Excellent prognosis following endoscopic resection of patients with rectal neuroendocrine tumors despite the frequent presence of lymphovascular invasion

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<ABSTRACT>

Background Endoscopic resection (ER) has been increasingly used for the treatment of rectal neuroendocrine tumors (NETs); however, only limited data are available on its long-term outcomes. This study analyzed the long-term outcomes of rectal NETs treated by ER and characterized potential risk factors for metastasis in these cases, with emphasis on lymphovascular invasion.

Methods We retrospectively analyzed the clinicopathological features and outcomes of 86 patients with 90 rectal NETs who had been treated by ER. Lymphovascular invasion was re-evaluated using elastic staining and double staining immunohistochemistry.

Results En bloc resection with tumor-free margins was achieved in 87 lesions (96.7%). The median tumor size was 5 mm (range, 2–13), and all the lesions were confined to the submucosal layer. The Ki-67 index was less than 3% in all the lesions, which were therefore classified as NET G1. Elastic staining and double staining immunohistochemistry revealed the presence of lymphatic and venous invasion in 23 (25.6%) and 35 lesions (36.7%), respectively. Collectively, lymphatic and/or vascular invasion was identified in 42 lesions (46.7%). All cases were followed up without additional surgery, and no metastasis or recurrence was detected during the median follow-up period of 67.5 months.

Conclusions This study showed an excellent long-term prognosis following ER of patients with rectal NETs, confirming that ER is a valid treatment option for small rectal NETs. The present study also revealed highly prevalent lymphovascular invasion even in minute rectal NETs; this observation raises a question regarding its significance as a risk factor for metastasis.

INTRODUCTION

Neuroendocrine tumors (NETs) are low- to intermediate-grade neuroendocrine neoplasms, that were formerly termed carcinoid tumors [1-3]. In most cases, NETs of the rectum are small localized tumors that are often found incidentally during endoscopic examination. Because of the increased use of screening colonoscopy, small asymptomatic rectal NETs are being detected more and more frequently [4-7]. Also, endoscopic resection (ER) has been used increasingly for their treatment; however, only limited data are available on the long-term clinical outcomes following ER in patients with rectal NETs.

Currently, the selection of treatment for rectal NETs is principally based on the tumor size and the depth of invasion, both of which can be estimated preoperatively [2, 3]. Based on the fact that rectal NETs that are small and confined to the submucosal layer rarely metastasize, these lesions are usually treated by local excision, including ER [2, 3, 8-14]. However, small rectal NETs, including those less than 10 mm in size, can also metastasize [8-12, 14-17]; therefore, identification of additional clinicopathological factors that could influence the risk of metastasis may help determine the most appropriate management. Until date, in addition to tumor size and depth of invasion,

several other risk factors for metastasis have also been reported, including the patient age, presence of lymphovascular invasion, tumor proliferative activity, presence of perineural invasion, and presence of atypical surface characteristics (depression and ulceration) [9-14, 18-22]. Among these, lymphovascular invasion is the most well-established risk factor for metastasis of rectal NETs, and some studies suggest that presence of lymphovascular invasion could be a risk factor for metastasis even in tumors smaller than 10 mm in size [9-17, 20].

In this context, there is a potential issue regarding histological evaluation of lymphovascular invasion. Notably, the reported prevalence of lymphovascular invasion varied considerably, ranging from 0%–18%, even among studies of small rectal NETs treated by local resection; this implies a possibility that lymphovascular invasion is not always properly evaluated [23-28]. Previous studies on colorectal adenocarcinoma analyzed interobserver variation in the diagnosis of lymphovascular invasion and suggested the requirement of special and/or immunohistochemical staining for the reproducible diagnosis [29-31]. However, to the best of our knowledge, there have been no studies examining lymphovascular invasion in rectal NETs in a systematic manner using immuohistochemical and/or special staining. Moreover, since NET tumor cells

exhibit minute cytological atypia, unlike most other malignant neoplasms, tumor cells within small vessels are often difficult to identify.

In the present study, we analyzed the long-term clinical outcomes of patients with rectal NETs treated by ER, and characterized potential risk factors for metastasis, with particular emphasis on the significance of lymphovascular invasion. We employed double staining immunohistochemistry for endothelial and neuroendocrine markers; simultaneous immunolabeling of NET tumor cells and vascular/lymphatic endothelial cells allows sensitive and reproducible identification of lymphovascular invasion.

PATIENTS AND METHODS

This study was conducted with the approval of the ethical committee of the National Cancer Center, Tokyo, Japan. A total of 98 patients underwent ER for rectal NETs at our institution between January 1997 and December 2011. Among these, 4 patients who underwent colectomy for colorectal cancer after the ER of rectal NETs and 8 patients who could be followed up for less than 1 year were excluded from this analysis. Finally, a total of 86 patients with 90 NETs were included in this study. The medical records were retrospectively reviewed for the endoscopic and pathological findings and the

clinical outcomes of the patients.

The tumor specimens resected by ER were routinely fixed with formalin and embedded in paraffin. Four-micrometer sections were stained with hematoxylin and eosin (HE) and the histopathological features, including the histopathological type, tumor size, depth of invasion, and resection margin, were evaluated. The resection margin was evaluated on the basis of the TNM classification [32]

The tumor cell proliferative activity was evaluated using Ki-67 immunohistochemistry (clone MIB-1; Dako, Glostrup, Denmark). For staining, we used an automated stainer (Dako, Glostrup, Denmark) according to the vendor's protocol. At least 500 tumor cells were counted to determine the percentage of cells that were positive for Ki-67, and the tumors were classified into G1 (<3% positive cells) and G2 (3%–20% positive cells) according to the WHO 2010 classification and the NANETS guidelines [1, 2]. Double staining immunohistochemistry to evaluate lymphovascular invasion was performed using a previously described procedure [33]. Anti-synaptophysin antibody (clone 27G12; Nichirei, Tokyo, Japan) was used to detect the NET tumor cells, in combination with anti-podoplanin (clone D2-40; Signet Laboratory, Dedham, MA, USA) or

anti-CD31 (clone JC70A; Dako, Glostrup, Denmark) antibody, to detect lymphatic or vascular endothelial cells, respectively [29, 34-37]. Lymphovascular invasion was deemed positive when synaptophysin-positive tumor cells were present within vascular spaces lined by podoplanin- or CD31-positive endothelial cells. Elastica van Gieson staining was used to identify the muscular venules, and tumor venous invasion was regarded as positive when tumor cells were identified inside the elastic lamina [30, 31].

Statistical analysis

To compare the clinicopathologic variables between tumors with and without lymphovascular invasion, Fisher's exact test or the chi-square test was used for categorical variables and the Mann-Whitney U test was used for continuous variables. P values below 0.05 were considered as denoting statistical significance. Overall survival time was measured from the date of ER to the date of death or the date of the latest confirmation of survival, and the overall survival rates was calculated by Kaplan–Meier analysis. All the statistical analyses were performed using the Statistical Package for the Social Sciences, version 20.0 (SPSS, Chicago, Illinois, USA), and the statistical program R, version 3.1.2 (http://cran.r-project.org).

RESULTS

Patient and tumor characteristics

The clincopathological features of the 86 patients with 90 rectal NETs treated by ER are summarized in Table 1. The male/female ratio was 1.3:1 and the median patient age at the time of ER was 57 years. The ER procedures included polypectomy/endoscopic mucosal resection (EMR) (n = 3), endoscopic submucosal resection with a ligation device (ESMR-L) (n = 83), and endoscopic submucosal dissection (ESD) (n = 4). The median tumor size was 5 mm, and 8 lesions were 10 mm or larger in size. The median size of the tumors treated by each of the ER procedures was as follows: polypectomy/EMR, 4.0 mm (range, 3–4); ESMR-L, 5.0 mm (2–11); ESD, 11.5 mm (10–13). The depth of invasion was limited to the submucosal layer in all 90 lesions. Ulceration or a depressed area was observed on the surface in three lesions. The Ki-67 index was less than 3% in all the lesions, which were therefore classified as NET G1 [1, 2]. Podoplanin/synaptophysin double staining immunohistochemistry revealed lymphatic invasion in 23 lesions (25.6%). Venous invasion was detected in 9 lesions (10.0%) by CD31/synaptophysin double staining, and in 33 lesions (36.7%) by elastic staining; both stainings revealed venous invasion in 7 lesions, thus, venous invasion was detected in 35 lesions in total (38.9%) (Fig. 1). Collectively, lymphovascular invasion

was identified in a total of 42 lesions (46.7%) by at least one of these staining procedures. On the other hand, the original diagnoses based on HE staining identified no case with lymphatic invasion and only one case with positive venous involvement.

Comparison of the clinicopathological features between cases with and without lymphovascular invasison

The clinicopathological features of cases with and without lymphovascular invasion are shown in Table 2. The size of the NETs with lymphovascular invasion was slightly larger (median, 5 mm; range, 3–13) than that of the NETs without lymphovascular invasion (median, 4 mm; range, 2–10; P = 0.02, Mann–Whitney U test), and lymphovascular invasion was found even in minute lesions. No significant differences of any of the other clinicopathological features, including the Ki-67 labeling index, were found between the two groups.

Endoscopic treatment-related short-term outcomes

En bloc resection was achieved for all lesions, except two treated by ESMR-L. En bloc resection with tumor-free margins (R0) was achieved in all except 3 lesions. The resection margin could not be assessed (RX) in two lesions treated by ESMR-L, because

of piecemeal resection. The resection margin was histologically positive (R1) in one patient treated by ESD. One of the patients with a lesion unevaluable for the resection margin underwent additional ER using ESMR-L and hot biopsy, and pathological examination detected the presence of residual tumor. No major complications were observed in any of the patients, except one who developed delayed bleeding after ESMR-L.

Long-term clinical outcomes

Original pathological diagnosis based on HE-stained sections detected lymphovascular invasion in one case. In addition, 3 patients who underwent RX or R1 resection were potentially eligible for additional surgery. However, all the four patients were followed-up without additional surgery after discussion and agreement. Thus, all 86 patients were followed-up without surgery.

The median follow-up period of the patients was 67.5 months (range, 12.2–175.2 months) and none of the patients developed recurrence or metastasis during the follow-up period. During the follow-up period, 78 patients (90.7%) underwent abdominal computed tomography (CT) and/or abdominal ultrasonography (US) for

surveillance against metastatic disease; in further detail, 70 patients underwent abdominal CT (1–20 times, median of 3 times), and 39 patients underwent abdominal US (1–39 times, median of 2 times). In addition, 79 patients (91.9%) underwent follow-up colonoscopy for surveillance against local recurrence (1–10 times, median of 3 times). Five patients died of other causes; the 5-year overall survival rate was 96.0% (Fig. 2). The median follow-up period of the patients with tumors showing positive lymphovascular invasion was 76.0 months (range, 19.1–139.4; n = 42).

DISCUSSION

The present study showed an excellent prognosis of patients with rectal NETs treated by ER. All the lesions were confined to the submucosal layer and were classified as G1 according to the Ki-67 labeling index. Eighty-two lesions were smaller than 10 mm in size, fulfilling the criteria for the application of endoscopic resection recommended by several recent guidelines [2, 3]. Eight tumors ≥10 mm in size (10–13 mm) also did not show evidence of recurrent disease; however, the number of these intermediate-sized NETs was too small to endorse the validity of ER for such lesions.

Currently, several different techniques are available for ER of rectal NETs [23-28,

38-41]. We have reported an excellent R0 resection rate of ESMR-L as compared with the rate obtained with polypectomy and conventional EMR [39, 40]. Since the large majority of the lesions were treated by ESMR-L in our series, a reasonable comparison of the outcomes among the different resection procedures was difficult. However, this study clearly showed the excellent outcomes of ESMR-L, including an R0 resection rate of 96.7%, low incidence of complications, and an excellent long-term prognosis.

Our study revealed an unexpectedly high prevalence of lymphovascular invasion in rectal NETs. This is likely to be due to the use of elastic staining and double staining immunohistochemistry, which are more sensitive methods for the evaluation of lymphovascular invasion. To the best of our knowledge, there have been no studies in which special and/or immunohistochemical staining for the detection of lymphovascular invasion has been carried out in a systematic manner. Thus, we speculate that most previous studies might have underestimated the prevalence of lymphovascular invasion because of the limited detection sensitivity of the conventional staining methods used. Indeed, our original diagnosis based on routine HE-stained sections identified venous invasion in only one case and lymphatic invasion in none of the cases.

Many previous studies have reported the presence of lymphovascular invasion in rectal NETs as a strong risk factor for metastasis [9-17]. However, the fact that nearly a half of the small rectal NETs could be positive for lymphovascular invasion raises a question about the clinical significance of this histopathologic variable. Furthermore, during the long-term follow-up period, none of the patients with tumors that were positive for lymphovascular invasion developed recurrence or metastasis. Based on these findings, we suggest that the presence of lymphovascular invasion may not directly indicate the risk of metastasis, particularly when the histological evaluation is performed using special and/or immunohistochemical staining.

Of note, there have been a few reported cases of recurrent disease developing after a long latency period [28, 42]. Thus, while the follow-up period in the present study was longer than in most previous analyses, a still longer follow-up period may be desirable. Also, as none of the patients in the present study underwent surgical resection, the regional lymph nodes were not histologically evaluated; therefore, we cannot exclude the presence of clinically undetectable minute metastatic lesions in the patients. Considering these findings, we think that the present results are insufficient to conclude that minute rectal NETs are free from the risk of metastasis. Further studies involving a

larger number of patients with extended follow-up periods would be required to draw broad conclusions for recommending the optimal follow-up schedule for patients undergoing endoscopic resection of rectal NETs. Another important limitation concerns the evaluation of lymhovascular invasion. Even though we employed sensitive detection methods for the detection of lymphovascular invasion, we analyzed only single representative sections; analysis of multiple sections may further increase the chance of detection of lymphovascular invasion.

The present study showed an excellent long-term prognosis following endoscopic resection of patients with rectal NETs, confirming that ER is a valid treatment option for small rectal NETs. Importantly, none of the patients developed clinically detectable metastatic disease despite the presence of lymphovascular invasion in nearly half of the lesions. While further studies would be required to reach definitive conclusions, our observations raise a question on the clinical significance of lymphovasucalar invasion in small rectal NETs.

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Conflict of interest All the authors have no conflict of interest.

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<Tables>

Table 1 Clinicopathological features of 86 patients with 90 rectal neuroendocrine tumors

Age (year-old), median (range)	57.0 (16-85)
Gender, n (%)	
Male	49 (57.0%)
Female	37 (43.0%)
Endoscopic resection procedure, n (%)	
Polypectomy/ EMR	3 (3.3%)
ESMR-L	83 (92.2%)
ESD	4 (4.4%)
Tumor size (mm), median (range)	5.0 mm (2-13)
Tumor depth, n (%)	
Submucosa	90 (100%)
Ulceration/depression, n (%)	
Present	3 (3.3%)
Absent	87 (96.7%)
Ki-67 labeling index (%), median (range)	0.9 (0.1-2.9)
Tumor grade, n (%)	
Grade 1	90 (100%)
Lymphatic invasion, D2-40/synaptophysin, n (%)	
Positive	23 (25.6%)
Negative	67 (74.4%)
Venous invasion, CD31/synaptophysin, n (%)	
Positive	9 (10.0%)
Negative	81 (90.0%)
Venous invasion, elastic staining, n (%)	
Positive	33 (36.7%)
Negative	57 (63.3%)
Cut margin of the resected specimen, n (%)	
R0	87 (96.7%)
R1	2 (2.2%)
RX	1 (1.1%)

Table 2 Clinicopathological features of rectal neuroendocrine tumors with and without lymphovascular invasion

	Lymphovascular invasion (+) n=42	Lymphovascular invasion (-) n=48	P value
Age (year-old), median (range)	57.5 (33-85)	56.0 (16-75)	0.86
Gender, n (%)			0.59*
Male	26 (61.9%)	27 (56.2%)	
Female	16 (38.1%)	21 (43.8%)	
Tumor size (mm), median (range)	5.0 (3-13)	4.0 (2-10)	0.02
Tumor depth			
Submucosa	42 (100%)	48 (100%)	
Ki-67 labeling index (%), median (range)	1.0 (0.3-2.9)	0.9 (0.1-2.0)	0.09
Ulceration/depression, n (%)			0.45**
Present	2 (4.8%)	1 (2.1%)	
Absent	40 (95.2%)	47 (97.9%)	

*, Chi-square test; **, Fisher's exact test.

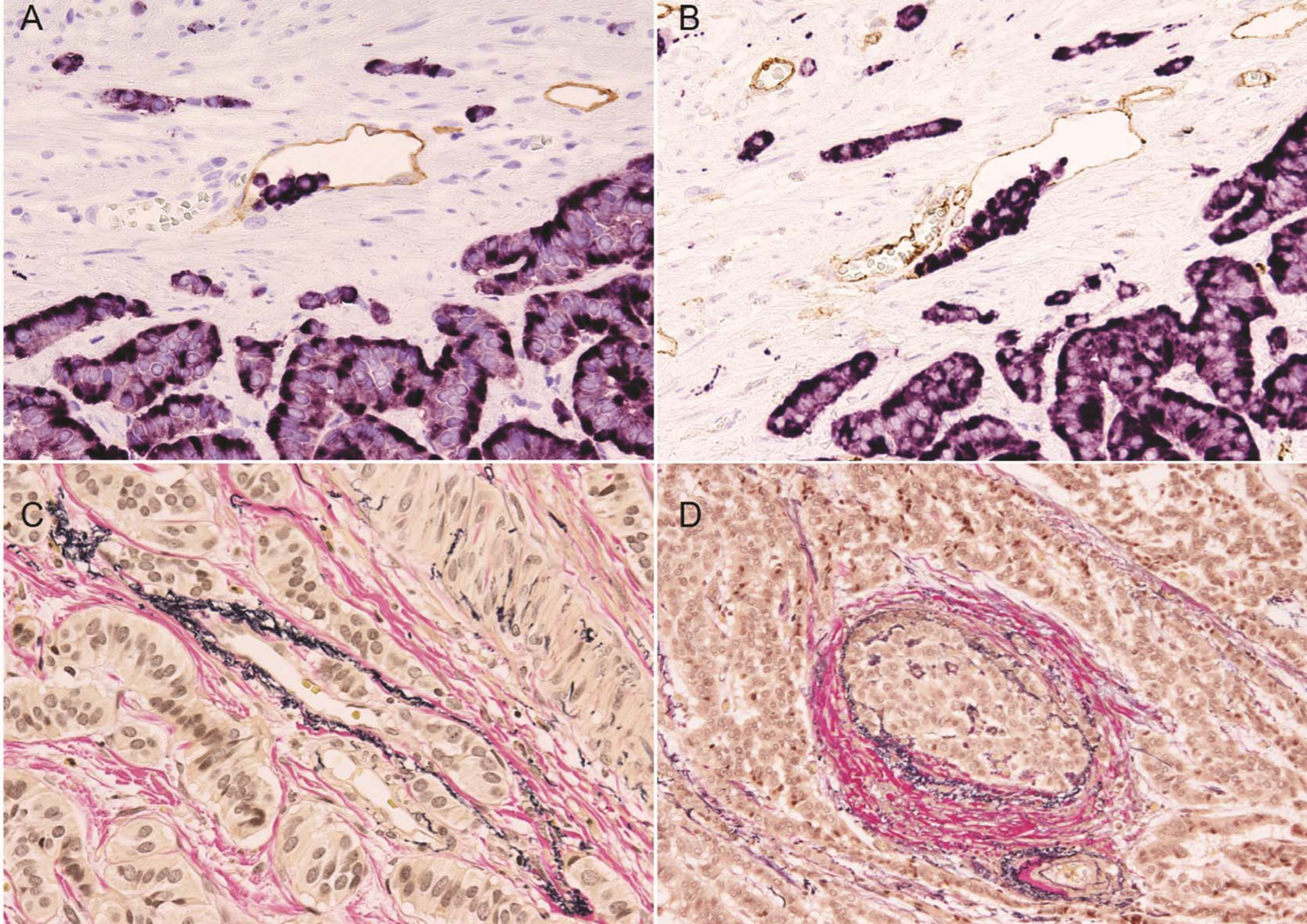
Fig 1. Lymphatic and venous invasion detected by the special staining

(a) Lymphatic invasion detected by podoplanin/synaptophysin double staining immunohistochemistry. Synaptophysin-positive tumor cells (purple) are present in a lymphatic vessel lined by podoplanin-positive endothelial cells (brown).

(b) Venous invasion detected by CD31/synaptophysin double staining immunohistochemistry. Synaptophysin-positive tumor cells (purple) are detected in a vascular space lined by CD31-positive endothelial cells (brown).

(c, d) Venous invasion detected by elastic staining. The elastic staining highlights elastic lamina of muscular venules (black). The vascular lumen is narrowed (c) or occluded (d).

Fig 2. Kaplan-Meier analysis for estimation of the overall survival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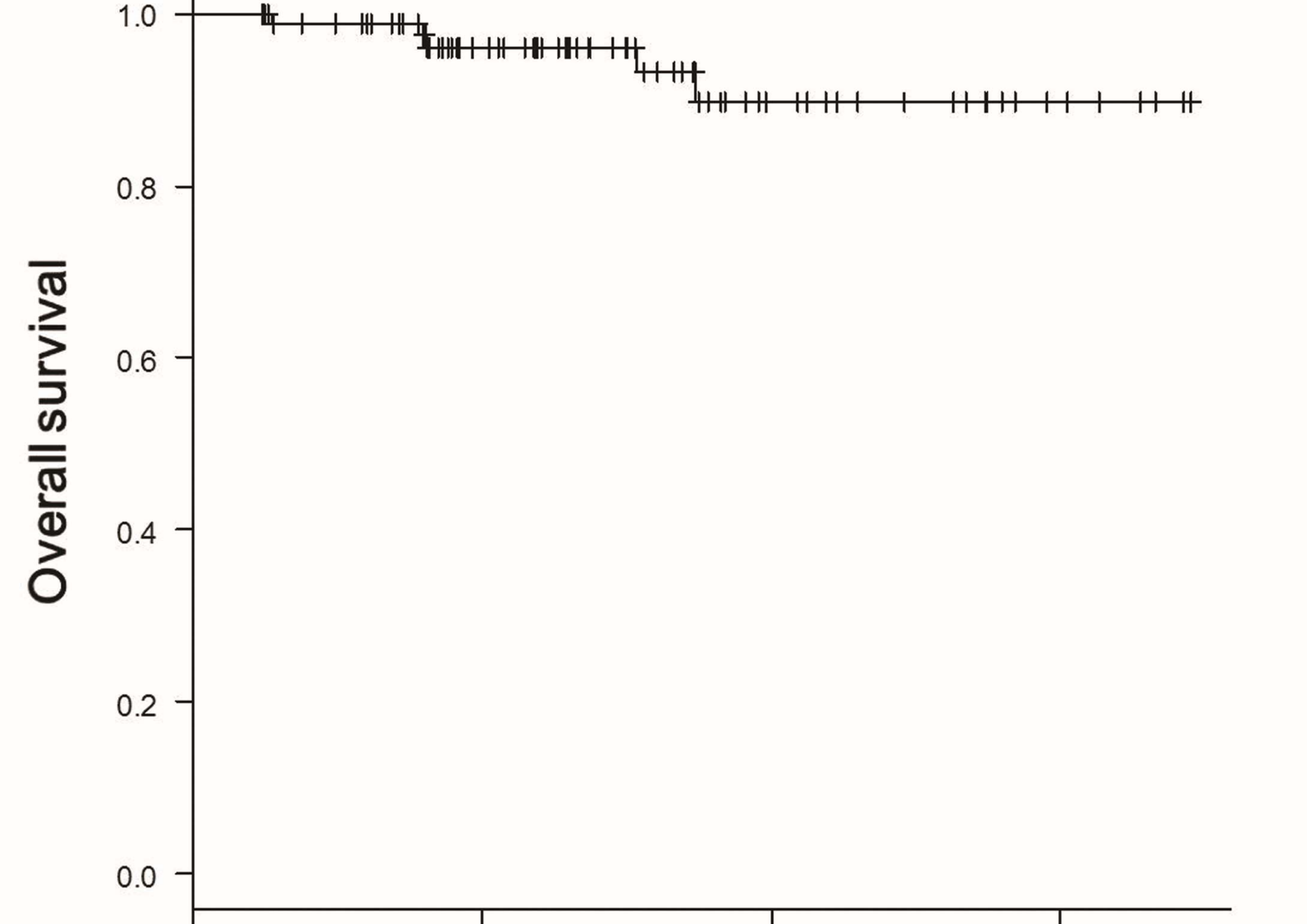
Overall survival
1.0
0.8
0.6
0.4
0.2
0.0