In our study 83.6% of patients had tumor size ≥ 5cm. This matched with Zu et al. study that documented that most of their studied cases had large tumor size[24]. This can be explained by the fact that the stomach is a wide luminal structure so the tumor can grow without the appearance of any symptoms so most of cases diagnosed with large size.

Regarding to tumor site, gastric pylorus was the commonest site. This finding compatible with the Egyptian study done by Darwish et al, Which noted that the majority of patients had tumors located in the lower third of the stomach[23]. However, Magdy et al revealed that the most frequent primary tumor location was the body of the stomach (39.3%), followed by pylorus (36.1%), and GEJ (24.6%)[19].

Most of the studied cases (91.8%) had lympho-vascular invasion (LVI), and about (72%) have peri-neural invasion (PNI), that as advanced and metastatic stage were significantly related to PNI and LVI positivity and most of cases in our study were advanced and metastatic stage. This result matched with the study done by Kim et al who founded that (80.6 %) of patients with PNI positive were in stage III [25]. A study done by Magdy et al showed marked difference from our findings, as LVI was noted in only 9.8% of their cases and PNI was present in 11.5% of their studied cases [19] This may be due to the most of cases at Magdy et al. study were early and locally advanced not metastatic.

By studying the association between TLI and clinicopathologic variables, we observed that both tumor and stromal TLI scores were significantly higher among patients with positive nodal metastasis. Also, Lee et al found a significant association between TLI density in both tumor epithelium and stroma and the presence of regional lymph node metastasis. So, TLI density may have a role in prediction of lymph node metastasis in gastric adenocarcinoma [26]. Conversely, a study done by Zhang et al showed that TLI score were significantly correlated with negative LN metastasis as most of cases at his study were not nodal metastatic [14].

Pyloric tumor location was significantly associated higher both tumor and stromal TLI (type 1, type 3 & type 5 scores) in the present work. However, the current finding is inconsistent with Jiang et al who found a high level TLI estimation was significantly at the tumor located in the body of the stomach[27].

In our study TLI scores in tumor and stroma TLI (type 1, 3, 4 and 5 scores) were significantly higher in patients suffering from LVI compared to patients who didn't develop LVI. This agrees with the Kang et al study that has proven TLI were significantly associated with the presence of lymphatic invasion [28]. While contrary to result of Zhang et al study, at which The TLI were significantly correlated with negative tumor embolism[14].

Interestingly, both tumor and stromal TLI in our study was associated with the clinical response to chemotherapy, as we observed that the mean TLI scores

(TLI type 1, 3, 4, and 5 scores) were significantly higher among patients with stationary followed by regressive disease course compared to patients with progressive disease course. Also, Lee et al and Wang et al reported that high TLI expression predicts sensitive therapeutic responsiveness to chemotherapy in gastric cancer. That may be explained by chemotherapy can enhance the efficacy of host functions by reducing tumor burdens [29], and also chemotherapeutic agents destroy tumor cells and release tumor-associated antigens that enhance the immunity which play a major role as anti-tumor factor[30].

Regarding survival analysis, grade 1 tumors, early stage and gastric cardia tumor site were associated with favorable OS in the studied cases. This result was in congruent with a study done by Becker et al which documented that the early stage, lower pN stage and proximal tumor location were associated with significant tumor regression and better OS[31].

In our study patients with high tumor TLI (type 3 score) showed better OS in comparison to low density tumor TLI and this agreement with a Meta-analysis of 2941 cases, which provides a quantitative assessment of the prognostic value of TLI in gastric cancer patients, revealed a significant association between high TLI levels in tumor tissue and improved survival [32].

On the other hand, Fukuda et al reported that TLI infiltration had no significant impact on OS These variation in the results may attributed to different methods for TLI evaluation and interpretation[33].

The current study revealed that the high tumor TLI density (score 3) in early-stage tumor patients exhibited prolonged OS in comparison to low TLI density. These outcomes were coincide with Zhang et al. results which Low and high levels of TLI had significant prognostic value for pTN stage I-III patients, and better prognosis was present with TLI high patients[14]. This finding can help to classify the patients into prognostic groups according to TLI density.

In a study done by Zhang et al, found that high TLI score correlates with a low rate of cancer metastasis and better patient survival which agreed with our finding [14].

The poor prognostic parameters in the present research like perineural invasion, LVI, high tumor grade, and advances TNM stage were found to be significantly associated with low TLI and poor OS. This was compatible with Dai et al results[34].

These results suggested that TLI density was associated with good prognosis in patients with gastric cancer which underlines the importance of TLI as a predictor of clinical outcome.

Future study with large numbers of cases in addition with typing of TLI by using immunohistochemistry is advisable and its impact on immunotherapy.

Conclusion:

In conclusion, TLI assessment is an easily applicable, cheap method, and it gave us satisfactory results. The current study documented that evaluation of score 3 TLI which corresponding to intratumoral lymphocytes giving significant prognostic impact on survival and advised in routinely diagnosis. Furthermore, high TLI, is a potential biomarkers and accurate predictor of good prognosis in patients with gastric cancer e.g. high TLI density may prevent tumor progression. TLI evaluation can help us foretell clinical outcome and determine patient subgroups with an unfavorable prognosis in gastric cancer. Our observations made during this study may give information concerning the effective immunotherapies and help to know of patients who are more probably to benefit from the immunotherapy.

List of abbreviations

TLI	Tumor lymphocytic infiltration
PD-1	Programmed death-1
CTLA-4	Cytotoxic T-lymphocyte- associated antigen 4
LVI	Lympho-vascular invasion
PNI	Peri-neural invasion
T	The tumor
N	Node
M	Metastasis
OS	Overall survival
PFS	Progression free survival
CR	complete response
PR	Partial response
PD	progressive disease

Acknowledgements:

None

References:

[1] Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49.

[2] Goto A, Nishikawa J, Hideura E, et al. Lymph node metastasis can be determined by just tumor depth and lymphovascular invasion in early gastric cancer patients after endoscopic submucosal dissection. Eur J Gastroenterol Hepatol 2017;29:1346.

[3] Rohr-Udilova N, Klinglmüller F, Schulte-Hermann R, et al. Deviations of the immune cell landscape between healthy liver and hepatocellular carcinoma. Sci Rep 2018;8:1–11.

[4] Corrales L, Glickman LH, McWhirter SM, et al. Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. Cell Rep 2015;11:1018–30.

[5] Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 2015;26:259–71.

[6] Müller P, Rothschild SI, Arnold W, et al. Metastatic spread in patients with non-small cell lung cancer is associated with a reduced density of

tumor-infiltrating T cells. Cancer Immunol Immunother 2016;65:1–11.

[7] Kollmann D, Ignatova D, Jedamzik J, et al. Expression of programmed cell death protein 1 by tumor-infiltrating lymphocytes and tumor cells is associated with advanced tumor stage in patients with esophageal adenocarcinoma. Ann Surg Oncol 2017;24:2698–706.

[8] Fanale D, Dimino A, Pedone E, et al. Prognostic and predictive role of Tumor-Infiltrating Lymphocytes (TILs) in ovarian cancer. Cancers (Basel) 2022;14:4344.

[9] Hu Q, Nonaka K, Wakiyama H, et al. Cytolytic activity score as a biomarker for antitumor immunity and clinical outcome in patients with gastric cancer. Cancer Med 2021;10:3129–38.

[10] Hong SA, Hwang HW, Kim MK, et al. High endothelial venule with concomitant high CD8+ tumor-infiltrating lymphocytes is associated with a favorable prognosis in resected gastric cancer. J Clin Med 2020;9:2628.

[11] Lei X, Lei Y, Li J-K, et al. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. Cancer Lett 2020;470:126–33.

[12] Dyck L, Mills KHG. Immune checkpoints and their inhibition in cancer and infectious diseases. Eur J Immunol 2017;47:765–79.

[13] Taube JM, Galon J, Sholl LM, et al. Implications of the tumor immune microenvironment for staging and therapeutics. Mod Pathol 2018;31:214–34.

[14] Zhang D, He W, Wu C, et al. Scoring system for tumor-infiltrating lymphocytes and its prognostic value for gastric cancer. Front Immunol 2019;10:71.

[15] Saldanha G, Flatman K, Teo KW, et al. novel numerical scoring system for melanoma tumour infiltrating lymphocytes has better prognostic value than standard scoring. Am J Surg Pathol 2017;41:906.

[16] Fang X, Xu J, Jin K, et al. Combining of immunotherapeutic approaches with chemotherapy for treatment of gastric cancer: Achievements and limitations. Int Immunopharmacol 2023;118:110062.

[17] Gaballah A, Moawad M, Yassin M, et al. RM-015 Clinicopathological, epidemiological and outcome of treatment of advanced gastric cancer in Egypt: single institution experience. Ann Oncol 2016;27:ii89.

[18] Ibrahim AS, Khaled HM, Mikhail NNH, et al. Cancer incidence in Egypt: results of the national population-based cancer registry program. J Cancer Epidemiol 2014;2014.

[19] Magdy M, Hussein T, Ezzat A, et al. Pre-treatment peripheral neutrophil-lymphocyte ratio as a prognostic marker in gastric cancer. J Gastrointest Cancer 2019;50:763–8.

[20] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7–34.

[21] Zeeneldin AA, Ramadan H, El Gammal MM, et al. Gastric carcinoma at Tanta Cancer Center: a comparative retrospective clinico-pathological study of the elderly versus the non-elderly. J Egypt Natl Canc Inst 2014;26:127–37.

[22] Akl MF, Ibrahem MA, Khater A, et al. Etiologic and clinicopathological correlates of gastric carcinoma in the Egyptian delta. Indian J Surg Oncol 2018;9:472–6.

[23] Darwish H, Sakr A, Basaam W, et al. 10 years Experience in the Treatment of Gastric Cancer: A Single Egyptian Cancer Center (NEMROCK). Pan Arab J Oncol 2016;9.

[24] Zu H, Wang F, Ma Y, et al. Stage-stratified analysis of prognostic significance of tumor size in patients with gastric cancer. PLoS One 2013;8:e54502.

[25] Kim JY, Kim WG, Kwon CH, et al. Differences in immune contextures among different molecular subtypes of gastric cancer and their prognostic impact. Gastric Cancer 2019;22:1164–75.

[26] Lee HE, Chae SW, Lee YJ, et al. Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer. Br J Cancer 2008;99:1704–11.

[27] Jiang N, Deng J-Y, Liu Y, Ke B et al. Incorporation of perineural invasion of gastric carcinoma into the 7th edition tumor–node–metastasis staging system. Tumor Biol 2014;35:9429–36.

[28] Kang BW, Seo AN, Yoon S, et al. Prognostic value of tumor-infiltrating lymphocytes in Epstein–Barr virus-associated gastric cancer. Ann Oncol 2016;27:494–501.

[29] Lake RA, Robinson BWS. Immunotherapy and chemotherapy—a practical partnership. Nat Rev Cancer 2005;5:397–405.

[30] Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:105–13.

[31] Becker K, Langer R, Reim D, et al. Significance of histopathological tumor regression after neoadjuvant chemotherapy in gastric adenocarcinomas: a summary of 480 cases. Ann Surg 2011;253:934–9.

[32] Lee JS, Won HS, Hong JH, et al. Prognostic role of tumor-infiltrating lymphocytes in gastric cancer: a systematic review and meta-analysis. Medicine (Baltimore) 2018;97.

[33] Fukuda K, Tsujitani S, Maeta Y, et al. The expression of RCAS1 and tumor infiltrating lymphocytes in patients with T3 gastric carcinoma. Gastric Cancer 2002;5:220–7.

[34] Dai C, Geng R, Wang C, et al. Concordance of immune checkpoints within tumor immune contexture and their prognostic significance in gastric cancer. Mol Oncol 2016;10:1551–8.