



Retrospective Study

Prognostic significance of tumor budding, desmoplastic reaction, and lymphocytic infiltration in patients with gastric adenocarcinoma

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Abstract

BACKGROUND

Recent studies have shown that the tumor microenvironment significantly influences the behavior of solid tumors. In this context, Accumulated data suggests that pathological evaluation of tumor budding (TB), desmoplastic reaction (DR), and tumor-infiltrating lymphocytes (TILs) may be crucial in determining tumor behavior in the gastrointestinal tract. Regarding gastric adenocarcinoma (GAC), although some results suggest that TB and TILs may be effective in determining the course of the disease, the data do not agree. Moreover, very few studies have investigated the relationship between DR and survival. At present, the associations between tumor TB, DR and TILs in GAC patients have not been determined.

AIM

To establish the relationships between TB, DR, and TILs in patients with GAC and to assess their influence on prognosis.

METHODS

Our study group comprised 130 patients diagnosed with GAC. The definition of TB was established based on the International TB Consensus Conference. The DR was categorized into three groups according to the level of tumor stroma maturation. The assessment of TILs was conducted using a semiquantitative approach, employing a cutoff value of 5%. The statistical analysis of the whole group and 100 patients with an intestinal subtype of GAC was performed using SPSS version 27.

RESULTS

A significant correlation between peritumoral budding (PTB) and intratumoral budding (ITB) was noted ($r = 0.943$). Tumors with high PTBs and ITBs had a greater incidence of immature DRs and low TILs ($P < 0.01$). PTB and ITB were associated with histological subtype, lymph node metastasis (LNM), and stage ($P < 0.01$). ITB, PTB, LNM, DR, and stage were significant risk factors associated with poor prognosis. The multivariate Cox regression analysis identified ITB, PTB, and LNM as independent prognostic variables ($P < 0.05$). In intestinal-type adenocarcinomas, a positive correlation between PTB and ITB was noted ($r = 0.972$). While univariate analysis revealed that LNM, stage, PTB, ITB, and DR were strong parameters for predicting survival ($P < 0.05$), only PTB and ITB were found to be independent prognostic factors ($P < 0.001$).

CONCLUSION

TB may be a potential prognostic marker in GAC. However, further studies are needed to delineate its role in pathology reporting protocols and the predictive effects of DR and TILs.

Key Words: Gastric cancer; Tumor budding; Desmoplastic stroma; Tumor-infiltrating lymphocytes; Prognosis

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Core Tip: This study investigated the relationships between tumor budding, desmoplastic reaction (DR), and tumor-infiltrating lymphocytes (TILs) in patients with gastric adenocarcinomas (GAC) and assessed their influence on prognosis. Our results demonstrated that TB is a promising prognostic factor in GAC. While it could also be valuable in determining survival in patients with unresectable tumors, further studies are needed to draw a conclusion. Although the DR and TILs were not observed as independent parameters, their close association with TB in patients with GAC suggests their value in predicting tumor behavior merits further research to clarify their roles better.

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INTRODUCTION

Gastric adenocarcinoma (GAC), the sixth most common tumor in the world, are among the most lethal types of cancer worldwide and exhibit significant rates of recurrence even after curative surgical procedures[1,2]. While the tumor-node-metastasis classification is often preferred for predicting high risk, heterogeneity in the survival of patients at the same stage has necessitated the search for new prognostic indicators to better determine tumor behavior[3-5]. In recent years, much evidence has shown that epithelial-mesenchymal transition (EMT) plays a vital role in the aggressiveness of many cancers[6-8]. In this context, tumor budding (TB), which reflects EMT in particular, has been used in routine reporting protocols as an independent prognostic parameter in colorectal cancer (CRC) patients[9-11]. In GAC, although there is evidence that TB is associated with tumor behavior[12-14], the data do not reach an agreement[15-17]. Besides, different studies use different methods to evaluate TB, which limits the determination of the importance of this parameter in these tumors.

Recently, studies have demonstrated that the tumor microenvironment (TME) plays a more active role in tumor progression, contrasting with previous opinions that consider the formation of excessive fibrous or connective tissue, or, in other words, desmoplasia (DR), around a tumor as a simple host-related factor[18-20]. Therefore, the DR has been noted to be a determinant of tumor behavior in solid cancers, including CRC[21-23]. However, studies evaluating this parameter in GAC are rare[24-26].

Moreover, immune cells that constitute a part of the TME, especially lymphocytes infiltrating the tumor, may play a role in determining tumor behavior in GAC, as noted in other organ tumors[27].

Recently, few studies in GAC have pointed to the association of high TB with immature stroma and tumor-infiltrating lymphocytes (TILs)[26,28]. Nonetheless, in patients with GAC, the interplay between these parameters and their efficiency in determining tumor behavior and survival have yet to be compared.

Therefore, this study aimed to investigate the relationships among DR, TB, TILs, clinicopathological parameters, and prognosis in GAC.

MATERIALS AND METHODS

Patient selection

This retrospective study included patients diagnosed at the Department of Pathology, Akdeniz University Medical School, Antalya, Türkiye, who underwent total or partial gastrectomy for GAC between 2004 and 2019. One hundred thirty patients were selected after excluding patients with other cancers, who underwent neoadjuvant therapy, or who had incomplete clinicopathological data. All patient-related data were collected and revised. Follow-up data were retrieved from patient records from the Department of Oncology of our institution. Tumor subtyping was performed according to the Lauren classification[29]. All patients were staged based on the eighth edition of the American Joint Committee on Cancer manual[30].

The study protocol was based on the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethical Committee of Akdeniz University.

Histopathological evaluation of TB, DR, and TILs

Hematoxylin and eosin (H&E)-stained slides from tumor blocks were reevaluated using light microscopy, and slides with low maturation of the tumor stroma, high tumor bud density, and high lymphocytic infiltration were selected for further analysis.

The assessment of peritumoral budding (PTB) and intratumoral budding (ITB) in this study followed the International TB Consensus Conference (ITBCC) guidelines[31]. In brief, a single tumor cell or a cluster of up to four tumor cells at the invasive front and within the primary tumor body were considered PTB and ITB, respectively. The count was determined in a standardized field area of 0.785 mm² at 200 × total magnification, and both PTB and ITB were categorized into three grades: grade 1 (0-4 TB), grade 2 (5-9 TB), and grade 3 (> 10 TB) (Figure 1).

DRs were evaluated and classified into three groups based on the maturation of the tumor stroma, as described by Ueno *et al*[32]. Mature-type DR comprised fine collagen fibers in multiple layers (DR1). While intermediate-type DR contained keloid-like collagen (DR2), immature-type DR constituted from the myxoid stroma (DR3) and occupied more than a 40 × objective lens field on slides (Figure 1).

The evaluation of TILs was performed semiquantitatively based on a 5% cutoff value on H&E-stained slides at a magnification of 200 ×[33]. Lymphatic infiltrates outside the tumor borders were excluded from the evaluation (Figure 1).

Statistical analysis

The data were analyzed with SPSS 27.0. Spearman's correlation test was used to evaluate the relationship between PTB and the ITB. The categorical data were examined by the chi-square test. Univariate survival analysis was performed with the Kaplan-Meier method, and the log-rank test was used to compare survival rates. A multivariate Cox proportional hazards regression model was applied to predict parameters influencing patient prognosis[34]. A *P* value < 0.05 indicated statistical significance. Furthermore, similar tests were also performed in patients with intestinal-type GAC, which allowed the application of these analyses (100 patients).

RESULTS

Clinicopathological and prognostic findings in the whole cohort

The clinicopathological characteristics of the patients in the study group are presented in Table 1. In brief, the mean age was 62.14 years ± 12.00 years (range 28 years to 89 years), and 53 females and 77 males were included. Patients were categorized into two groups for further analysis based on their mean age and mean tumor diameter (1.86 cm ± 1.02 cm, range 1.0 cm to 6.8 cm); regarding the level of invasion, a great majority of patients were classified as having tumors limited to the subserosa (40.0%), followed by tumors limited to the muscularis propria (30.0%), and tumors with invasion beyond the serosa and adjacent organs (24.6%). Invasion of the mucosa and submucosa was observed in 5.4% of the patients. Lymph node metastasis (LNM) was observed in 39.6% of the patients. The median follow-up period was 39 months (2-120 months, mean 42.44 months).

The patients were divided into three groups according to their PTB status, which resulted in 26 patients (20.0%) being classified as PTB1, 42 patients (32.3%) as PTB2, and 62 patients (47.7%) as PTB3. The ITB groups were categorized as follows: 31 patients (23.8%) were classified as ITB1, 25 patients (19.2%) as ITB2, and 74 patients (56.9%) as ITB3.

According to the DR classification, a total of 58 (44.6%) patients were classified as DR1, 38 (29.2%) patients as DR2, and 34 (26.2%) patients as DR3. The number of patients with TILs less than the cutoff value (68 patients, 52.3%) outnumbered that with higher lymphocytic infiltration (62 patients, 47.7%).

The relationships between clinicopathological parameters and PTB, ITB, DR, and TILs are presented in Table 1. There was a positive correlation between PTB and invasion and distant metastasis (*P* < 0.05). Higher PTB and ITB were more frequently observed in patients with LNM (*P* < 0.001). Similarly, both parameters were associated with the disease stage (*P* < 0.001). Compared with those with intestinal carcinomas, patients with higher PTB and ITB were more likely to have diffuse and mixed subtypes (*P* < 0.01).

Spearman correlation analysis revealed a strong correlation between PTB and ITB (*r* = 0.943, Figure 2). In patients with either PTB or ITB, immature stroma (DR3) and low TILs were more frequent (*P* < 0.01) (Table 1).

In the total cohort, the median OS was 36.5 ± 14.26 (ranging from 2 to 120 months). According to the univariate analysis, histologic subtype, ITB, PTB, LNM, DR, and stage were identified as risk factors for poor prognosis (*P* < 0.01).

Table 1 Tumor budding, desmoplastic reaction and tumor-infiltrating lymphocytes in relation to clinicopathological parameters in the whole group

Parameters	<i>n</i>	PTB1 (%)	PTB2 (%)	PTB3 (%)	ITB1 (%)	ITB2 (%)	ITB3 (%)	DR1 (%)	DR2 (%)	DR3 (%)	TILs (%)	TILs (%)
Age, yr												
< 62.14 ± 12.00	64	11 (42.3)	25 (59.5)	28 (45.2)	15 (48.4)	14 (56.0)	35 (47.3)	29 (50.0)	20 (52.6)	15 (44.1)	34 (50.0)	30 (48.4)
≥ 62.14 ± 12.00	66	15 (57.7)	17 (40.5)	34 (54.8)	16 (51.6)	11 (44.0)	39 (52.7)	29 (50.0)	18 (47.4)	19 (55.9)	34 (50.0)	32 (51.6)
Gender												
Male	77	16 (61.5)	26 (61.9)	35 (56.5)	18 (58.1)	19 (76.0)	40 (54.1)	43 (74.1)	23 (60.5)	11 (32.4) ^a	36 (52.9)	41 (66.1)
Female	53	10 (38.5)	16 (38.1)	27 (43.5)	13 (41.9)	6 (24.0)	34 (45.9)	15 (25.9)	15 (39.5)	23 (67.6)	32 (47.1)	21 (33.9)
Diameter												
< 1.86 ± 1.02	84	16 (61.5)	29 (69.0)	39 (62.9)	16 (51.6)	19 (76.0)	49 (66.2)	34 (58.6)	28 (73.7)	22 (64.7)	47 (69.1)	37 (59.7)
≥ 1.86 ± 1.02	46	10 (38.5)	13 (31.0)	23 (37.1)	15 (48.4)	6 (24.0)	25 (33.8)	24 (41.4)	10 (26.3)	12 (35.3)	21 (30.9)	25 (40.3)
Invasion												
T1	7	5 (19.2)	2 (4.8)	0 ^b	4 (12.9)	1 (4.0)	2 (2.7)	3 (5.2)	3 (7.9)	1 (2.9)	4 (5.9)	3 (4.8)
T2	39	4 (15.4)	15 (35.7)	20 (32.3)	12 (38.7)	8 (32.0)	19 (25.7)	22 (37.9)	9 (23.7)	8 (23.5)	18 (26.5)	21 (33.9)
T3	52	11 (42.3)	15 (35.7)	26 (41.9)	10 (32.3)	6 (24.0)	36 (48.6)	25 (43.1)	16 (42.1)	11 (32.4)	27 (39.7)	25 (40.3)
T4	32	6 (23.1)	10 (23.8)	16 (25.8)	5 (16.1)	10 (40.0)	17 (23.0)	8 (13.8)	10 (26.3)	14 (41.2)	19 (27.9)	13 (21.0)
LNM												
Absent	82	23 (88.5)	29 (69.0)	30 (48.4) ^a	29 (93.5)	16 (64.0)	37 (50.0) ^a	43 (74.1)	23 (60.5)	16 (47.1) ^c	38 (55.9)	44 (71.0)
Present	48	3 (11.5)	13 (31.0)	32 (51.6)	2 (6.5)	9 (36.0)	37 (50.0)	15 (25.9)	15 (39.5)	18 (52.9)	30 (44.1)	18 (29.0)
Metastasis												
Absent	106	26 (100.0)	31 (73.8)	49 (79.0) ^c	21 (67.7)	23 (92.0)	62 (83.8)	49 (84.5)	33 (86.8)	24 (70.6)	55 (80.9)	51 (82.3)
Present	24	-	11 (26.2)	13 (21.0)	10 (32.3)	2 (8.0)	12 (16.2)	9 (15.5)	5 (13.2)	10 (29.4)	13 (19.1)	11 (17.7)
Stage												
I	21	11 (42.3)	9 (21.4)	1 (1.6) ^a	10 (32.3)	6 (24.0)	5 (6.8) ^a	13 (22.4)	6 (15.8)	2 (5.9)	13 (19.1)	8 (12.9)
II	38	6 (23.1)	12 (28.6)	20 (32.3)	8 (25.8)	4 (16.0)	26 (35.1)	20 (34.5)	11 (28.9)	7 (20.6)	18 (26.5)	20 (32.3)
III	48	8 (30.8)	11 (26.2)	29 (46.8)	3 (9.7)	14 (56.0)	31 (41.9)	16 (27.6)	15 (39.5)	17 (50.0)	24 (35.3)	24 (38.7)
IV	23	1 (3.8)	10 (23.8)	12 (19.4)	10 (32.3)	1 (4.0)	12 (16.2)	9 (15.5)	6 (15.8)	8 (23.5)	13 (19.1)	10 (16.1)
Subtype												

Intestinal	100	24 (92.4)	39 (92.9)	37 (59.7) ^a	30 (96.8)	21 (84.0)	49 (66.2) ^b	47 (81.0)	28 (73.7)	25 (73.5)	52 (76.5)	48 (77.4)
Diffuse	19	1 (3.8)	3 (7.1)	15 (24.2)	1 (3.2)	2 (8.0)	16 (21.6)	8 (13.8)	4 (10.5)	7 (20.6)	10 (17.7)	9 (14.5)
Mixed	11	1 (3.8)	0	10 (16.1)	0	2 (8.0)	9 (12.2)	3 (5.2)	6 (15.8)	2 (5.9)	6 (8.8)	5 (8.1)
LVI												
Absent	89	14 (53.8)	28 (66.7)	47 (75.8)	16 (51.6)	19 (76.0)	54 (73.0)	39 (67.2)	24 (63.2)	26 (76.5)	45 (66.2)	44 (71.0)
Present	41	12 (46.2)	14 (33.3)	15 (24.2)	15 (48.4)	6 (24.0)	20 (27.0)	19 (32.8)	14 (36.8)	8 (23.5)	23 (33.8)	18 (29.0)
PNI												
Absent	94	16 (61.5)	28 (66.7)	50 (80.6)	18 (58.1)	19 (76.0)	57 (77.0)	43 (74.1)	25 (65.8)	26 (76.5)	52 (76.5)	42 (67.7)
Present	36	10 (38.5)	14 (33.3)	12 (19.4)	13 (49.0)	6 (24.0)	11 (23.0)	15 (25.9)	13 (34.2)	8 (23.5)	16 (23.5)	20 (32.3)
Survival												
Deceased	100	9 (34.6)	29 (69.0)	62 (100.0) ^a	8 (25.8)	20 (80.0)	72 (97.3) ^a	40 (69.0)	29 (76.3)	31 (91.2)	56 (82.4)	44 (71.0)
Alive	30	17 (65.4)	13 (31.0)	0	23 (74.2)	5 (20.0)	2 (2.7)	18 (31.0)	9 (23.7)	3 (8.8)	12 (17.6)	18 (29.0)
PTB												
PTB1	26	-	-	-	17 (54.8)	3 (12.0)	6 (8.1) ^a	15 (25.9)	8 (21.1)	3 (8.8) ^d	10 (14.7)	16 (25.8) ^b
PTB2	42	-	-	-	14 (45.2)	18 (72.0)	10 (13.5)	24 (41.4)	12 (31.6)	6 (17.7)	17 (25.0)	25 (40.3)
PTB3	62	-	-	-	0	4 (16.0)	58 (78.4)	19 (32.8)	18 (47.4)	25 (73.5)	41 (60.3)	21 (33.9)
ITB												
ITB1	31	-	-	-	-	-	-	21 (36.2)	8 (21.1)	2 (5.9) ^d	9 (13.2)	22 (35.5) ^d
ITB2	25	-	-	-	-	-	-	13 (22.4)	6 (15.8)	6 (17.6)	13 (19.2)	12 (19.4)
ITB3	74	-	-	-	-	-	-	24 (41.4)	24 (63.2)	26 (76.5)	46 (67.6)	28 (45.1)
DR												
DR1	58	-	-	-	-	-	-	-	-	-	22 (32.4)	36 (58.0) ^b
DR2	38	-	-	-	-	-	-	-	-	-	24 (35.2)	14 (22.6)
DR3	34	-	-	-	-	-	-	-	-	-	22 (32.4)	12 (19.4)

^a*P* < 0.001.^b*P* < 0.01.^c*P* < 0.05.^d*P* < 0.01.

N: Number of cases; PTB: Peritumoral budding; ITB: Intra-tumoral budding; DR: Desmoplastic reaction; TILs: Tumor-infiltrating lymphocytes; LVI: Lymphovascular invasion; PNI: Perineural invasion; LNM: Lymph node metastasis.

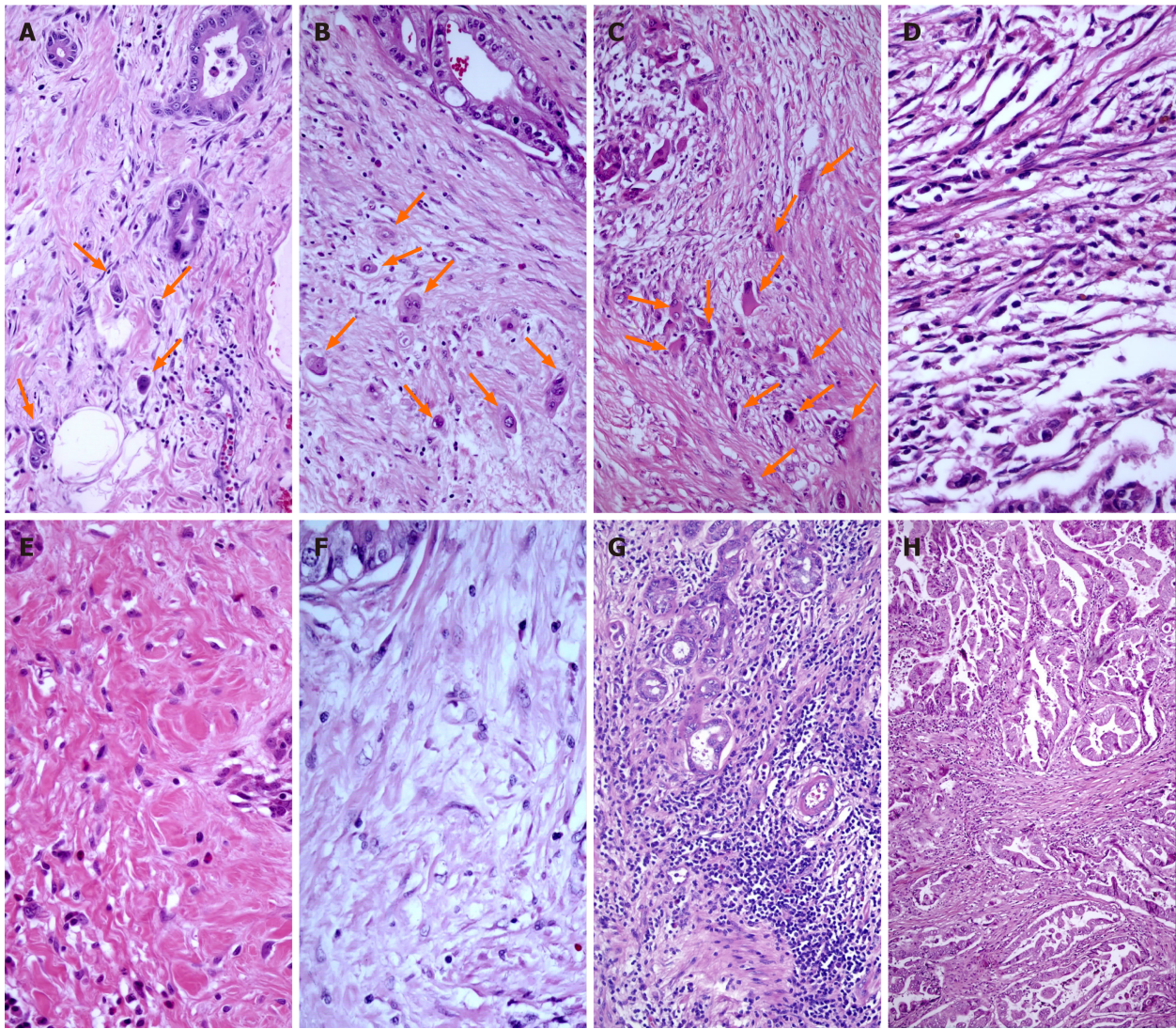


Figure 1 Tumor budding grades assessed according to International Tumor Budding Consensus Conference recommendations, desmoplastic reaction, and tumor-infiltrating lymphocytes. A: TB1 (1-4 tumor bud/hot spot), 200 ×; B: TB2 (5-9 tumor bud/hot spot), 200 ×; C: TB3 (10 tumor bud/hot spot), 200 ×; D: Desmoplastic reaction 1 (DR1), mature stroma composed of tightly packed collagen fibers, 400 ×; E: DR2, intermediate stroma, consisting of areas of collagen that resemble keloids, 400 ×; F: DR3, immature stroma with myxoid alterations; G: Gastric carcinoma with high TILs in tumor stroma ($\geq 5\%$), 100 ×; H: Gastric carcinoma with low TILs ($< 5\%$), 100 ×. Hematoxylin-eosin, black arrows indicate tumor buds.

(Table 2, Figure 3). The relationships between age, sex, and tumor diameter and these features and outcomes were not significantly different ($P > 0.05$).

According to the multivariate Cox regression analysis, ITB, PTB, and LNM were found to be independent prognostic factors ($P < 0.05$, Table 3).

Clinicopathological and prognostic findings in the intestinal subtype

In this cohort, higher PTB, higher ITB and immature stroma were more common in patients with LNM ($P < 0.003$). DR was also associated with male predominance (Table 4).

There was a positive correlation between PTB and ITB with the stage and grade ($P < 0.01$). In addition, PTB was related to invasion ($P < 0.05$). While PTB was positively associated with DR, an inverse relationship was observed between higher TILs and these parameters ($P < 0.006$, Table 4). Besides, Spearman correlation analysis revealed a strong correlation between PTB and ITB ($r = 0.972$, Figure 2).

In this group, the median survival ranged from 33.8 to 42.1 months (median: 38.0 ± 2.1). Kaplan-Meier analysis revealed that LNM ($P < 0.001$), stage ($P < 0.04$), PTB ($P < 0.001$), ITB ($P < 0.001$), and DR ($P < 0.001$) were powerful indicators of the disease course (Table 4, Figure 4). According to the multivariate analysis, PTB and ITB were found to be independent prognostic parameters ($P < 0.001$, Table 3).

Table 2 Clinicopathological parameters associated with survival in all cases and patients with intestinal tumors (Log-rank test)

Parameters	All cases		Intestinal tumors	
	mean \pm SE (95%CI)	Median \pm SE (95%CI)	mean \pm SE (95%CI)	Median \pm SE (95%CI)
Age				
< 62.14 \pm 12.00	48.2 \pm 4.5 (39.4-56.9)	37.0 \pm 1.1 (34.8-39.1)	53 \pm 5.9 (41.4-64.5)	36.0 \pm 1.2 (33.4-38.5)
\geq 62.14 \pm 12.00	50.8 \pm 4.8 (41.3-60.3)	39.0 \pm 9.5 (20.1-57.8)	55.7 \pm 5.7 (44.4-66.9)	60.0 \pm 16.2 (28.2-91.7)
Gender				
Male	50.1 \pm 4.7 (41.3-59.0)	36.0 \pm 2.6 (30.8-41.1)	54.7 \pm 5.5 (43.7-65.6)	38.0 \pm 3.3 (31.5-44.5)
Female	44.9 \pm 5.2 (39.1-58.7)	38.0 \pm 1.6 (34.9-41.0)	54.5 \pm 6.3 (42-67.1)	38.0 \pm 9.6 (19.1-56.8)
Diameter				
< 1.86 \pm 1.02	47.3 \pm 3.7 (40.0-54.6)	38.0 \pm 1.8 (3.4-41.5)	50.2 \pm 4.5 (41.3-59.1)	38.0 \pm 2.7 (32.6-43.3)
\geq 1.86 \pm 1.02	52.3 \pm 6.3 (39.8-64.7)	36.0 \pm 4.6 (26.8-45.1)	61.4 \pm 8.2 (45.2-77.6)	38.0 \pm 13.4 (11.6-64.3)
Invasion				
T1	65.1 \pm 7.1 (51.2-79.1)	78.0 \pm 22.1 (34.5-121.4)	65.1 \pm 7.1 (51.1-79.1)	78.0 \pm 22.1 (34.5-121.4)
T2	54.5 \pm 6.6 (41.5-67.5)	38.0 \pm 2.6 (32.9-43.0)	63.5 \pm 8.6 (46.6-80.4)	52.0 \pm 14.4 (23.7-80.2)
T3	44.8 \pm 4.8 (35.4-54.3)	36.0 \pm 4.3 (27.5-44.5)	46.4 \pm 5.7 (35-57.7)	35.0 \pm 5.5 (24.1-45.8)
T4	44.0 \pm 5.4 (32.3-53.6)	30.0 \pm 2.8 (24.4- 35.5)	48.7 \pm 7.0 (35-62.5)	36.0 \pm 3.1 (29.7-42.2)
LN				
Absent	51.6 \pm 4.8 (52.1-71.0)	46.0 \pm 5.5 (35.1-56.8) ^a	66.1 \pm 5.5 (55.3-77.1)	62.0 \pm 13.2 (35.8-88.1) ^a
Present	30.6 \pm 3.0 (24.6-36.6)	24.0 \pm 2.2 (19.5-28.4)	32.1 \pm 4.5 (23.3-40.8)	24.0 \pm 7.7 (8.7-39.2)
Metastasis				
Absent	48.3 \pm 3.2 (41.9-54.8)	38.0 \pm 1.5 (34.9-41.0)	53.4 \pm 4.1 (45.2-61.6)	39.0 \pm 4.8 (29.4-48.5)
Present	51.7 \pm 9.7 (32.6-70.7)	24.0 \pm 8.3 (7.6-40.3)	55.1 \pm 10.8 (33.8-76.5)	36.0 \pm 12.0 (12.4-59.5)
Stage				
I	69.1 \pm 6.9 (55.5-82.7)	72.0 \pm 11.1 (50.3-93.6) ^b	71.5 \pm 6.8 (58.1-85.1)	72.0 \pm 10.9 (50.5-93.4) ^c
II	45.1 \pm 5.3 (34.7-55.5)	37.0 \pm 2.2 (32.6-41.3)	52.3 \pm 7.2 (38.1-66.5)	39.0 \pm 2.5 (34.0-43.9)
III	38.6 \pm 3.9 (30.8-46.4)	33.0 \pm 3.4 (26.2-39.7)	40.0 \pm 5.8 (28.5-51.4)	29.0 \pm 4.9 (19.2-38.7)
IV	31.3 \pm 10.8 (37.1-59.5)	30.0 \pm 12.3 (11.8-60.1)	38.3 \pm 10.8 (27.1-48.7)	30.0 \pm 12.3 (29.8-42.1)
Subtype				
Intestinal	55.1 \pm 4.4 (46.6-63.7)	38.0 \pm 2.7 (32.9-42.1) ^d	-	-
Not intestinal	32.5 \pm 2.2 (18.8-36.7)	30.0 \pm 1.2 (6.2-17.7)	-	-
Grade				
Low	-	-	61.9 \pm 5.7 (50.6-73.2)	52.0 \pm 11.6 (29.1-74.8)
Moderate	-	-	49.9 \pm 10.6 (29.0-70.8)	32.0 \pm 4.7 (22.7-41.2)
High	-	-	43.2 \pm 6.8 (29.9-56.5)	29.0 \pm 12.3 (4.7-53.2)
LVI				
Absent	54.3 \pm 5.8 (43.0-65.7)	41.0 \pm 2.2 (36.5-45.4)	59.0 \pm 6.9 (45.4-72.7)	41.0 \pm 7.0 (27.0-54.9)
Present	46.6 \pm 4.0 (38.6-54.6)	36.0 \pm 3.4 (29.1-42.8)	51.4 \pm 5.1 (41.2-61.5)	36.0 \pm 2.9 (30.2-41.7)
PNI				
Absent	55.2 \pm 6.5 (42.5-67.9)	38.0 \pm 3.9 (30.2-45.7)	60.4 \pm 7.7 (45.3-75.5)	38.0 \pm 9.8 (18.6-57.3)
Present	46.9 \pm 3.8 (39.4-54.4)	36.0 \pm 2.2 (31.6-40.3)	51.7 \pm 4.8 (42.3-61.2)	38.0 \pm 2.5 (33-42.9)
PTB				
PTB1	88.3 \pm 5.2 (78.0-98.6)	73.5 \pm 12.7 (71.6-92.5) ^a	92.4 \pm 4.7 (83.3-101.9)	88.2 \pm 7.6 (56.9-91.6) ^a

PTB2	61.8 ± 6.4 (48.6-73.6)	38.0 ± 2.8 (33.5-42.5)	63.0 ± 6.5 (50.2-75.8)	41.0 ± 3.1 (34.8-47.1)
PTB3	26.7 ± 2.4 (21.9-31.5)	22.0 ± 1.5 (19.1-24.9)	22.7 ± 3.6 (15.6-29.8)	16.0 ± 2.1 (33.8-42.1)
ITB				
ITB1	101.1 ± 6.7 (87.8-114.4)	91.2 ± 5.2 (81.4-112.5) ^a	103.8 ± 6.4 (91.1-116.4)	63.0 ± 8.3 (88.7-97.4) ^a
ITB2	47.7 ± 4.6 (38.6-56.7)	41.0 ± 4.2 (32.8-49.2)	50.1 ± 5.2 (39.9-60.2)	42.0 ± 6.3 (29.0-52.9)
ITB3	29.9 ± 2.4 (25.3-34.6)	26.0 ± 1.9 (22.2-29.8)	28.5 ± 3.4 (21.8-35.2)	22.0 ± 4.0 (13.9-30.0)
DR				
DR1	60.4 ± 5.4 (49.8-70.9)	41.0 ± 7.9 (25.4-56.6) ^d	67.2 ± 6.2 (55.1-79.3)	72.0 ± 18.1 (36.4-107.5) ^a
DR2	48.0 ± 6.6 (35.1-61.0)	36.0 ± 4.5 (27.3-44.8)	53.5 ± 8.4. (36.9-70.1)	38.0 ± 9.1 (20.0-55.9)
DR3	27.0 ± 4.1 (18.8-35.2)	18.0 ± 3.6 (10.8-25.1)	31.8 ± 5.3 (21.3-42.2)	24.0 ± 6.7 (10.9-37.0)
TILs				
TILs	53.8 ± 4.3 (45.3-62.4)	39.0 ± 4.8 (29.4-48.5)	59.6 ± 5.2 (49.3-70.0)	53.0 ± 14.7 (24.0-81.9)
TILs	43.9 ± 4.6 (34.9-52.9)	35.0 ± 4.5 (26.2-43.7)	47.8 ± 5.7 (36.5-59.1)	35.0 ± 5.1 (24.9-45.0)

^a*P* < 0.001.^b*P* < 0.01.^c*P* < 0.05.^d*P* < 0.01.

SE: Standard error; CI: Confidence interval; LNM: Lymph node metastasis; LVI: Lymphovascular invasion; PNI: Perineural invasion; PTB: Peritumoral budding; ITB: Intratumoral budding; DR: Desmoplastic reaction; TILs: Tumor-infiltrating lymphocytes.

Table 3 Multivariate analysis including parameters associated with prognosis in the Log-rank test in the entire group and tumors of the intestinal subtype

Parameters	All group			<i>P</i> value	Intestinal tumors			<i>P</i> value
	HR	Lower (95%CI)	Upper (95%CI)		HR	Lower (95%CI)	Upper (95%CI)	
ITB	2.06	1.40	3.01	< 0.001	3.32	2.34	4.72	< 0.001
PTB	1.83	1.29	2.59	< 0.001	2.01	1.32	3.05	< 0.001
LNM	1.53	1.00	2.33	0.04	1.09	0.59	2.04	0.760
Stage	1.06	0.81	1.40	0.63	1.12	0.80	1.58	0.480
Subtype	0.76	0.50	1.17	0.22	-	-	-	-
DR	1.11	0.87	1.42	0.39	1.17	0.86	1.60	0.290

HR: Hazard ratio; CI: Confidence interval; LNM: Lymph node metastasis; PTB: Peritumoral budding; ITB: Intratumoral budding; DR: Desmoplastic reaction; TILs: Tumor infiltrating lymphocytes.

DISCUSSION

TB has been investigated in numerous studies of CRC and is currently used in pathological reporting protocols due to its prognostic importance in low-grade tumors[31,35]. However, TB has yet to be studied extensively in GAC. This may be because GAC is less frequently observed than CRC, especially in Western countries[26,36]. Moreover, a standard evaluation method for this variable has yet to be determined. For example, studies investigating the role of TB in predicting LNM in early gastric carcinoma (EGC) patients have indicated that detecting the presence of TB may be effective[37-39]. Yim *et al*[40] recently observed a strong association between TB and LNM metastasis with three different evaluation methods in EGC. However, only the presence of TB was an independent prognostic factor. The limited number of EGC patients in our series did not allow a separate analysis of this group. However, these results suggest that the presence of TB is an effective marker for predicting LNM metastasis and patient prognosis, at least in EGC.

Recently, in studies that included gastric cancer (GC) patients of all stages and histopathological subtypes, TB was observed to be an independent prognostic factor, which is consistent with our findings[12-14,41,42]. Interestingly, although different categorizations were used in the statistical analysis to determine the predictive role of TB in the course of the disease, the evaluation methods applied in most of these studies were based on the ITBCC, similar to our research [13,14,28,42]. In our study, the survival of patients with TB3 was significantly lower than that of patients with TB2 or TB1. Taken together, these data point to the value of the ITBCC-recommended evaluation of TB in GAC patients.

Table 4 Tumor budding, desmoplastic reaction, and tumor-infiltrating lymphocytes in relation to clinicopathological parameters in the intestinal group

Parameters	n	PTB1 (%)	PTB2 (%)	PTB3 (%)	ITB1 (%)	ITB2 (%)	ITB3 (%)	DR1 (%)	DR2 (%)	DR3 (%)	TILs (%)	TILs (%)
Age, yr		24	39	37	30	21	49	47	28	25	52	48
< 62.14 ± 12.00	47	10 (41.7)	24 (61.5)	13 (35.1)	14 (46.7)	11 (52.4)	22 (44.9)	21 (44.7)	15 (53.6)	11 (44.0)	25 (48.1)	22 (45.8)
≥ 62.14 ± 12.00	53	14 (58.3)	15 (38.5)	24 (63.9)	16 (53.3)	10 (47.6)	27 (55.1)	26 (55.3)	13 (46.4)	14 (56.0)	27 (51.9)	26 (54.2)
Gender												
Male	60	15 (62.5)	24 (61.5)	21 (56.8)	18 (60.0)	15 (71.4)	27 (55.1)	34 (72.3)	16 (57.1)	10 (40.0) ^a	27 (51.9)	33 (68.8)
Female	40	9 (37.5)	15 (38.5)	16 (43.2)	12 (40.0)	6 (28.6)	22 (44.9)	13 (27.7)	12 (42.9)	15 (60.0)	25 (48.1)	15 (31.3)
Diameter												
< 1.86 ± 1.02	67	16 (66.7)	26 (66.7)	25 (67.6)	15 (50.0)	16 (76.2)	36 (73.5)	28 (59.6)	22 (78.6)	17 (68.0)	38 (73.1)	29 (60.4)
≥ 1.86 ± 1.02	33	8 (33.3)	13 (33.3)	12 (32.4)	15 (50.0)	5 (23.8)	13 (26.5)	19 (40.4)	6 (21.4)	8 (32.0)	14 (26.9)	19 (39.6)
Invasion												
T1	7	5 (20.8)	2 (5.1)	0	4 (13.3)	1 (4.8)	2 (4.1)	3 (6.4)	3 (10.7)	1 (4.0)	4 (7.7)	3 (6.3)
T2	28	4 (16.7)	15 (38.5)	9 (24.3)	12 (40.0)	7 (33.3)	9 (18.4)	14 (29.8)	9 (32.1)	5 (20.0)	12 (23.1)	16 (33.3)
T3	42	10 (41.7)	13 (33.3)	19 (51.4)	9 (30.0)	6 (28.6)	27 (55.1)	23 (48.9)	12 (42.9)	7 (28.0)	23 (44.2)	19 (39.6)
T4	23	5 (20.8)	9 (23.1)	9 (24.3)	5 (16.7)	7 (33.3)	11 (22.4)	7 (14.9)	4 (14.3)	12 (48.0)	13 (25.0)	10 (20.8)
LNМ												
Absent	69	22 (91.7)	29 (74.4)	18 (48.6) ^b	29 (96.7)	14 (66.7)	26 (53.1) ^b	39 (83.0)	19 (67.9)	11 (44.0) ^c	32 (61.5)	37 (77.1)
Present	31	2 (8.3)	10 (25.6)	19 (51.4)	1 (3.3)	7 (33.3)	23 (46.9)	8 (17.0)	9 (32.1)	14 (56.0)	20 (38.5)	11 (22.9)
Metastasis												
Absent	79	24 (100.0)	29 (74.4)	26 (70.3) ^d	21 (70.0)	20 (95.2)	38 (77.6)	39 (83.0)	23 (82.1)	17 (68.0)	40 (76.9)	39 (81.3)
Present	21	0	10 (25.6)	11 (29.7)	9 (30.0)	1 (4.8)	11 (22.4)	8 (17.0)	5 (17.9)	8 (32.0)	12 (23.1)	9 (18.7)
Stage												
I	20	11 (45.8)	9 (23.2)	0	10 (33.3)	5 (23.8)	5 (10.2) ^c	12 (25.5)	6 (21.4)	2 (8.0)	12 (23.1)	8 (16.7)
II	26	6 (25.0)	10 (25.6)	10 (27.0)	7 (23.3)	4 (19.0)	15 (30.6)	15 (31.9)	9 (32.1)	2 (8.0)	12 (23.1)	14 (29.3)
III	32	6 (25.0)	10 (25.6)	16 (43.2)	3 (10.0)	11 (52.4)	18 (36.7)	12 (25.5)	7 (25.0)	13 (52.0)	15 (28.8)	17 (35.2)
IV	22	1 (4.2)	10 (25.6)	11 (29.7)	10 (33.4)	1 (4.8)	11 (22.4)	8 (17.0)	6 (21.4)	8 (32.0)	13 (25.0)	9 (18.8)
Grade												

Low	54	19 (79.2)	21 (53.8)	14 (37.8) ^a	22 (73.3)	12 (57.1)	20 (40.8) ^a	28 (59.6)	16 (57.1)	10 (40.0)	29 (55.8)	25 (52.1)
Moderate	29	2 (8.3)	13 (33.4)	14 (37.8)	3 (10.0)	6 (28.6)	20 (40.8)	11 (23.4)	10 (35.7)	8 (32.0)	15 (28.8)	14 (29.2)
High	17	3 (12.5)	5 (12.8)	9 (24.3)	5 (16.7)	3 (14.3)	9 (18.4)	8 (17.0)	2 (7.2)	7 (28.0)	8 (15.4)	9 (18.7)
LVI												
Absent	67	13 (54.2)	26 (66.7)	28 (75.7)	15 (50.0)	15 (71.4)	37 (75.5)	34 (72.3)	14 (50.0)	19 (76.0)	32 (61.5)	35 (72.9)
Present	33	11 (45.8)	13 (33.3)	9 (24.3)	15 (50.0)	6 (28.6)	12 (24.5)	13 (27.7)	14 (50.0)	6 (24.0)	20 (38.5)	13 (27.1)
PNI												
Absent	71	14 (58.3)	26 (66.7)	31 (83.8)	18 (60.0)	15 (71.4)	38 (77.6)	34 (72.3)	16 (57.1)	21 (84.0)	37 (71.2)	34 (70.8)
Present	29	10 (41.7)	13 (33.3)	6 (16.2)	12 (40.0)	6 (28.6)	11 (22.4)	13 (27.7)	12 (42.9)	4 (16.0)	15 (28.8)	14 (29.2)
Survival												
Deceased	71	7 (29.2)	27 (69.2)	37 (100.0) ^b	7 (23.3)	16 (76.2)	48 (98.0) ^b	29 (61.7)	20 (71.4)	22 (88.0)	41 (78.8)	30 (62.5)
Alive	29	17 (70.8)	12 (30.8)	0	23 (76.7)	5 (23.8)	1 (2.0)	18 (38.3)	8 (28.6)	3 (12.0)	11 (21.2)	18 (37.5)
PTB												
PTB1	24	-	-	-	17 (56.7)	3 (14.3)	4 (8.2) ^b	14 (29.8)	8 (28.6)	2 (8.0) ^c	10 (19.2)	14 (29.2) ^d
PTB2	39	-	-	-	13 (43.3)	18 (85.7)	8 (16.3)	23 (48.9)	11 (39.3)	5 (20.0)	16 (30.8)	23 (47.9)
PTB3	37	-	-	-	0	0	37 (75.5)	10 (21.3)	9 (32.1)	18 (72.0)	26 (50.0)	11 (22.9)
ITB												
ITB1	30	-	-	-	-	-	-	21 (44.7)	8 (28.6)	1 (4.0) ^c	9 (17.3)	21 (43.8) ^c
ITB2	21	-	-	-	-	-	-	12 (25.5)	3 (10.7)	6 (24.0)	9 (17.3)	12 (25.0)
ITB3	49	-	-	-	-	-	-	14 (29.8)	17 (60.7)	18 (72.0)	34 (65.4)	15 (31.2)
DR												
DR1	47	-	-	-	-	-	-	-	-	-	17 (32.7)	30 (62.5) ^d
DR2	28	-	-	-	-	-	-	-	-	-	17 (32.7)	11 (22.9)
DR3	25	-	-	-	-	-	-	-	-	-	18 (34.6)	7 (14.6)

^a $P < 0.05$.^b $P < 0.001$.^c $P < 0.008$.^d $P < 0.01$.

N: Number of cases; PTB: Peritumoral budding; ITB: Intratumoral budding; DR: Desmoplastic reaction; TILs: Tumor-infiltrating lymphocytes; LNM: Lymph node metastasis.

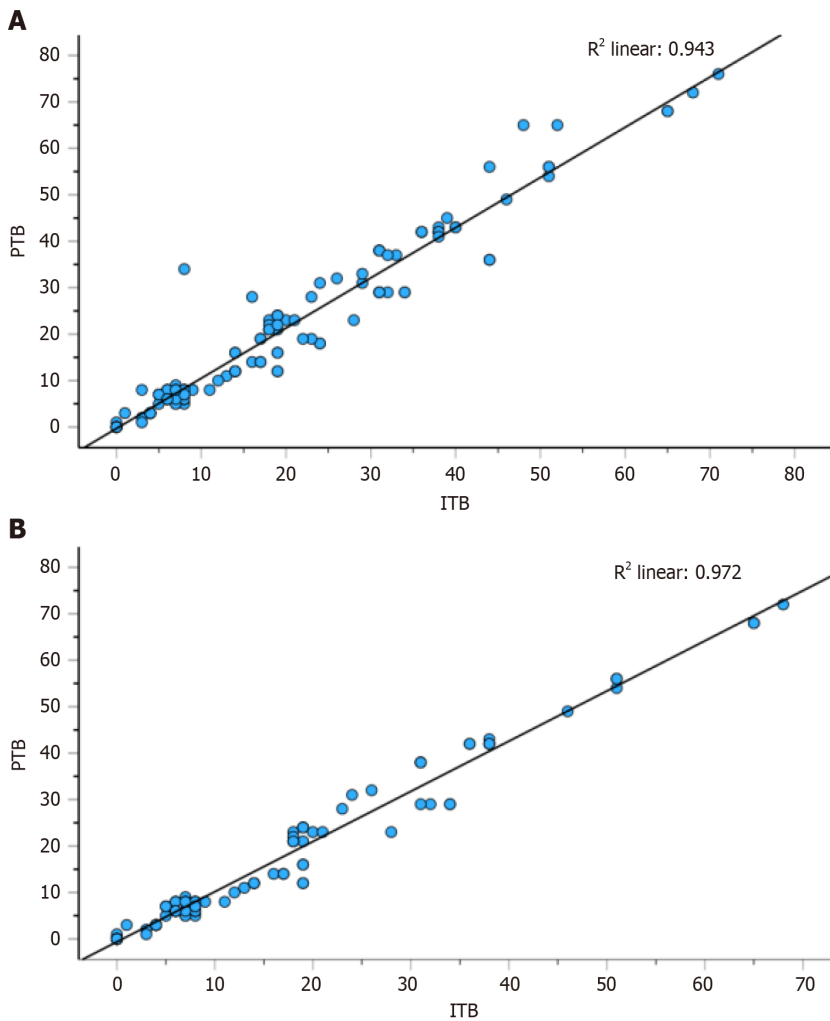


Figure 2 Scatter plot of Spearman's rank correlation between peritumoral budding (vertical axis) and intratumoral budding (horizontal axis). A: Whole cohort B: Intestinal subgroup. ITB: Intratumoral budding.

When adenocarcinoma subtypes in GC were considered separately, TB was observed to be associated with tumor behavior in the intestinal type of GAC but not in diffuse tumors. Although no further analysis of this subtype could be performed in our study group due to the limited number of patients with nonintestinal tumors, TB was observed to be related to survival in patients with intestinal-type GAC according to the log-rank analysis. Moreover, multivariate analysis revealed that the TB score is an independent prognostic parameter. Although TB incidence has been correlated with intestinal-type GAC behavior and survival in many studies, the results of multivariate analyses have yet to be consistent. While in some studies, the evaluation of TB was observed to be a decisive parameter in determining the course of the disease[13,42,43], such an effect was not noted in others[15,16,26]. These different findings may be due to diversity in the number of cases and data categorization among studies. Our findings are consistent with those of studies in which TB was observed to be a strong prognostic parameter in intestinal-type GAC patients and emphasize the need for additional research to establish the value of TB in GAC reporting guidelines.

Another notable finding of our study was that in addition to the whole cohort, PTB and ITB were found to be independent prognostic factors for the intestinal subtype, and their correlation with each other was strong. To our knowledge, only one study has evaluated TB separately in intestinal GAC patients. Qi *et al*[43] observed a strong association between ITB and PTB; both were found to be independent prognostic parameters for predicting survival. Although these findings need to be supported by further studies, the independent prognostic value of TB in both topographic areas support the idea that TB can be evaluated to stratify patients with intestinal-type GAC for prognosis[4, 5]. Furthermore, given the substantial correlation between the ITB and PTB, TB could be used as a predictive parameter for determining tumor behavior, especially in patients who are unsuitable for surgical resection.

Although DR in GAC was associated with survival according to univariate analysis in this study, it was not an independent prognostic factor when other parameters related to tumor behavior and prognosis were analyzed. To our knowledge, very few studies have investigated the effectiveness of DR in determining the survival of patients with GAC [24-26]. In these tumors, examination of the thickness of collagen fibers by second-generation harmonic imaging indicated that the presence of large desmoplastic collagen fibers was associated with poor prognosis[24]. In an elegant study in which DR was categorized into two groups (mature and immature), Kemi *et al*[25] reported that DR was an independent parameter for determining the course of disease in patients with GAC. They also noted that DR was associated with 5-

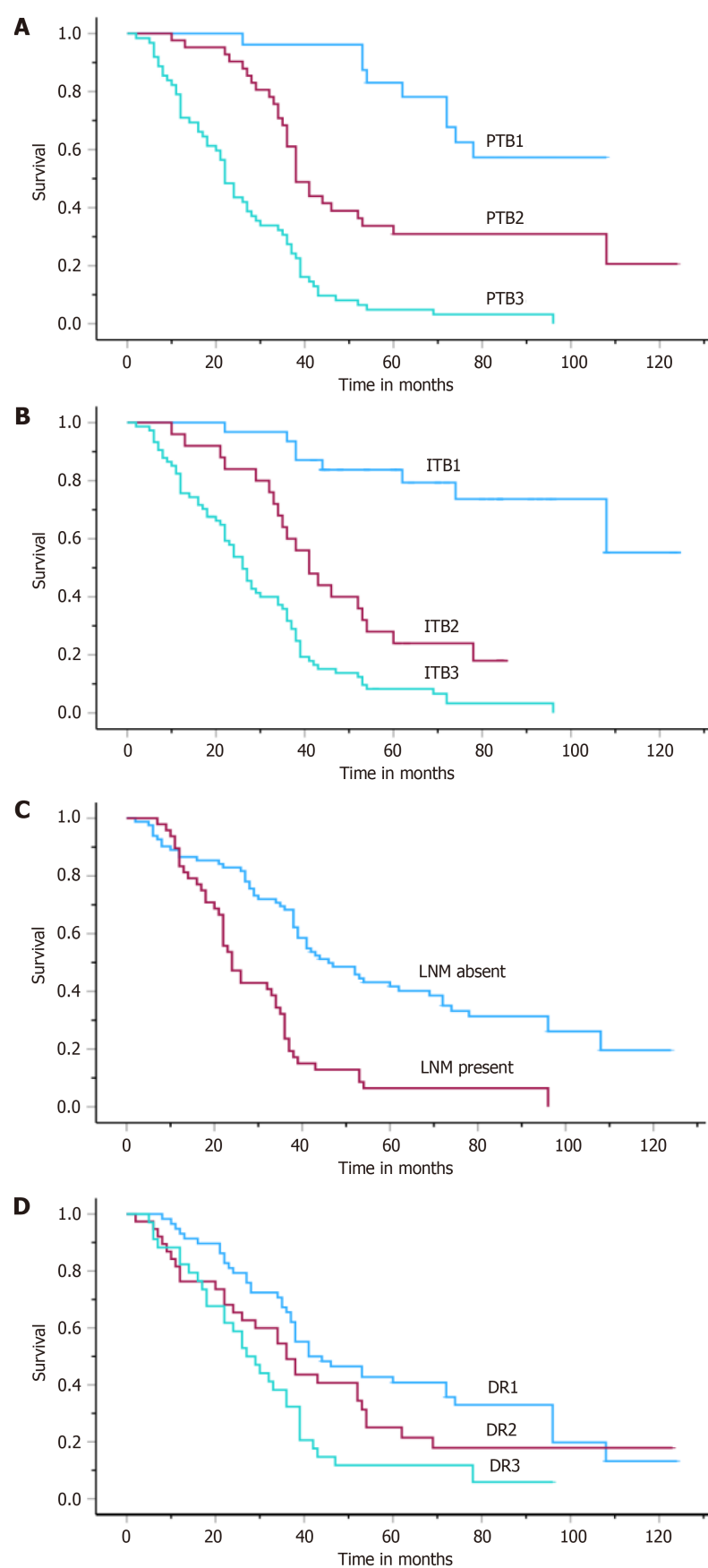


Figure 3 Kaplan-Meier curves of survival analyses in the total cohort. A: Peritumoral budding (log-rank test $P < 0.001$); B: Intratumoral budding (log-rank test $P < 0.001$); C: The presence of lymph node metastasis (log-rank test $P < 0.002$); D: Desmoplastic reaction (log-rank test $P < 0.002$). PTB: Peritumoral budding; ITB: Intratumoral budding; LNM: Lymph node metastasis; DR: Desmoplastic reaction.

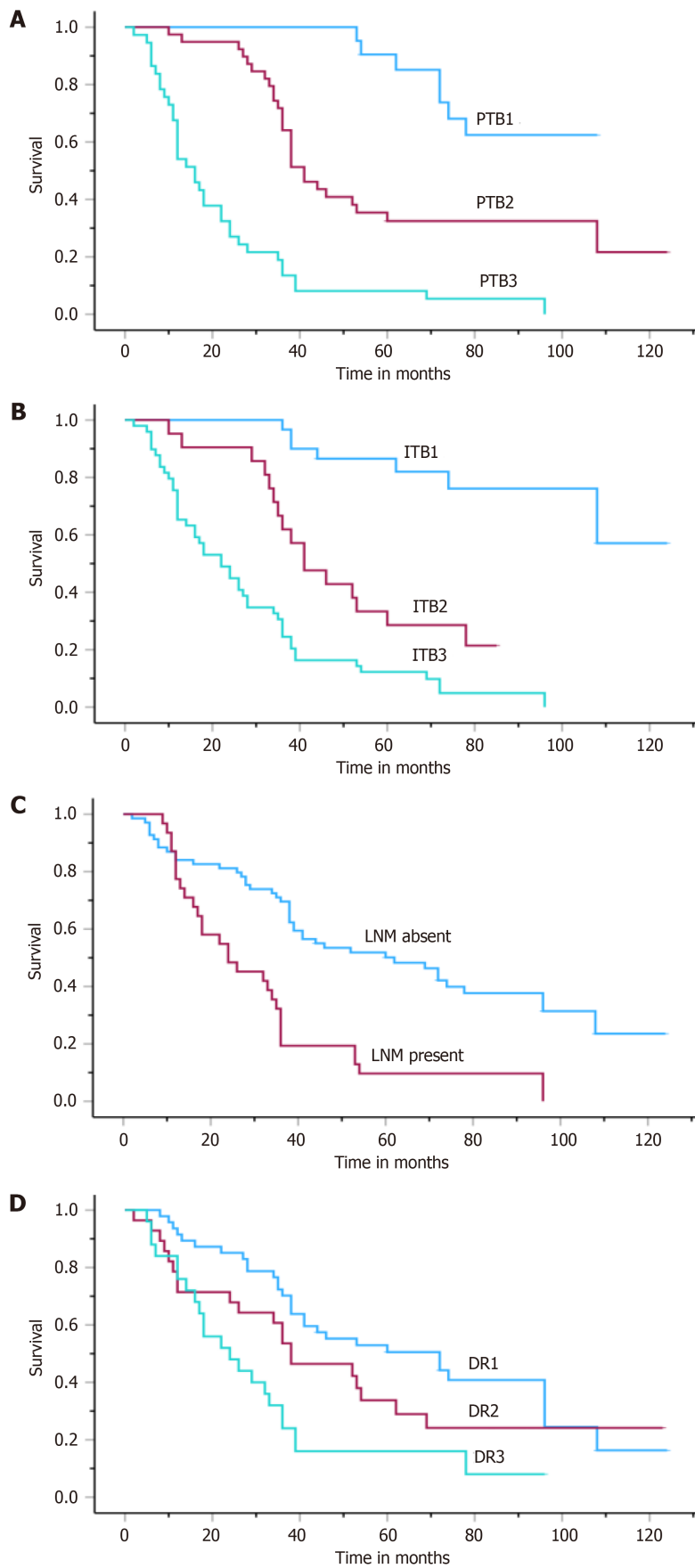


Figure 4 Kaplan-Meier curves of survival analyses in patients with an intestinal subtype of gastric adenocarcinoma. A: Peritumoral budding (log-rank test, $P < 0.001$); B: Intratumoral budding (log-rank test, $P < 0.001$); C: Desmoplastic reaction (log-rank test, $P < 0.001$); D: The presence of lymph node metastasis (log-rank test, $P < 0.001$). PTB: Peritumoral budding; ITB: Intratumoral budding; LNM: Lymph node metastasis; DR: Desmoplastic reaction.

year survival in the intestinal subgroup, whereas no such association was observed for diffuse carcinomas.

On the other hand, Pun *et al*[26] did not detect such a relationship in intestinal-type adenocarcinomas. In both studies, DR was evaluated both in the invasive tumor area and in the main tumor mass. In our study, we investigated DR only on invasive edges according to the method applied in the assessment of DR in many studies, and we found that DR was not an effective prognostic parameter in either the whole group or intestinal tumors. These results emphasize that a different method should be applied to investigate the role of DR in GAC. Recently, Hacking *et al*[44] suggested a different approach for evaluating stromal maturity in patients with CRC. However, the prognostic impact of DR in GAC remains to be investigated *via* this method. In brief, further studies comparing different evaluation methods and categorizations in large patient series are needed to determine the value of DR as a parameter in pathology protocols for these tumors.

We observed a strong positive relationship between DR and TB in the study group. In parallel with these data, a recent study demonstrated the association of high TB with immature stroma in GAC[26]. Moreover, our research revealed an inverse correlation between DR and TILs. To our knowledge, no study has investigated the relationship between these three parameters in patients with GAC. Our findings support studies highlighting the importance of DR in the TME. Although it has not been determined to be an independent prognostic marker, further studies are needed to determine the potential of DR as a marker in GAC.

TILs, an essential component of the tumor environment, have been studied extensively in GAC, but the results are still controversial, even when evaluating lymphocyte subsets by immunohistochemistry. In this study, we did not observe TILs to be a significant predictive parameter for GAC prognosis. There are studies in which TILs were semiquantitatively investigated by H&E staining, similar to our method. A substantial correlation between TILs and survival has been noted [45,46]. Unfortunately, the topographical differences in the evaluation of TILs (intratumoral *vs* stromal) in these studies and the investigation of different types of GACs, such as EBV-associated GCs, limit the comparison of our data[47,48].

Regarding immunohistochemical studies on TILs in GAC, while one study linked higher CD8+ T-cell density in GAC to poor prognosis[49], another noted that higher numbers of CD8+ T cells and TILs improved overall survival (OS)[50]. Similarly, there is disagreement over the predictive importance of CD4+ T-cell tumor infiltration[27,51]. Different data were also obtained in past meta-analyses of GAG[51-53]. The presence of CD3+ lymphocytes was the highest predictive factor for OS (HR = 0.52)[51]. A significant relationship between CD8+ TILs and survival was demonstrated in another analysis[53]. The results also indicated that high intratumoral T-cell infiltration levels were associated with improved survival in GAC patients, and a high density of intratumoral FOXP3+ T cells was not closely associated with poor prognosis[28].

In our study, the strong association between TILs and TB suggested the potential role of TILs in tumor behavior in GAC. Parallel to this observation, in a recent study, Zhang *et al*[28], by double immunohistochemical staining, noted an inverse correlation between TILs and TB, predicting a favorable outcome. On the other hand, we did not observe TILs to be a significant predictive factor. The present study suggests that the method employed for assessing TILs has certain limitations. In other words, it is essential to emphasize that the finding that TILs were unrelated to survival in our study does not exclude the importance of recent research that has primarily investigated various lymphocyte subtypes by immunohistochemistry.

To our knowledge, the relationship between TILs and DR has yet to be described in GAC, and the present study revealed the inverse relationship between TILs and DR, suggesting that DR is an important component of tumor immune surveillance. Moreover, these data merit further investigations into the association of DR with different subsets of lymphocytes to better understand its role in the prediction of survival in GAC.

This study has several limitations. It is conducted within a single center, limiting the sample size to remain relatively small, which might restrict the power to detect more nuanced associations or differences, particularly when stratifying the analysis by adenocarcinoma subtypes or evaluating the interaction between different prognostic factors. Moreover, potential selection biases cannot be excluded due to the retrospective nature of the study, limiting the generalizability of the results to other populations and settings. Therefore, multicenter prospective studies and external validation are needed to confirm the findings.

Another limitation is the need for a standardized evaluation method for assessing TB, DR, and TILs in GAC, which might lead to variability in the results. Although we have employed methods consistent with current literature and guidelines, the need for universally accepted criteria for these histopathological features may affect the reproducibility and comparison of our findings with those of other studies. Additionally, the heterogeneous behavior of GAC necessitates a multifactorial analysis incorporating a wide range of potential prognostic markers. Our study focused on a select few, which, while important, do not encompass all the factors that could influence patient outcomes.

Despite these limitations, our study contributes valuable insights into the prognostic significance of TB, DR, and TILs in GAC, supporting the need for their consideration in future research and potential inclusion in pathological reporting protocols.

CONCLUSION

The findings support that the assessment of TB based on the ITBCC criteria can be used to categorize patients with GAC for treatment and prognosis. Although the strong relationship between PTB and ITB also suggests that these two variables can be used in determining the course of the disease in patients for whom surgical resection is not feasible, especially for those with the intestinal subtype, further studies are needed to delineate their role.

Although DR was related to TB in our series, it was not an independent parameter for predicting survival, suggesting that its value in determining GAC behavior merits further research.

Within the context of our findings, despite the emergence of recent discoveries, we did not notice TILs to be a significant predictive component in GAC. The present study suggested that the method employed for assessing TILs in these tumors has certain limitations. However, it is essential to note that this does not diminish the importance of recent research investigating various lymphocyte subtypes.

The relationships among TB, DR, and TILs in the tumor area observed in our study warrant further investigations with a more extensive patient cohort to determine the role of a scoring system consisting of these three parameters in determining the behavior of GC.

FOOTNOTES

Author contributions: Yavuz A and Elpek GOE designed and performed the research and wrote the paper; Simsek K, Alpsoy A, Altunay B, Gedik EO, Unal B, and Bassorgun CI are involved in the data curation, investigation, and resources; Tatli AM provided clinical advice; and all authors equally contributed to the analysis of the data.

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