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Original Article

Tumor Budding as an Additional Factor in Determining the Need for Surgery after Endoscopic Resection in Mucosal Invasive Gastric Cancer: A Retrospective Study from a Korean Tertiary Hospital

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Background/Aims: The presence of individual cancer cells at the invasive tumor front is referred to as tumor budding (TB). The purpose of this study was to assess the clinicopathological significance of TB in patients with early gastric cancer (EGC).

Methods: A total of 939 patients who received radical surgery for EGC were included in this retrospective study. We assessed clinicopathological features in relation to TB including the grade of histologic differentiation, the extent of invasion depth, the width of submucosal (SM) invasion, and the presence of lymphovascular invasion (LVI), lymph node metastasis (LNM) and perineural invasion (PNI).

Results: TB was identified in 59.5% of the patients with EGC, 38.7% of the patients with mucosal invasive cancer, and 80.4% of the patients with SM invasive cancers. TB showed significant association with male sex, undifferentiated tumor types, SM invasion, LVI, PNI, and LNM. The presence of SM invasion (odds ratio [OR], 8.750; p<0.001), TB (OR, 5.586; p<0.001), and an undifferentiated-type histology (OR, 2.648; p=0.0005) were found to be significantly associated with LNM/LVI. TB was the sole significant risk factor for LNM/LVI (OR, 7.181; p=0.0016) among the mucosal invasive cancers. In SM invasive cancers, three independent risk factors for LNM/LVI were identified: a tumor located in the lower third of the stomach (OR, 3.425; p=0.0061), an undifferentiated-type histology (OR, 2.320; p=0.0177), and an SM invasion width greater than 4,000 μm (OR, 2.849; p=0.0041).

Conclusions: TB may be an important factor associated with LNM, particularly in mucosal gastric cancer. **(Gut Liver, 2025;19:559-568)**

Key Words: Stomach neoplasm; Neoplasm invasiveness; Surgery; Prognosis

INTRODUCTION

Gastric cancer is globally recognized as the fifth most common type of cancer and hold the rank of the third leading cause of deaths due to cancer.[1] The advancement in diagnostic endoscopy and the widespread availability of health examinations have resulted in a rise in the detection of early gastric cancer (EGC).[2] Endoscopic resection (ER) is the preferred treatment choice for EGC due to its effectiveness in achieving high rates of *en bloc* resections and, thus, low local recurrence, achieving a 5-year survival rate of 92.6%.[3] It is important to identify pathological features that can predict lymph node metastasis (LNM) because accurate prediction of LNM is most important for appropriate curative treatment planning in EGC.

Tumor budding (TB) is particularly a prognostic fac-

tor of interest in colorectal cancer.[4] It is characterized by the existence of isolated, detached, or infiltrating single neoplastic cells or clusters of up to five tumor cells along the tumor's invasion front.[5,6] TB has been recognized as a significant risk factor for LNM in colorectal cancer, and it serves as a valuable early detection marker for predicting a poor prognosis and aggressive behavior in colorectal cancer cases.[7,8] The World Health Organization (WHO) colorectal cancer classification introduced TB as the second major grade criterion.[9] Pathologists are now required to routinely report TB using the consensus method for both pT1 and stage II colorectal carcinomas.[4] However, the clinical value of TB in EGC remains uncertain.

The objective of this study was to evaluate the predictive value of TB for LNM risk and its clinical relevance in EGC. Furthermore, we identified independent factors related to LNM risk in EGC, dividing it into mucosal and submucosal (SM) invasive cancer subgroups.

MATERIALS AND METHODS

1. Patients

We conducted a retrospective analysis by collecting the medical records and pathologic slides of 939 patients who had undergone radical gastrectomy for EGC between 2006 and 2018 at Gangnam Severance Hospital. All pathological slides were prospectively reviewed by an experienced pathologist. Of 939 patients, 407 were diagnosed with diffuse-type EGC, including poorly cohesive carcinoma according to the WHO criteria.[10] Since non-cohesiveness is a defining histopathologic feature of these types, inherently presenting extensive TB, we excluded them from our analysis to ensure a consistent and reliable evaluation of TB. Mixed types were also excluded to avoid potential confounding effects, and similar to other studies on TB in gastric cancer, our study focused on tubular adenocarcinomas to maintain consistency. Among the remaining 532 patients, 152 patients were also excluded because they were diagnosed with other rare histological subtypes, including papillary adenocarcinoma, mucinous adenocarcinoma, adenosquamous carcinoma, and carcinoma with lymphoid stroma, or because the pathologic slides were not available for re-review due to poor quality. We ultimately enrolled 380 patients who were diagnosed with tubular adenocarcinoma according to the WHO criteria,[10] differentiated and undifferentiated types by Japanese classification and analyzed the clinicopathological data. This study received approval from the Gangnam Severance Hospital Institutional Review Board (IRB number: 3-2022-0254). Informed consent was deemed unnecessary for this study since it relied exclusively on the use of anonymized patient records, ensuring the protection of patient privacy and confidentiality. The research was carried out in accordance with the guidelines specified in the Declaration of Helsinki, ensuring that ethical principles and standards were upheld throughout the study.

2. Clinicopathologic assessment

We reviewed patients' medical records from the time

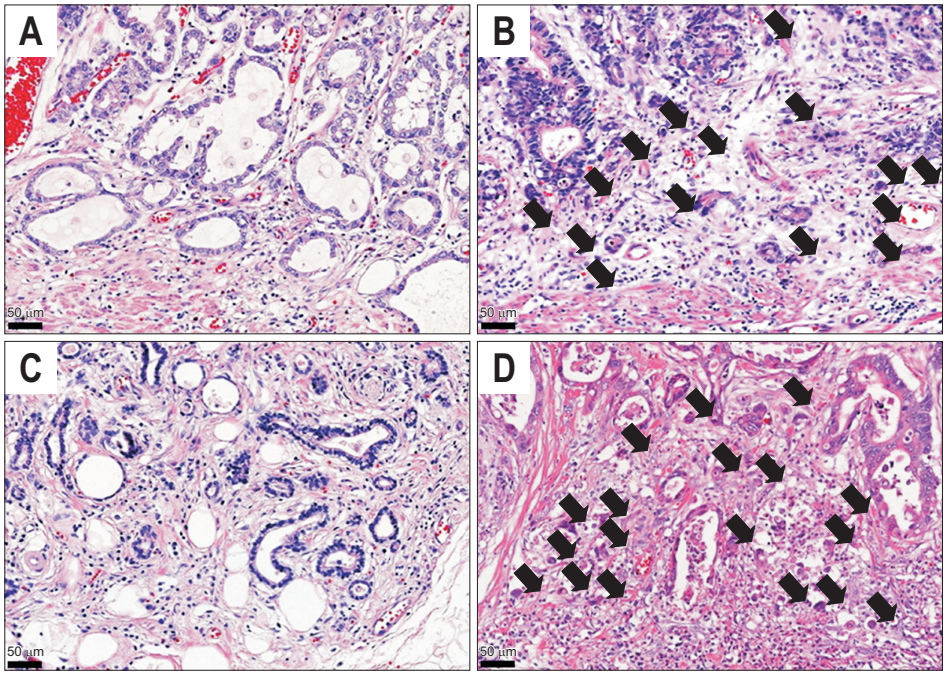

Fig. 1. Mucosal early gastric tubular adenocarcinoma (A) without tumor budding and (B) with tumor budding. Submucosal early gastric tubular adenocarcinoma (C) without tumor budding and (D) with tumor budding (hematoxylin and eosin staining, ×200). Arrows indicate tumor budding.

of diagnosis until 2020. We collected data for analysis, including patient demographic information, operation type, synchronous cancer multiplicity, tumor location, macroscopic type, and the presence or absence of ulceration. The patients were divided into three types according to the Japanese macroscopic classifications for gastric cancer: (1) elevated (type I and IIa), (2) flat (type IIb), and (3) depressed (type IIc and III).[11] Histology types were classified into well-differentiated, moderately differentiated, and poorly differentiated subtypes based on the WHO classification. In addition, pathological types were divided into two groups according to the Japanese classification: (1) differentiated (well and moderately differentiated adenocarcinoma by WHO classification), and (2) undifferentiated (poorly differentiated adenocarcinoma by WHO classification). Information regarding tumor size, T stage according to the extent of vertical invasion (mucosal vs SM), lymphovascular invasion (LVI), perineural invasion, and LNM were also evaluated. The invasion width and depth were measured to verify the correlation between SM width >4,000 μm and LNM.[12] Poorly cohesive and differentiated components of the SM layer were evaluated. TB was examined in the invasive components of the tumor through a series of microscopic sections. All slides were reassessed by an experienced gastrointestinal pathologist (S.J.S.). As described in a previous study, the invasive front of each tumor was analyzed at 40x magnification first, and two representative slides that displayed the highest density of tumor buds were selected for further analysis.[13] Subsequently, five areas with the greatest density of tumor bud were chosen from each slide, consisting of a total of 10 assessment fields per case. TB was counted at ×200 magnification (Fig. 1). We defined the maximum TB as the greatest number of tumor buds out of 10 fields, indicating a sole hotspot. The total number of TB was described as the sum of all tumor buds counted in 10 assessment fields, reflecting loosely dispersed but possibly more extensive TB in general. Clinicopathologic features were evaluated according to TB.

3. Statistical analysis

We employed various statistical methods in our study. For the analysis of categorical data, we chose between the chi-square test and Fisher exact test based on their applicability. When comparing continuous data, we used the independent two-sample t-test. We considered a p-value below 0.05 as indicative of statistical significance. To identify the most effective TB value for predicting LNM risk, we conducted analyses using receiver operating characteristic curves. Additionally, a multivariate logistic regression analysis was performed to ascertain the factors influencing the likelihood of LNM. All these statistical procedures were executed using version 9.4 of SAS (SAS Institute Inc., Cary, NC, USA).

RESULTS

1. Baseline characteristics

In total, 380 patients (286 men, 75.26%; 94 women, 25.74%) were eligible for the final analysis (Table 1). There were 191 (50.26%) mucosal invasive EGCs and 189 (49.74%) SM invasive EGCs; 112 (29.47%) well-differentiated and 199 (52.37%) moderately differentiated cases, categorized together into the differentiated type (n=311,

Table 1. Baseline Characteristics: Demographic, Clinical and Pathological Features

Characteristic	All patients (n=380)
Sex	
Male	286 (75.26)
Female	94 (24.74)
Age, median (IQR), yr	62 (55–71)
Operation type	
Total gastrectomy	47 (12.37)
Subtotal gastrectomy	333 (87.63)
Multiplicity	
Single EGC	367 (96.58)
Multiple EGCs	13 (3.42)
Tumor location	
Upper 1/3	41 (10.79)
Middle 1/3	89 (23.42)
Lower 1/3	250 (65.79)
WHO classification	
Well differentiated	112 (29.47)
Moderately differentiated	199 (52.37)
Poorly differentiated	69 (18.16)
Japanese classification	
Differentiated	311 (81.84)
Undifferentiated	69 (18.16)
Macroscopic type	
Elevated	107 (28.16)
Flat	107 (28.16)
Depressed	166 (43.68)
Ulceration	19 (5.00)
Size (long span), median (IQR), mm	24 (15–35)
Depth	
pT1a	191 (50.26)
pT1b	189 (49.74)
LVI	90 (23.68)
PNI	10 (2.63)
LNM	32 (8.42)
Tumor budding present	226 (59.47)

Data are presented as number (%) unless indicated otherwise.
IQR, interquartile range; EGC, early gastric cancer; WHO, World Health Organization; LVI, lymphovascular invasion; PNI, perineural invasion; LNM, lymph node metastasis.

Table 2. Comparison of TB in All Patients and Subgroup Analysis According to the Invasion Depth

Characteristics	All patients			pT1a patients			pT1b patients		
	TB negative (n=154)	TB positive (n=226)	p-value	TB negative (n=117)	TB positive (n=74)	p-value	TB negative (n=37)	TB positive (n=152)	p-value
Male sex	125 (81.17)	161 (71.24)	0.0276	91 (77.78)	48 (64.86)	0.0508	34 (91.89)	113 (74.34)	0.0213
Age, median (IQR), yr	63 (56–70)	62 (55–72)	0.7560	63 (56–70)	60 (51–71)	0.2280	63 (57–73)	62 (56–72)	0.9732
Multiple EGCs	4 (2.60)	9 (3.98)	0.4659	4 (3.42)	2 (2.70)	>0.9999	0	7 (4.61)	0.3487
Tumor location			0.2938			0.7235			0.4623
Upper 1/3	12 (7.79)	29 (12.83)		4 (3.42)	4 (5.41)		8 (21.62)	25 (16.45)	
Middle 1/3	38 (24.68)	51 (22.57)		27 (23.08)	15 (20.27)		11 (29.73)	36 (23.68)	
Lower 1/3	104 (67.53)	146 (64.60)		86 (73.50)	55 (74.32)		18 (48.65)	91 (59.87)	
WHO classification			<0.0001			0.0001			0.0002
Well differentiated	72 (46.75)	40 (17.70)		57 (48.72)	16 (21.62)		15 (40.54)	24 (15.79)	
Moderately differentiated	72 (46.75)	127 (56.19)		51 (43.59)	41 (55.41)		21 (56.76)	86 (56.58)	
Poorly differentiated	10 (6.50)	59 (26.11)		9 (7.69)	17 (22.97)		1 (2.70)	42 (27.63)	
Japanese classification			<0.0001			0.0027			0.0012
Differentiated	144 (93.51)	167 (73.89)		108 (92.31)	57 (77.03)		36 (97.30)	110 (72.37)	
Undifferentiated	10 (6.49)	59 (26.11)		9 (7.69)	17 (22.97)		1 (2.70)	42 (27.63)	
Ulceration	8 (5.19)	11 (4.87)	0.8856	6 (5.13)	3 (4.05)	>0.9999	2 (5.41)	8 (5.26)	>.9999
Size (long span), median (IQR), mm	24 (14–33)	24 (17–39)	0.0897	22 (12–33)	25 (15–40)	0.1694	25 (20–35)	24 (18–39)	0.7435
Depth, n(%)			<0.0001			-			-
pT1a	117 (75.97)	74 (32.74)		-	-		-	-	
pT1b	37 (24.03)	152 (67.26)		-	-		-	-	
LVI	12 (7.79)	78 (34.51)	<0.0001	2 (1.71)	12 (16.22)	0.0002	10 (27.03)	66 (43.42)	0.0682
PNI	0	10 (4.42)	0.0068	0	0	>0.9999	0	10 (6.58)	0.2143
LNM	3 (1.95)	29 (12.83)	0.0002	1 (0.85)	3 (4.05)	0.3006	2 (5.41)	26 (17.11)	0.0724
SM depth, median (IQR), mm	-	-	-	-	-	-	0.7 (0.3–2.0)	2.0 (1.0–3.0)	0.0002
SM width, median (IQR), mm	-	-	-	-	-	-	3.0 (1.5–10.0)	9.0 (5.0–13.5)	0.0014
SM width ≥4 mm	-	-	-	-	-	-	18 (48.65)	122 (80.26)	<.0001
Poorly cohesive in SM*	-	-	-	-	-	-	0	18 (11.92)	0.0262
Poorly differentiated in SM**	-	-	-	-	-	-	0	56 (37.09)	<0.0001
Maximum TB, median (IQR)	-	3.00 (2.00–7.00)	-	-	2.00 (1.00–3.00)	-	-	4.00 (2.00–9.00)	-
Total TB, median (IQR)	-	0.80 (0.30–2.60)	-	-	0.30 (0.20–0.90)	-	-	1.30 (0.40–3.80)	-
Recurrence	1 (0.65)	2 (0.88)	>0.9999	1 (0.85)	0	>0.9999	0	2 (1.32)	>0.9999

Data are presented as number (%) unless indicated otherwise.

TB, tumor budding; IQR, interquartile range; EGC, early gastric cancer; WHO, World Health Organization; LVI, lymphovascular invasion; PNI, perineural invasion; LNM, lymph node metastasis; SM, submucosa.

81.84%) according to the WHO criteria. LNM was identified in 32 patients (8.42%). LVI and perineural invasion were noted in 90 patients (23.68%) and 10 patients (2.63%), respectively. TB was present in 226 patients (59.47%).

2. Correlation between TB and clinicopathological features

Table 2 shows the comparison between the TB-negative and TB-positive cases. In all patients with EGC, moderately and poorly differentiated types were higher in proportion in the TB-positive group than in the TB-negative group. The proportion of undifferentiated types was also higher in the TB-positive group than in the TB-negative group. TB-positive tumors were more common in SM invasive cancers than in mucosal invasive cancers (67.26% vs 32.74%, p<0.0001). LVI was identified in 34.51% of TB cases; however, only 7.79% of cases were TB-negative. TB positive was significantly more common with LNM (29 cases, 12.83%) compared to TB-negative group (3 cases, 1.95%; p=0.0002).

Clinicopathological features were compared between the TB-negative and TB-positive groups according to tumor invasion depth. LVI was significantly more common in the TB-positive group for mucosal invasive cancers (pT1a).

The median SM invasion depth, SM invasion width, and width >4,000 μm were all significantly greater in the TB-positive group among the SM invasive cancers (pT1b). In addition, both poorly cohesive and poorly differentiated SM were significantly greater in the TB-positive group.

The relationship between TB and LVI or LNM was confirmed. Therefore, we used the receiver operating characteristic curve to select the cutoff value to accurately examine the effect of TB. The values of the three groups were selected because of their high sensitivity and specificity (<2, >21, and ≥21) (Supplementary Fig. 1).

3. Risk factors for LNM/LVI

We executed a multivariate logistic regression analysis to identify independent risk factors with potential predictive value for LNM/LVI. Risk prediction factors, including LNM/LVI, were analyzed because LVI is the strongest independent risk factor for representative LNM in EGC.

In the univariate analysis (Table 3), significant high-risk factors for LNM/LVI in all EGC patients included poor differentiation, undifferentiated type, SM invasion, and TB positivity. The rates of LNM/LVI increased in the TB group from low to high when TB was divided into three groups.

The multivariate analysis identified the following significant independent risk factors that significantly elevate the risk for LNM/LVI: undifferentiated type, SM invasive cancer, and TB. In the multivariate analysis of TB divided into

Table 3. Multivariate Analysis of Potential Risk Factors for LNM/LVI in All Patients

Variable	Univariable model		Multivariable model*		Multivariable model†	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Female sex	1.548 (0.931–2.573)	0.0921				
Age	1.008 (0.987–1.030)	0.4450				
Multiple EGCs	0.828 (0.223–3.075)	0.7777				
Tumor location						
Upper 1/3	Ref		Ref			
Middle 1/3	1.354 (0.545–3.364)	0.5141	1.944 (0.705–5.363)	0.1992		
Lower 1/3	1.604 (0.706–3.641)	0.2591	3.528 (1.409–8.830)	0.0071		
WHO classification						
WD	Ref					
MD	1.416 (0.798–2.512)	0.2351				
PD	3.333 (1.702–6.528)	0.0004				
Japanese classification						
Differentiated	Ref		Ref			
Undifferentiated	2.648 (1.535–4.569)	0.0005	1.999 (1.019–3.922)	0.044		
Size, long span	1.009 (0.997–1.022)	0.1440				
Depth (ref: pT1a)	8.750 (4.866–15.735)	<0.0001	7.267 (3.655–14.449)	<0.0001	5. 569 (2. 912–10.650)	<0.0001
Tumor budding	5.586 (3.076–10.146)	<0.0001				
Total tumor budding (3 groups)						
Low (<2)	Ref		Ref		Ref	
Intermediate (<21)	3.907 (2.131–7.162)	<0.0001	1.920 (0.967–3.816)	0.0625	2.333 (1.154–4.323)	0.0171
High (≥21)	12.599 (6.308–25.165)	<0.0001	3.673 (1.613–8.366)	0.0019	5.030 (2.353–10.748)	<0.0001

LNM, lymph node metastasis; LVI, lymphovascular invasion; OR, odds ratio; CI, confidence interval; EGC, early gastric cancer; WHO, World Health Organization; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; Ref, reference.
*A stepwise multivariate model with all variables; †A stepwise multivariate model including variables with a univariate p<0.2.

three groups, the risk of developing LNM/LVI increased proportionally with the degree of TB.

4. Risk factor for LNM/LVI according to the depth of invasion

In the univariate analysis of mucosal invasive cancers (Table 4), it was found that both female sex and TB were significantly linked with LNM/LVI. Further, multivariate analysis identified that TB represents an independent risk factor for LNM. Compared with the low TB group (<2), patients in the intermediate and high TB groups were also more likely to have LNM/LVI. In particular, the OR of LNM/LVI was approximately 27 times greater in the high-TB group when compared to the low-TB group.

In the univariate analysis of SM invasive cancers (Table 5), lower-third location, undifferentiated type, SM width, SM width >4,000 μm, poorly differentiated cells in SM, and high TB (≥21) were significantly associated with LNM/LVI. However, SM depth, which is already known to be associated with LNM in SM cancer, was not statistically associated with LNM/LVI in this study, but a statistical trend was identified.

Multivariate analysis showed that lower-third location, undifferentiated type, and SM width >4,000 μm were independently and significantly related to LNM/LVI in SM invasive cancers. TB was not independently related to LNM/LVI.

5. Cases of mucosal invasive cancer with LNM meeting ER criteria

In our study, LNM was identified in four patients with mucosal cancer. According to the ER guidelines published in Japan in 2018,[14] cases 3 and 4 had undifferentiated-type and lesions larger than 20 mm, which did not meet the ER criteria for EGC treatment. Cases 1 and 2 were of the differentiated type and absence of ulcers in cancer so ER can be attempted because they met the criteria of the current guidelines. However, these patients required additional surgical treatment even after ER, and TB was the only factor suggestive of preoperative LNM (Table 6), as LNM was found postoperatively in case 1.

DISCUSSION

ER has been performed extensively in the treatment of EGC only for lesions that have a negligible risk of LNM. Therefore, many studies have been conducted to estimate LNM risk factors, and current ER criteria have been established as a result of this. Additional surgery, including lymph node dissection, is necessary in the consideration of the LNM risk.

Therefore, a close pathological analysis after ER is important. Cases in which LNM is found have been report-

Table 4. Multivariate Analysis of Potential Risk Factors for LNM/LVI in pT1a Subgroup

pT1a subgroup	Univariable model		Multivariable model*		Multivariable model†	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Female sex	2.978 (1.055–8.405)	0.0393				
Age	1.031 (0.982–1.083)	0.2157	1.042 (0.993–1.093)	0.0943		
Multiple EGCs	2.359 (0.253–22.032)	0.4514				
Tumor location						
Upper 1/3	Ref					
Middle 1/3	1.505 (0.060–37.687)	0.8034				
Lower 1/3	1.785 (0.082–38.692)	0.7120				
WHO classification						
WD	Ref					
MD	1.643 (0.475–5.687)	0.4333				
PD	3.137 (0.724–13.596)	0.1266				
Japanese classification						
Differentiated	Ref					
Undifferentiated	2.318 (0.687–7.826)	0.1755				
Ulceration	0					
Size, long span	1.007 (0.982–1.034)	0.5700				
Tumor budding	7.181 (2.117–24.365)	0.0016				
Total tumor budding (3 groups)						
Low (<2)	Ref				Ref	
Intermediate (<21)	5.578 (1.719–18.095)	0.0042	6.240 (1.913–20.356)	0.0024	5.578 (1.719–18.095)	0.0042
High (≥21)	27.894 (4.351–178.820)	0.0004	37.221 (5.076–272.904)	0.0004	27.894 (4.351–178.820)	0.0004

LNM, lymph node metastasis; LVI, lymphovascular invasion; OR, odds ratio; CI, confidence interval; Ref, reference; EGC, early gastric cancer; WHO, World Health Organization; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; Ref, reference.
*A stepwise multivariate model with all variables; †A stepwise multivariate model including variables with a univariate p<0.2.

Table 5. Multivariate Analysis of Potential Risk Factors for LNM/LVI in pT1b Subgroup

pT1b subgroup	Univariable model		Multivariable model*		Multivariable model†	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Female sex	1.707 (0.856–3.404)	0.1292				
Age	0.993 (0.965–1.021)	0.6036				
Multiple EGCs	0.499 (0.094–2.644)	0.4136				
Tumor location						
Upper 1/3	Ref		Ref		Ref	
Middle 1/3	2.121 (0.791–5.687)	0.1353	1.972 (0.692–5.621)	0.2041	2.006 (0.714–5.639)	0.1865
Lower 1/3	3.425 (1.420–8.262)	0.0061	4.424 (1.697–11.535)	0.0024	4.414 (1.714–11.365)	0.0021
WHO classification						
WD	Ref					
MD	0.804 (0.382–1.691)	0.5650				
PD	1.979 (0.821–4.771)	0.1283				
Japanese classification						
Differentiated	Ref		Ref		Ref	
Undifferentiated	2.320 (1.158–4.652)	0.0177	3.093 (1.413–6.773)	0.0047	2.989 (1.383–6.461)	0.0054
Ulceration	0.519 (0.130–2.070)	0.3526				
Size, long span, median (mm)	1.007 (0.990–1.025)	0.4199				
SM depth						
<500 μm	Ref					
<1,000 μm	0.778 (0.250–2.417)	0.6640				
≥1,000 μm	2.031 (0.912–4.523)	0.0829				
SM width	1.068 (1.022–1.116)	0.0035	1.065 (1.018–1.114)	0.0062		
SM width ≥4 mm	2.849 (1.393–5.828)	0.0041	2.960 (1.401–6.252)	0.0044	2.960 (1.401–6.252)	0.0044
Poorly cohesive in SM‡	2.789 (0.999–7.782)	0.0502				
PD in SM§	1.908 (1.014–3.589)	0.0452				
Tumor budding	1.838 (0.863–3.914)	0.1147				
Total tumor budding (3 groups)						
Low (<2)	Ref					
Intermediate (<21)	1.562 (0.728–3.351)	0.2521				
High (≥21)	3.339 (1.484–7.514)	0.0036				

LNM, lymph node metastasis; LVI, lymphovascular invasion; OR, odds ratio; CI, confidence interval; EGC, early gastric cancer; WHO, World Health Organization; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; SM, submucosal; Ref, reference.
*A stepwise multivariate model with all variables; †A stepwise multivariate model including variables with a univariate p<0.2; ‡Presence of poorly cohesive carcinoma cells infiltrating the SM layer; §Presence of poorly differentiated adenocarcinoma component within the SM layer.

Table 6. Cases Summary of Patients with Mucosal Invasive Cancer with Lymph Node Metastasis

Case	Age, yr/sex	Location	Tumor size, mm	Ulcer	Gross	WHO	Lauren's	Japanese	LVI	Total number of TB	TB group
1	50/F	Lower 1/3	60×50	None	Elevated	MD	Intestinal	Differentiated	Absent	20	Intermittent
2	78/M	Lower 1/3	40×35	None	Flat	MD	Intestinal	Differentiated	Present	1	Low
3	66/F	Lower 1/3	23×17	None	Flat	PD	Mixed	Undifferentiated	Present	3	Intermittent
4	67/F	Lower 1/3	45×20	None	Flat	PD	Mixed	Undifferentiated	Absent	None	

WHO, World Health Organization; LVI, lymphovascular invasion; TB, tumor budding; M, male; F, female; MD, moderately differentiated; PD, poorly differentiated.

ed[15] even if they belong to the current ER criteria. Studies are currently being conducted to identify additional risk factors. This study also analyzed whether TB can be a factor that can accurately predict LNM in EGC, and our findings suggest that TB is a risk factor for LNM in mucosal invasive cancer.

The results of this study showed that TB is not rare finding in EGC. TB was identified in 59.5% of EGD, 38.7% of mucosal invasive cancer, and 80.4% of SM invasive cancers. However, the significance of TB in EGC has not yet been identified unlike in colorectal cancer. As a result, pathological findings of TB after gastric cancer resection have not been analyzed. Several previous studies have tried to evaluate the significance of TB in EGC. A study by Gulluoglu *et al.*[7] included 126 patients with EGC and reported that TB was one of the significant predictors of LNM in EGC.

However, this study had limitations in that the sample size was small and TB was divided into only two groups: absent and present. Ulase *et al.*[16] confirmed the efficacy and prognostic significance of TB according to the International Tumor Budding Consensus Conference criteria for a European gastric cancer cohort of 456 patients. Kemi *et al.*[17] studied the meaning of TB in gastric cancer. This paper analyzed 583 gastric adenocarcinoma patients by dividing into low-budding (<10) and high-budding (≥10) groups, and high TB was found to be an independent factor to predict intestinal type of gastric adenocarcinoma. Another study published by Szalai *et al.*[18] in 2022 analyzed 290 gastric cancer patients by dividing TB according to the criteria of the International Tumor Budding Consensus Conference, and found high TB as an independent predictor for LNM and an independent prognostic factor for survival in gastric cancer. Olsen *et al.*[13] conducted a study on 104 patients who received surgery for gastric cancer and revealed high TB scores in intestinal-type gastric adenocarcinoma are linked with increased T-stage, N-stage, grade, and recurrence probability. However, all these papers are different from our study because they included all stomach cancer subjects, not including EGC. So, the biggest strength of our study is not only including EGC patients in the study but also dividing EGC into mucosal and SM invasive cancers to confirm the importance of TB, and by dividing TB into three groups, we discovered that the risk of LNM increased proportionally.

In this study, mucosal and SM invasive cancers were analyzed separately. Differences were observed between the two groups. TB was the only risk factor for LNM in mucosal invasive cancers and was not a significant factor in SM invasive cancers. In SM invasive cancers, accurate analysis of the burden of SM invasion rather than TB may be important for an accurate prediction of LNM, and a meaningful SM width of ≥4,000 μm was also previously suggested as an accurate predictor of LNM of SM invasive cancer.[13]

Thus, it is important to identify new LNM risk factors in mucosal invasive cancer. Recently, the Japanese guidelines revised mucosal invasive cancer as an absolute indication for ER, and there was no size limitation for differentiated cancer confined to mucosal cancers without ulcers.[16] However, in our study, when four cases of LNM among mucosal cancers were reviewed, one case could not be predicted as LNM using the current ER criteria, but 20 cases of TB were observed, so LNM could be predicted by TB. Therefore, TB can be an additional factor that can determine additional surgery after ER in patients with mucosal invasive cancer. Currently, LVI is known to be the strongest risk factor for LNM, but since the mucosal layer lacks lymphatics compared to the SM layer, LVI cannot be a strong predictor of SM cancer. In this study, TB was found to be the sole factor capable of predicting the risk of LNM in patients with mucosal invasive cancer.

Our study was subject to several limitations. First, this retrospective study may have inherent selection bias, and external validation is necessary. We plan to address this in future research through multicenter studies to validate our findings. Second, this particular cohort had a small sample size, especially when it came to the number of LNM cases, including mucosal cancer compared with SM cancer. In this study, LVI and LNM were defined as composite outcomes due to their combined clinical significance in predicting tumor aggressiveness and guiding management in EGC. Sensitivity analysis revealed that LVI, as a strong independent risk factor for LNM, masked the predictive power of TB in multivariate analysis, likely due to the small number of LNM cases (Supplementary Table 1). Further evaluations with a larger sample size in the future study are expected to yield more consistent results. Third, the prognosis of patients with confirmed TB positivity could not be determined. However, since LNM is an important marker indicating a poor prognosis in EGC,[19,20] TB will also be a helpful factor in evaluating the prognosis with EGC patients. Fourth, the lack of a standardized cutoff value for TB in EGC and the use of classification systems derived from colon cancer staging present inherent limitations due to the unique histologic characteristics of gastric cancer. A standardized classification system specific to EGC is needed for more accurate prognostic assessment and individualized treatment. Future multicenter studies are necessary to establish a validated and widely accepted cutoff value for TB in EGC. Finally, interobserver variation is not calibrated because only one pathologist assessed TB on the slides. For future studies, we plan to involve multiple pathologists and conduct blinded reviews to improve reliability and reduce potential bias.

Regardless of this limitation, our study shows a novel finding that TB can be a predictor of LNM, even in the absence of LVI, in differentiated-type mucosal invasive cancers currently included in the absolute indication guidelines for ER. If TB is found after ER of a mucosal invasive cancer, it may be necessary to consider additional surgery.

In conclusion, TB is a significant factor associated with LNM, particularly in mucosal-invasive gastric cancer. However, its role as an independent predictor of LNM is limited when other established risk factors, LVI, are considered. While TB may provide additional insights for predicting LNM in mucosal-invasive cancers, further large-scale, multicenter studies are needed to validate its prognostic value and establish its role in clinical decision-making.

CONFLICTS OF INTEREST

J.H.K. is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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AUTHOR CONTRIBUTIONS

Study concept and design: Y.J., Y.K. S.J.S., J.H.K. Data acquisition: S.J.S., J.H.K. Data analysis and interpretation: Y.J., Y.K., J.H.K., J.C., Y.H.Y., H.P., S.H.N., I.G.K. Drafting of the manuscript: Y.J., Y.K., S.J.S., J.H.K. Critical revision of the manuscript for important intellectual content: Y.K., J.H.K. Statistical analysis: G.P. Obtained funding: J.H.K. Administrative, technical, or material support; study supervision: S.J.S., J.H.K. Approval of final manuscript: all authors.

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SUPPLEMENTARY MATERIALS

Supplementary materials can be accessed at https://doi.org/10.5009/gnl240352.

DATA AVAILABILITY STATEMENT

The supporting data for the results of this research can be obtained from the corresponding author if requested appropriately.

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