Shirouzu *et al.*
World Journal of Surgical Oncology (2024) 22:274
https://doi.org/10.1186/s12957-024-03556-6

World Journal of Surgical Oncology

RESEARCH

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The clinical significance of elastic lamina invasion in patients with pStage II colorectal cancer: a notable prognostic indicator

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Abstract

Background Some colorectal cancers (CRCs) are clinically diagnosed as cT4a with serosal invasion (SI). However, the cT4a is most often underdiagnosed pathologically as pT3 without SI by hematoxylin–eosin (H&E) staining alone. Using Elastica van Gieson (EVG) staining, some pT3 tumors invade the elastic lamina (EL), which extends just below the serosal layer. Recently, EL invasion (ELI) has been described as a poor prognostic factor for disease-free survival (DFS) and overall survival (OS) in patients with pStage II CRC. However, its clinicopathological significance remains unclear due to the limited number of studies and poor understanding of ELI.

Objective This study investigated the association between the ELI and patient prognosis.

Methods After 1982, pathological diagnosis was routinely performed using H&E and EVG staining methods, and long-term follow up was performed until 2016. All clinicopathological features including ELI were prospectively registered into our computer and 569 patients with pStage II CRC were collected from the database. Based on the ELI status, pT3 was divided into three pathological categories: pT3ELI − was defined as pT3a, pT3ELI + as pT3b and unidentified EL (pT3EL −) as pT3u.

Results Using H&E staining alone, gross cT4a was most often pathologically underdiagnosed as pT3 (93.8%) and very rarely as pT4a, resulting in a large diagnostic discrepancy. Using EVG staining, 60.7% of the cT4a tumors were diagnosed as pT3b. The 10-year DFS and OS rates were similar for pT3a and pT3u patients. However, the 10-year DFS and OS rates of pT3b patients were significantly lower than those of pT3a patients (75.6% vs. 95.6%, $p < 0.0001$ and 58.4% vs. 70.6%, $p = 0.0024$, respectively) but did not differ from those of pT4a patients (70.6%, $p = 0.5799$ and 52.0%, $p = 0.1116$, respectively). Multivariate analysis revealed that the ELI was the strongest independent risk factor for recurrence and CRC-specific death ($p < 0.0001$).

Conclusions A better understanding of the ELI allows us to reconsider the diagnostic discrepancy of serosal invasion, i.e., pT3b should be considered pT4a. The ELI-based subclassification of pT3 is expected to be incorporated into the TNM staging system in the future. The ELI is a notable prognostic indicator in patients with pStage II CRC.

Keywords Elastic lamina invasion, Colorectal cancer, Serosal invasion, Survival

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Introduction

Colorectal cancer (CRC) is one of the most common cancers in the world [1]. Some CRCs are diagnosed clinically as cT4a with serosal invasion (SI). With hematoxylin–eosin (H&E) staining alone, cT4a is most often underdiagnosed pathologically as pT3without SI, i.e., invasion into subserosa or non-peritonealized pericolic/perirectal tissue, and very rarely as pT4a with high risk potential for peritoneal recurrence [2–5]. There is a large discrepancy in the diagnosis of SI [6]. The distinction between pT3 and pT4a tumors continues to be a source of diagnostic confusion [7–13]. One of the reasons for the diagnostic difficulty appears to be the diagnosis based on H&E staining alone, as noted by Lu [14]. However, according to the results of Elastica van Gieson (EVG) staining, some pT3 tumors invade the elastic lamina (EL), which extends just below the serosal layer. Therefore, some authors have reported that pT3 disease with ELI + is considered to be occult pT4a disease [3, 8, 15]. In addition, Liang [16] proposed that pT3 with ELI + should be considered pT4a. Moreover, the ELI + has been recently described as a poor prognostic factor in patients with pStage II CRC [7, 17–20]. In lung cancer, the 7th edition of the TNM staging system [21] defines ELI as positive pleural invasion. Several studies have reported that the ELI is associated with poor prognosis in lung cancer, gastric cancer [22–24], and pT3N0/N (+) CRC [7, 16–20, 25]. However, the TNM staging system 8th edition [26] has not yet defined the ELI in CRC. This may be because the clinicopathological significance of the ELI remains unknown. The pathological features of ELI were previously studied by the author (KS) and were briefly described in the research article published in 1984 [27]. On this occasion, the author reevaluated its clinicopathological significance and addressed issues that have long been questioned by surgeons. The present study improves the understanding of the ELI and highlights the prognostic differences in patients with ELI-based subclassification of pT3 tumors. The diagnostic discrepancy between gross and pathological SI (cT4a/pT4a) is also discussed from the perspective of the ELI.

Methods

Study design and inclusion/exclusion criteria

This was a retrospective cohort study. The data were collected from the computerized original database initiated by the author (KS) in 1982 [28–32]. Prospectively registered records from January 1982 to December 2013 were reviewed. A total of 3364 patients with CRC underwent surgical treatment. Of these, 569 patients with pStage II (pT3/pT4a) disease had a primary CRC and underwent curative surgery. Any cT4b/pT4b cases were not included in this study. All the tumors were located proximal to the peritoneal reflection, where the intestine is surrounded by the serosal membrane. Patients with multiple primary cancers, those who received neoadjuvant chemotherapy, and those who died in the hospital were excluded. Fourteen patients (2.5%) were lost to follow-up and were excluded from this study.

Tumor location and surgical procedures

There were 198 patients in the right colon, 295 in the left colon and 76 in the intraperitoneal rectum. In accordance with Japanese clinical practice guidelines [33], D3 lymph node (LN) *en-bloc* dissection [34] was performed in 476 patients, but D2/D1 LN dissection was performed in 93 patients depending on their preoperative health status. Prior to 1995, 545 patients underwent open surgery. Subsequently, 24 patients underwent laparoscopic surgery.

Processing of resected specimens

The processing of resected specimens has been previously reported [28–32]. Briefly, the serosal surface of fresh resected specimens was carefully observed to diagnose cT3 or cT4a tumors. The mesenteric LNs were harvested immediately after surgery. Pericolic/perirectal LNs close to the main tumor were never harvested to avoid damaging the serosal membrane and extramural adipose tissue. After formalin fixation for 1 week, the entire tumor mass, including serosa and extramural adipose tissue, was cut longitudinally along the long axis of the bowel into large step-shaped sections of 5–6 mm thickness, including the oral and anal edges of the tumor. Depending on the tumor situation, the tumor mass was cut perpendicular to the long axis of the bowel to confirm the serosal invasion. All blocks were then embedded in paraffin after alcohol dehydration. Thin 4–5 μm sections were cut from the paraffin-embedded blocks. All thin sections were routinely stained with both H&E and EVG since 1982.

Typical ELI and definition of pT3 classification

The author (KS) was responsible for the final pathological diagnosis of each patient from 1982 until 2014, when he retired. All pathological findings including the ELI were sketched in detail on an ISO B4 size paper as previously reported [32]. The elastic lamina (EL) is occasionally breached or disrupted by tumor or desmoplastic reaction, but can be traced by identifying fragments of elastic fibers. A typical ELI is shown in Fig. 1. H&E and EVG stained micrographs of the same case were shown in a vertical row. ELI − indicates that the tumor invasion does not exceed the EL, ELI + indicates that the tumor invasion exceeds the EL, and EL − indicates that the EL is unidentified. pT3 was sub-classified into three groups: pT3ELI − was defined as pT3a (Fig. 1a, EVG), pT3ELI + as

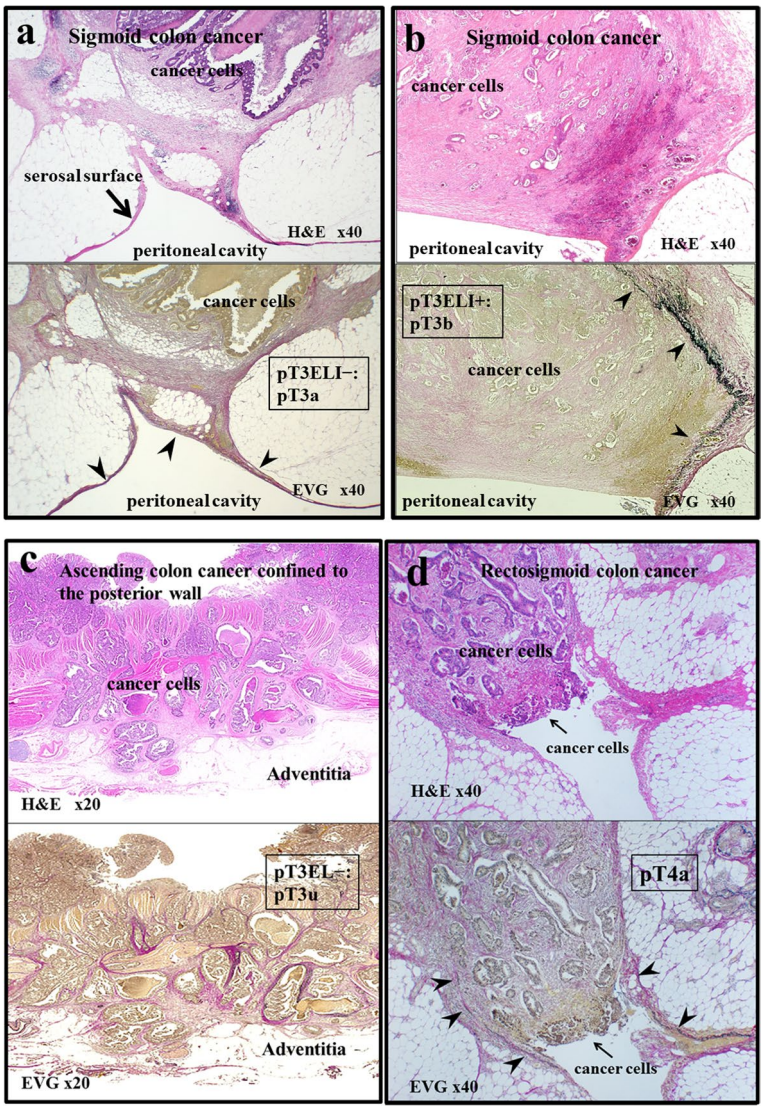

Fig. 1 Typical Elastic Lamina Invasion and Definition of pT3 Classification. H&E and EVG stained micrographs of the same case were shown in a vertical row. Arrow heads: elastic lamina (EL); ELI − : negative elastic lamina invasion; ELI + : positive elastic lamina invasion; EL − : unidentified elastic lamina. **a** Sigmoid colon cancer; Cancer cells do not invade the EL, i.e. pT3ELI − is defined as pT3a. **b** Sigmoid colon cancer; Cancer cells invade beyond the EL, i.e. pT3ELI + is defined as pT3b. **c** Ascending colon cancer confined to the posterior wall; EL is not identified in the adipose tissue of the adventitia, i.e. pT3EL − is defined as pT3u. **d** Rectosigmoid colon cancer: Cancer cells are exposed to the serosal surface and peritoneal cavity, defined as pT4a

pT3b (Fig. 1b, EVG), and pT3EL − as pT3u (Fig. 1c, EVG). pT4a was defined as cancer cells exposed to the serosal surface or in the peritoneal cavity (Fig. 1d, H&E and EVG), according to the TNM staging system 8th edition [26].

Criteria of histologic differentiation, venous invasion, lymphatic invasion, perineural invasion and tumor budding

Histologic differentiation was briefly described according to Japanese classification of colorectal carcinoma [35]. Well differentiated adenocarcinoma was characterized by distinct and large gland formation and moderately differentiated adenocarcinoma was composed of medium to small glands, which is intermediate between well and poorly differentiated adenocarcinoma. Poorly differentiated adenocarcinoma was characterized as cancers with indistinct gland formation. Mucinous adenocarcinoma was characterized by cancer cells that produce substantial amount of mucus outside the cells forming mucus nodules or lakes. The criteria of venous invasion, lymphatic invasion, perineural invasion, and budding

were previously reported. Briefly, venous invasion was assessed by EVG staining and was defined as cancer cells within veins with an unequivocal elastic fiber [28]. Lymphatic invasion was defined as cancer cells floating within a lymphatic endothelial-lined lumen, distinct from those within the space due to the tissue shrinkage artifact. [29]. Perineural invasion was defined as cancer cells invading the perineural space of extramural nerve bundles or cancer cells invading Auerbach's plexus [30]. The budding was defined as isolated undifferentiated cancer cells at the tumor invasive front. The term "budding" was coined by the author K.S. in the 1980s, and reported by his colleague Morodomi in 1989 [36]. This nomenclature was introduced to the world by Western researchers in 2012 and 2016 [37, 38].

In addition, pN0 cases with tumor deposits are currently defined as pN1, i.e. pStage III. Therefore, these cases were not included in this study.

Postoperative adjuvant chemotherapy

Postoperative adjuvant chemotherapy with oral 5-Fu-based regimens was administered to 240 patients (42.2%).

Follow-up examination

The date of the outpatient visit was recorded as the date of survival confirmation. The routine program included serum tumor marker measurements, chest radiography and abdominal ultrasound every three months for the first three years, every six months for the next two years and annually thereafter. When recurrence was suspected, a definitive diagnosis was made using computed tomography and/or magnetic resonance imaging. Local recurrence was defined as tumors occurring in the peritoneal cavity or pelvis within the field of the initial surgery. Distant metastases were defined as the tumors observed in the liver, lung, or other hematogenous organs. Other recurrences included peritoneal dissemination or intra/extra-abdominal LN metastases. Follow-up began in 1983 and was performed annually [28–32]. The author (KS) was involved in the follow-up work until the end of September 2016.

Statistical analysis

Statistical analyses were performed using StatView software (version 5.0 J; SAS Institute, Inc. Cary NC, USA). Pearson's chi-square (χ^2) test and logistic regression analyses were performed to estimate correlations and the risk of recurrence for categorical variables. Univariate and multivariate Cox regression analyses were performed to identify independent prognostic factors. The Kaplan–Meier method was used to calculate time-dependent survival probabilities. The log-rank (LR) test was performed to estimate the differences in survival. All the statistical tests were two-tailed, and $p < 0.05$ was considered to indicate statistical significance, with confidence intervals (CIs) of 95% for hazard ratios (HRs) and odds ratios (ORs).

Survival analyses

Disease-free survival (DFS) was defined as the time from the date of surgery to the date of the first confirmed tumor recurrence or CRC-related death. Overall survival (OS) was defined as the time from the date of surgery to the date of death from any cause. All deaths were considered events.

Results

Identification of EL and ELI

A total of 4910 block sections were obtained from the resected specimens of 569 patients (mean 8.6, standard deviation [SD] 3.2; range 4–20, median 8.0). EL was identified in 91.4% (520/569), and was unidentified in 8.6% (49/569) (Tables 1a, b). EL was well identified in sigmoid colon (S) and rectosigmoid colon (Rs) cancers. EL was not identified in 8–9% of cecum (C) and transverse colon (T) cancers, and was difficult to identify in 11–15% of ascending colon (A), descending colon (D), and intraperitoneal rectal (Ra) cancers (Table 1a). The combined categories A + D + Ra and C + T + S + Rs were significantly correlated with the frequency of EL identification ($p = 0.014$). In addition, EL was not identified in a significantly greater percentage (34.7%) of tumors < 3 cm in size ($p < 0.0001$, Table 1b). Regarding the circumferential distribution of A, D and Ra cancers, the unidentified rate was significantly higher for the posterior wall tumors (44.4%) than for other circumferential distributions ($p < 0.0001$, Table 1c). As shown in Table 1d, ELI + was detected in 46.7% (254/544) of the pT3 cases and in 100% (25/25) of all pT4a cases. ELI − was detected in 44.3% (241/544) of the pT3 cases. A significant correlation was found between cT and pT categories ($p < 0.0001$).

Gross and pathological findings of resected specimens

Fresh resected specimens are shown in Fig. 2a-c. H&E and EVG stained micrographs of the same case were shown in a vertical row. Gross findings of the serosa were unequivocal cT4a. As previously reported [13], the pathological diagnoses by H&E staining were pT3, pT3, and pT4a, respectively (Fig. 2d-f), whereas the diagnoses by EVG staining were pT3a/ELI −, pT3b/ELI +, and pT4a/ELI +, respectively (Fig. 2j-l). In the cases in Fig. 2a and b, the gross and pathological diagnoses were not concordant, but in the case of Fig. 2c, the diagnoses were compatible. This study included 356 cases with cT4a (Table 1d). Of these, 334 cases were diagnosed as pT3 (93.8%) and 22 cases (6.2%) were diagnosed as pT4a by H&E staining

Table 1 Identification of Elastic Lamina (EL) and Elastic Lamina Invasion (ELI)

a. EL and Tumor Location

EL	Tumor Location								
	C	A	T	D	S	Rs	Ra	*p*-value	Total
Identified	29	87	62	32	157	88	65	0.014*	520 (91.4)
Unidentified (%)	3 (9.4)	11 (11.2)	6 (8.8)	4 (11.1)	9 (5.4)	5 (5.4)	11 (14.5)		49 (8.6)

C cecum, *A* ascending colon, *T* transverse colon, *D* descending colon, *S* sigmoid colon, *Rs* recto-sigmoid colon, *Ra* intraperitoneal rectum above the peritoneal reflection; *A/D/Ra vs. C/T/S/Rs: Chi-square test showed a significant correlation between tumor location and EL

b. EL and Tumor Size

EL	Tumor size					
	< 3 cm	3-5 cm	≥ 5 cm	Unknown	*p*-value	Total
Identified	32	192	293	3	< 0.0001*	520 (91.4)
Unidentified (%)	17 (34.7)	27 (12.3)	5 (1.7)	0		49 (8.6)

*Chi-square test showed a significant correlation between tumor size and EL

c. EL and Circumferential Distribution at A, D, and Ra

EL	Circumferential Distribution						
	Posterior	Anterior	Lateral	Whole circumference	Unknown	*p*-value	Total
Identified	20	61	20	82	1	< 0.0001*	184 (87.6)
Unidentified (%)	16 (44.4)	4 (6.2)	4 (16.7)	2 (2.4)	0		26 (12.4)

A ascending colon, *D* descending colon, *Ra* intraperitoneal rectum above the peritoneal reflection
*Chi-square test showed a significant correlation between circumferential distribution and EL

d. ELI and cT

cT	pT3, *n* = 544, (%)			pT4a, *n* = 25, (%)	*p*-value	Total
	pT3a [ELI −]	pT3u [EL −]	pT3b [ELI +]	[ELI +]		
cT3	128 (60.1)	44 (20.7)	38 (17.8)	3 (2.8)	< 0.0001*	213
cT4a	113 (31.7)	5 (1.4)	216 (60.7)	22 (6.2)		356
Total	241	49	254	25		569

*Chi-square test showed a significant correlation between cT and pT categories

alone. There was a large discrepancy between the gross and pathological diagnoses of SI. Based on the ELI status, 113 cases (31.7%) were diagnosed as pT3a and 216 cases (60.7%) were diagnosed as pT3b.

Causes of death during long-term follow-up

The last survey for surviving patients was completed at the end of September 2016. Eligible surviving and censored patients were followed up for a mean of 127 months (range, 2–351, SD 73, median 116 months). Of these patients, 6 (2.0%) were followed up for less than 3 years, 21 (7.0%) for 3–5 years, 109 (36.1%) for 5–10 years, 135 (44.7%) for 10–20 years, and 31 (10.3%) for more than 20 years. A total of 134 patients had died of malignant disease by the end of September 2016. Of these, 72 patients (52.9%) died of primary CRC. The remaining 62 patients died mainly from second primary malignancies of the liver, stomach, or lung. Regarding non-malignant diseases, 133 patients died mainly from senility/dementia, cardiovascular, cerebrovascular or pulmonary diseases.

Postoperative recurrence

Postoperative recurrence occurred in 80 (14.1%) of the 569 patients (Table 2a). Recurrence and the pT category were significantly correlated ($p < 0.0001$, χ^2 test). The rate of recurrence did not differ between pT3a and pT3u (4.6% vs. 6.1%, respectively) and between pT3b and pT4a (23.2% vs. 28.0%, respectively). Cumulative recurrence rates of 5- and 10-year did not differ between pT3a and pT3u and between pT3b and pT4a ($p = 0.552$, $p = 0.580$, respectively). Logistic regression analysis revealed significantly greater ORs for pT3b and pT4a than for pT3a and pT3u ($p = 0.0001$, OR = 6.3, 95%CI = 3.2–12.4 and $p = 0.0001$, OR = 8.1, 95%CI = 2.8–23.5, respectively). Among these, recurrence sites were found in 105 lesions

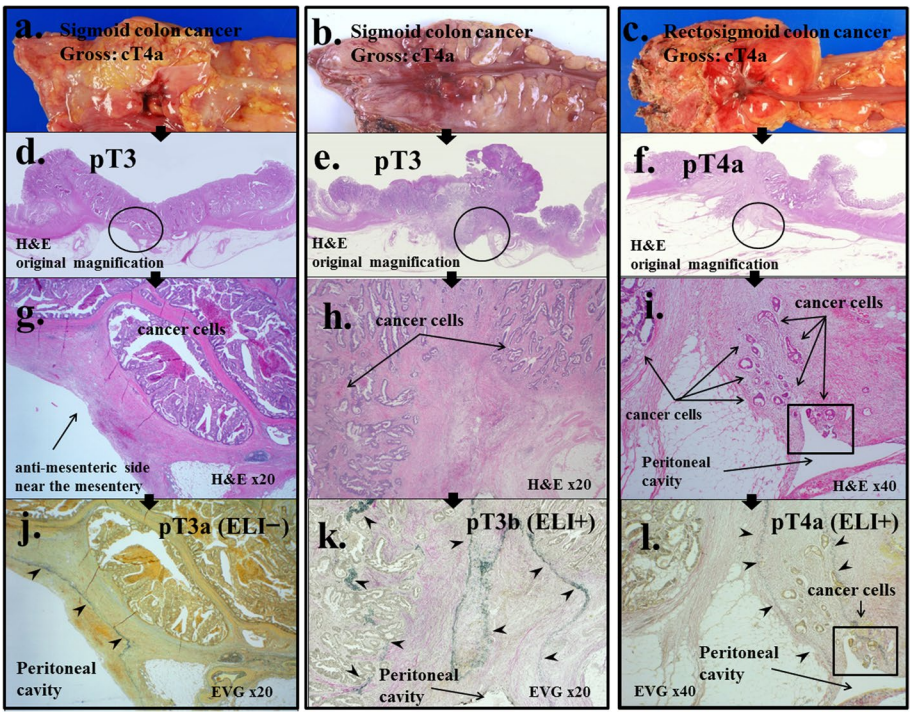

Fig. 2 Differences between Gross and Pathological Diagnoses. H&E and EVG stained micrographs of the same case were shown in a vertical row. Arrow heads: elastic lamina (EL); **a**) **b**) **c**) Fresh resected specimens; gross diagnosis of serosal invasion was cT4a. **d**) **e**) **f**) H&E staining of cT4a area at low-power view. **g**) **h**) **i**) H&E staining of the black circle areas at high-power view. **d**) **e**) **g**) **h**) The pathological diagnosis was pT3 by H&E staining, with a discrepancy between the gross and pathological diagnoses of serosal invasion. **i**) Several cancer cells in the rectangle area are exposed to the serosal surface and peritoneal cavity. The pathological diagnosis was pT4a by H&E staining alone, which was compatible with the gross diagnosis. **j**) **k) l**) EVG staining of the H&E staining areas; j) Cancer cells do not invade the EL, i.e. pT3a (ELI −), k) Fragmented ELs are seen on the left. Cancer cells invade beyond the ELs, i.e. pT3b (ELI +), l) Cancer cells invade beyond the EL and several cancer cells in the rectangle area are exposed to the serosal surface and peritoneal cavity, i.e. pT4a (ELI +)

including the liver and lung, followed by the peritoneum, local sites and others (Table 2b). To avoid statistical bias caused by the diversity of the sites, associations between the site of recurrence and the pT category were not analyzed. Distant metastasis was found at a high rate in patients in each ELI-based pT category, but the correlation was not significant ($p=0.3901$; Table 2c). However, peritoneal recurrence occurred significantly more often in patients with pT3b and pT4a (26.1% and 31.8%, respectively) than in those with pT3a and pT3u (0% and 0%, respectively, $p=0.0206$).

Survival results

The 10-year DFS did not differ between pT3a and pT3u patients (95.6% vs. 93.8%, respectively; $p=0.5523$; Fig. 3a). The 10-year OS also did not differ between the two categories (70.6% vs. 64.1%, respectively; $p=0.9429$, Fig. 3b). However, as shown in Fig. 3c, patients with pT3b had a lower 10-year DFS than those with pT3a (75.6% vs. 95.6%, respectively; $p<0.0001$) but were similar to those with pT4a (75.6% vs. 70.6%, respectively; $p=0.5799$). They also had a lower OS than those with pT3a (58.4% vs. 70.6%, respectively; $p=0.0024$, Fig. 3d), but were similar to those with pT4a (58.4% vs. 52.0%, respectively; $p=0.1116$).

Univariate and multivariate analyses

Univariate analysis indicated that tumor budding, perineural invasion, and the ELI were significant risk factors for postoperative recurrence (Table 3a). After multivariate analysis, the ELI was found to be the strongest independent risk factor ($p<0.0001$, HR = 4.89, 95%CI = 2.718–8.819). Similarly, these factors were found to be significant predictors of CRC-specific death (Table 3b), and on multivariate analysis, the ELI was the strongest independent predictor ($p<0.0001$, HR = 6.26, 95%CI = 3.171–12.347).

Discussion

This retrospective cohort study clarified clinical and pathological issues, i.e., diagnostic discrepancy between cT4a and pT4a tumors, and demonstrated the close relationship between the ELI and prognosis in patients with pStage II CRC.

Table 2 Postoperative recurrence

a. Recurrence in each pT Category

pT	Recurrence		Cumulative Recurrence Rate				Logistic Regression Analysis	
	Presence n, (%)	Absence n, (%)	Total	5-year n*, (%)	10-year n*, (%)	*p*-value	OR (95%CI)	*p*-value
pT3a (EL −)	11 (4.6)	230 (95.4)	241	6 (2.7)	9 (4.4)	0.552	1 (reference)	-
pT3u (EI −)	3 (6.1)	46 (93.9)	49	3 (6.2)	3 (6.2)		1.4 (0.37–5.1)	0.6440
pT3b (ELI +)	59 (23.2)	195 (76.8)	254	53 (21.8)	58 (24.4)	0.580	6.3 (3.2–12.4)	0.0001
pT4a (ELI +)	7 (28.0)	18 (72.0)	25	7 (29.4)	7 (29.4)		8.1 (2.8–23.5)	0.0001
Total	80 (14.1)	489 (85.9)	569					

ELI − negative elastic lamina invasion, *EL* − unidentified elastic lamina, *ELI* + positive elastic lamina invasion, *OR* Odds Ratio; Recurrence and pT category were significantly correlated ($p < 0.0001$, χ^2 test). The rate of recurrence did not differ between pT3a and pT3u and between pT3b and pT4a. Cumulative recurrence rate did not differ between pT3a and pT3u and between pT3b and pT4a. ORs were significantly higher for 3b and pT4a than for pT3a and pT3u. *cumulative number of recurrence

b. Recurrence Sites, including Multiple Sites of Recurrence

pT	Liver	Lung	Peritoneum	Local	Bone	Others	Total
pT3a (ELI −), (%)	4 (36.4)	4 (36.4)	0 (0)	2 (18.2)	0	1 (0.9)	11
pT3u (EI −), (%)	3 (100)	0	0 (0)	0	0	0	3
pT3b (ELI +), (%)	29 (42.0)	10 (14.5)	18 (26.1)	9 (13.0)	2 (2.9)	1 (1.4)	69
pT4a (ELI +), (%)	6 (27.3)	5 (22.7)	7 (31.8)	3 (13.6)	1 (4.5)	0	22
Total (%)	42 (40)	19 (18.1)	25 (23.8)	14 (13.3)	3 (2.9)	2 (1.9)	105

ELI − negative elastic lamina invasion, *EL* − unidentified elastic lamina, *ELI* + positive elastic lamina invasion; To avoid statistical bias caused by the diversity of the sites, associations between the site of recurrence and pT category were not analyzed

c. Distant Metastasis and Peritoneal Recurrence

pT	Distant Metastasis			Peritoneal Recurrence		
	Presence	Absence	Total	Presence	Absence	Total
pT3a (EL −), (%)	8 (72.7)	3 (27.3)	11	0 (0)	11 (100)	11
pT3u (EI −), (%)	3 (100)	0 (0)	3	0 (0)	3 (100)	3
pT3b (ELI +), (%)	41 (59.4)	28 (40.6)	69	18 (26.1)	51 (73.9)	69
pT4a (ELI +), (%)	12 (54.5)	10 (45.5)	22	7 (31.8)	15 (68.2)	22
Total (%)	64 (54.5)	41 (45.5)	105	25 (23.8)	80 (76.2)	105

ELI − negative elastic lamina invasion, *EL* − unidentified elastic lamina, *ELI* + positive elastic lamina invasion; Distant metastasis was found at a high rate in each ELI-based pT category without correlation ($p = 0.3901$, χ^2 test). Peritoneal recurrence was significantly associated with patients with pT3b and pT4a than in those with pT3a and pT3u ($p = 0.0206$, χ^2 test)

First of all, the serosal surface of fresh resected specimens should be carefully examined [8]. The serosal surface of cT4a often appears as whitish stripes/streaks, flattened indentation, or deep depression in fresh resected specimens [8, 15, 39] (Fig. 2a-c). Microscopically, desmoplastic reaction, isolated small glandular foci, and budding cells are usually observed on the tumor invasion front. Puppa [3] reported that when some advanced CRCs invade the serosa, the cap of fibrosis caused by a fibro-inflammatory reaction on the invasive front of the carcinoma is likely to replace areas of earlier peritoneal invasion. These serosal findings are confidently diagnosed as cT4a with SI by many surgeons at the time of surgery or on a fresh resected specimen, whereas the cT4a is most often underdiagnosed as pT3 without SI according to the TNM classification [26]. Many surgeons are surprised by this under-diagnosis. Several authors have noted the significant discrepancy between the gross and pathological diagnoses of SI [3, 6]. This diagnostic discrepancy may be due not only to diagnostic ambiguity based on H&E staining alone [13, 14, 40], but also to differences in interpretation of the clinical and pathological definitions of SI. The reported frequency of pT4a varies widely, ranging from 13.4% to 67.7% among 8 Japanese institutions [41] and the diagnostic difficulties are similar to those in other countries [9, 10]. On the other hand, when using EVG staining, some pT3 tumors invaded the EL (Fig. 2k). However there were differences in the

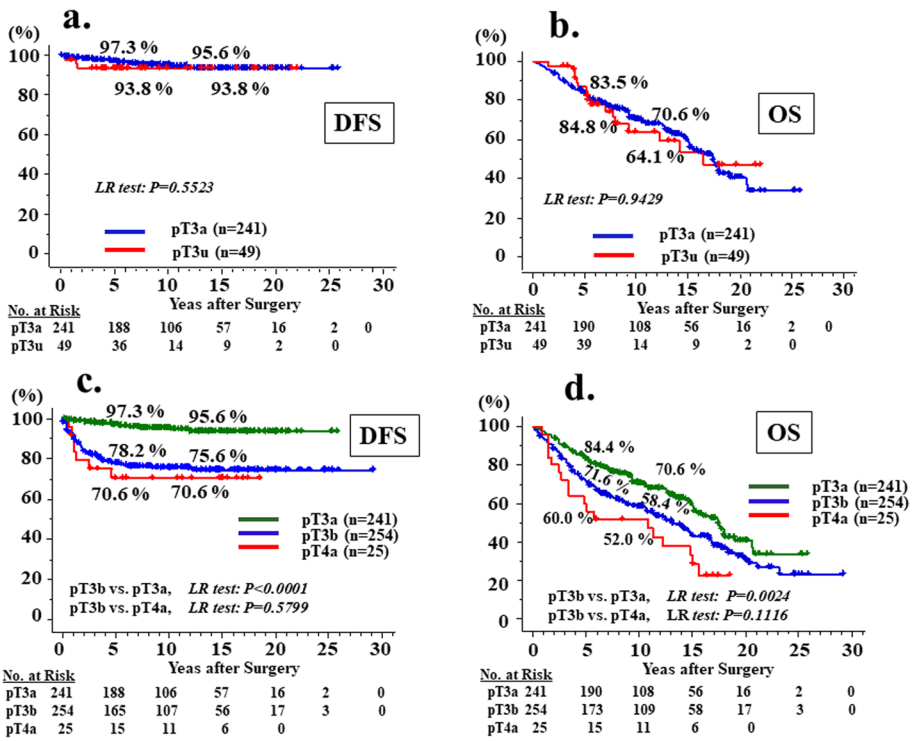

Fig. 3 Cumulative survival according to the pT3 subclassification based on the ELI. DFS: disease-free survival; OS: overall survival; LR: log-rank; **a**) **b**) There were no significant differences in DFS or OS between pT3a and pT3u patients. **c**) **d**) The DFS and OS rates of patients with pT3b disease were significantly lower than those of patients with pT3a disease, but they did not differ from those of patients with pT4a disease

EL identification according to tumor location and size (Tables 1a, b). Moreover, when some tumors are confined to the posterior wall, it may be difficult to identify the EL by longitudinal sectioning of the tumor in the ascending/descending colon cancer and intraperitoneal rectal cancer in which the posterior wall is not covered by serous membrane (Table 1c). In those cases, ELI can be assessed by sectioning perpendicular to the long axis to the bowel including serosa. If the tumor is sectioned in this manner, pT3u may be equivalent to pT3a. In addition, pT3u should be classified as pT3a based on the oncologic results, as described below. Several authors reported that EL was detected less frequently in right-sided tumors than in left-sided tumors [6, 42, 43], which is similar to the results found in our study. Therefore, the likelihood of EL involvement depends not only on the degree of extramural invasion, but also on the tumor location, tumor size, and circumferential distribution [44]. This problem is also more likely to occur when the number of tumor block sections is small, because the serosa may not be included. In the present study, a mean of 8.6 block sections were stained with both H&E and EVG. Lu [14] also reported that the rate of unidentified EL was 18.5%, with a mean of 3.2 sections with EVG staining. Liang [18] used only 1 EVG section per case and reported that the EL could not be identified in 58% of cases. These unidentified rates were higher than our results (8.6%, Tables 1a, b). In particular, small tumors < 3 cm should be carefully sectioned due to the high incidence of unidentified EL (34.7%, Table 1b). We believe that at least 4–5 block sections, including the serosal layer, are required to identify the EL.

Regarding the ELI, previous studies have reported that the frequency of pT3b tumors ranged from 16.7% to 52.6% [7, 17–20, 42]. Our results showed a similar frequency of 46.7% (254/544, Table 1d) as described above. In addition, as shown in Figs. 1b and 2k, the majority of cT4a cases were diagnosed as pT3b with ELI + (60.7%, Table 1d). When pT3 tumors closely infiltrate the serosal surface beyond the EL, even if a desmoplastic reaction occurs, there may be a moment when cancer cells are exfoliated into the peritoneal cavity [15]. Serosal cytologic smears demonstrated cancer cells in 19–26% of pT3 tumors [44, 45]. In other words, pT3b may be occult pT4a [3, 8, 15]. Liang [16] suggested that if ELI is present, the tumor should be considered SI positive and the stage should be pT4a. We agree with his opinion. This idea could resolve the diagnostic discrepancy between gross and pathological SI. EVG staining may be the best way to eliminate this discrepancy.

Table 3 Univariate and multivariate Cox's regression analysis for independent prognostic factors

a. Postoperative Recurrence in pT3 Diseases

Independent variable		Univariate analysis HR (95%CI)	*p*-value	Multivariate analysis HR (95%CI)	*p*-value
Histologic differentiation	well others	1 (reference) 1.42 (0.879–2.292)	0.1518	–	–
Lymphatic invasion	no yes	1 (reference) 1.79 (0.986–3.272)	0.0555	–	–
Venous invasion	no yes	1 (reference) 1.53 (0.617–3.798)	0.3583	–	–
Tumor budding	no yes	1 (reference) 1.77 (1.026–3.041)	0.0400	1 (reference) 1.44 (0.834–2.483)	0.1906
Perineural invasion	no yes	1 (reference) 4.60 (1.995–10.618)	0.0003	1 (reference) 3.06 (1.320–7.102)	0.0091
Postoperative adjuvant chemotherapy	no yes	1 (reference) 1.0 (0.626–1.602)	0.9959	–	–
ELI	negative positive	1 (reference) 5.31 (2.967–9.517)	< 0.0001	1 (reference) 4.89 (2.718–8.819)	< 0.0001

well well differentiated adenocarcinoma, *others* moderately or poorly differentiated adenocarcinoma or mucinous adenocarcinoma, *ELI* elastic lamina invasion, *HR* hazard ratio, *CI* confidence interval; Multivariate analysis identified ELI as the strongest independent risk factor for postoperative recurrence

b. CRC-specific Death in pT3 Diseases

Independent variable		Univariate analysis HR (95%CI)	*p*-value	Multivariate analysis HR (95%CI)	*p*-value
Histologic differentiation	well others	1 (reference) 1.40 (0.845–2.338)	0.1891	–	–
Lymphatic invasion	no yes	1 (reference) 1.86 (0.992–3.474)	0.0529	–	–
Venous invasion	no yes	1 (reference) 1.84 (0.740–4.596)	0.1888	–	–
Tumor budding	no yes	1 (reference) 1.84 (1.047–3.240)	0.0341	1 (reference) 1.49 (0.845–2.625)	0.1678
Perineural invasion	no yes	1 (reference) 4.74 (2.043–10.974)	0.0003	1 (reference) 1.58 (1.305–7.079)	0.0100
Postoperative adjuvant chemotherapy	no yes	1 (reference) 0.77 (0.464–1.277)	0.3119	–	–
ELI	negative positive	1 (reference) 6.85 (3.489–13.429)	< 0.0001	1 (reference) 6.26 (3.171–12.347)	< 0.0001

CRC colorectal cancer, *well* well differentiated adenocarcinoma, *others* moderately or poorly differentiated adenocarcinoma or mucinous adenocarcinoma, *ELI* elastic lamina invasion, *HR* hazard ratio, *CI* confidence interval; Multivariate analysis identified ELI as the strongest independent predictor for CRC-specific death

The oncological significance of the ELI has been reported to be closely associated with prognosis in lung or gastric cancer [22–24]. Several studies have also reported that ELI is a poor prognostic factor for pT3 CRC [7, 16–20, 25]. Conversely, Grin [42] reported that ELI was not a negative prognostic factor in pStage II CRC. The small number of cases may also have contributed to this difference. Liang [18] noted that some previous studies included pStage II and III disease, which did not show uniformity and introduced bias [7, 14, 16, 17, 19]. The present study specifically focused on patients with pStage II CRC to avoid any bias caused by differences in pStages [18, 42]. In addition, Japanese D3 LN *en-bloc* dissection [34], which is equivalent to complete mesocolic or total mesorectal excision [46, 47], appears to improve prognostic outcomes in patients with pT3a and pT3u tumors. However, patients with pT3b and pT4a had a higher risk of recurrence than those with pT3a or pT3u ($p=0.0001$, OR = 6.3 and $p=0.0001$, OR = 8.1, respectively; Table 2a). Cumulative recurrence rates appear to be similar between pT3a and pT3u and between pT3b and pT4a. ELI + had a strong effect on postoperative recurrence [7, 17, 18]. The top four sites of recurrence were the liver, lung, peritoneum, and local site (Table 2b), similar to the finding of other studies [7, 17, 19]. However, there are few reports on the association between distant metastasis and ELI [7, 17, 48]. Shinto [17] and Kojima [7] reported that distant metastasis occurred more frequently in the

pT3b and pT4a groups than in the pT3a group. El-Aziz [48] also reported that distant metastasis rates differed significantly according to ELI status. However, in the present study, distant metastasis and the ELI-based pT category were not associated ($p=0.3901$, Table 2c). On the other hand, pT4a, i.e., pathological SI, is known to be a marker for predicting peritoneal recurrence. [3–5]. It is easy to understand that pT4a is prone to peritoneal dissemination, because cancer cells are exposed to the serosal surface and/or exfoliated into the peritoneal cavity. To our knowledge, there are few reports on the association between the ELI and peritoneal dissemination. The present study showed that peritoneal recurrence was more strongly associated with pT3b and pT4a than with pT3a and pT3u ($p=0.0206$, Table 2c), similar to the results of other studies [7, 20]. We can better understand the idea that pT3b may be occult pT4a [3, 8, 15].

In addition to the effect of D3 dissection, both pT3a and pT3u tumors appear to be low malignant, and the prognosis appears to be favorable [14, 18] (Fig. 3a, b). Conversely, the malignant potential of pT3b is considered to be much higher than that of pT3a/pT3u and very similar to that of pT4a [7, 17–20] (Fig. 3c, d). pT3b tumors appear to be a heterogeneous entity of pT3. Therefore, patients with pT3b disease should be upgraded to pT4a disease and intensive adjuvant chemotherapy is recommended. However, some authors [42, 49] have found no prognostic significance between pT3a and pT3b in terms of DFS or OS, which differed from the results of our study (Fig. 3c and d).

According to univariate and multivariate analyses, the ELI was found to have a strong effect on recurrence and CRC-specific death in patients with pT3 disease (Tables 3a, b). Other studies have shown that the ELI is an independent prognostic factor for postoperative survival [17] and is an independent risk factor for recurrence [7]. The ELI appears to be a better prognostic indicator than traditional prognostic factors such as venous [28], lymphatic [29], and perineural invasion [30]. In patients with pStage II disease, the ELI is very useful for identifying high-risk individuals, and confirming the diagnosis of SI. EVG staining is needed for identifying the ELI.

Conclusions

In conclusion, a better understanding of the ELI allows us to reconsider the diagnostic discrepancy of serosal invasion, i.e., pT3b should be considered pT4a. The ELI-based subclassification of pT3 is expected to be incorporated into the TNM staging system in the future. The ELI is a notable prognostic indicator in patients with pStage II CRC.

Abbreviations

CRC Colorectal cancer
EL Elastic lamina
ELI Elastic lamina invasion
H&E Hematoxylin- eosin
EVG Elastica van Gieson
DFS Disease-free survival
OS Overall survival
SI Serosal invasion
LN Lymph node
5-Fu 5-Fluorouracil
LR Log-rank
CI Confidence interval
HR Hazard ratio
OR Odds ratio
SD Standard deviation

Acknowledgements

The present study was supported by colorectal surgeons. They were involved in the surgery, long-term follow-up, and processing of resected specimens.

Authors' contributions

K.S. was responsible for obtaining and analyzing data, drafting manuscripts, and making critical revisions. K.S. and T.H. contributed to the pathological analyses and evaluations. F.F. participated in the drafting of the manuscript and development of study concept and design. T.H., T.Y. and K.K. were responsible for patient follow-up and related the collection of clinicopathological information. All authors contributed to the critical review of this manuscript and approved its submission.

Funding

The authors have not received any external funding.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

In this study, retrospective anonymized clinicopathological data collected from our computer were used. This study was exempted from the need for informed consent and approved by the Ethics Committee of the Kurume University School of Medicine (Approval No 21187).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 14 August 2024 Accepted: 6 October 2024
Published online: 14 October 2024

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN Estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424. https://doi.org/10.3322/caac.21492.
2. Lennon AM, Mulcahy HE, Hyland JMP, et al. Peritoneal involvement in stage II colon cancer. Am J Clin Pathol. 2003;119:108–13. https://doi.org/10.1309/J6BD-TWM2-M792-TN2V.
3. Puppa G, Shepherd NA, Sheahan K, et al. Peritoneal elastic lamina invasion in colorectal cancer: the answer to a controversial area of pathology?

Am J Surg Pathol. 2011;35:465–8. https://doi.org/10.1097/PAS.0b013e31820ac84a.
4. Shepherd NA, Baxter KJ, Love SB. The prognostic importance of peritoneal involvement in colonic cancer: a prospective evaluation. Gastroenterology. 1997;112:1096–102. https://doi.org/10.1016/s0016-5085(97)70119-7.
5. Kyang LS, Valle SJ, Alzahrani NA, et al. Prevention of peritoneal recurrence in high-risk colorectal cancer and evidence of T4 status as a potential risk factor. ANZ J Surg. 2018;88:975–81. https://doi.org/10.1111/ans.14428.
6. Compton CC. Colorectal carcinoma: diagnostic, prognostic, and molecular features. Mod Pathol. 2003;16:376–88. https://doi.org/10.1097/01.MP.0000062859.46942.93.
7. Kojima M, Nakajima K, Ishii G, et al. Peritoneal elastic invasion of colorectal cancer. The diagnostic utility and clinicopathological relationship. Am J Surg Pathol. 2010;34:1351–60. https://doi.org/10.1097/PAS.0b013e3181ecfe98.
8. Maguire A, Sheahan K. Controversies in the pathological assessment of colorectal cancer. World J Gastroenterl. 2014;20:9850–61. https://doi.org/10.3748/wjg.v20.i29.9850.
9. Klaver CEL, Bulkmans N, Drillenburg P, et al. Interobserver, intraobserver, and interlaboratory variability in reporting pT4a colon cancer. Virchows Arch. 2020;476:219–30. https://doi.org/10.1007/s00428-019-02663-0.
10. Naso JR, Yang HM, Scheffer DF. Variability in synoptic reporting of colorectal cancer pT4a category and lymphovascular invasion. Arch Pathol Lab Med. 2021;145:343–51. https://doi.org/10.5858/arpa.2020-0124-OA.
11. Karamchandani DM, Chetty R, King TS, et al. Challenges with colorectal cancer staging: results of an international study. Mod Pathol. 2020;33:153–63. https://doi.org/10.1038/s41379-019-0344-3.
12. Vasques RP, Arslan ME, Lee H, et al. T3 versus T4a staging challenges in deeply invasive colonic adenocarcinomas and correlation with clinical outcomes. Mod Pathol. 2021;34:131–40. https://doi.org/10.1038/s41379-020-0622-0.
13. Panarelli NC, Hammer STG, Lin J, et al. Reproducibility of AJCC criteria for classifying deeply invasive colon cancers is suboptimal for consistent cancer staging. Am J Surg Pathol. 2020;44:1381–8. https://doi.org/10.1097/PAS.0000000000001510.
14. Lu J, Hu X, Meng Y, et al. The prognosis significance and application value of peritoneal elastic lamina invasion in colon cancer. PloSONE. 2018;13(4):e0194804. https://doi.org/10.1371/journal.pone.0194804.
15. Ludeman L, Shepherd HA. Serosal involvement in gastrointestinal cancer: its assessment and significance. Histopathology. 2005;47:123–31. https://doi.org/10.1111/j.1365-2559.2005.02189.x.
16. Liang WY, Wang YC, Hsu CY, et al. Staging of colorectal cancers based on elastic lamina invasion. Human Pathol. 2019;85:44–9. https://doi.org/10.1016/j.humpath.2018.10.019.
17. Shinto E, Ueno H, Hashiguchi Y, et al. The subserosal elastic lamina: an anatomic landmark for stratifying pT3 colorectal cancer. Dis Colon Rectum. 2004;47:467–73. https://doi.org/10.1007/s10350-003-0083-9.
18. Liang WY, Chan WC, Hsu CY, et al. Retrospective evaluation of elastic stain in the assessment of serosal invasion of pT3N0 colorectal cancers. Am J Surg Pathol. 2013;37:1565–70. https://doi.org/10.1097/PAS.0b013e31828ea2de.
19. Yokota M, Kojima M, Noumra S, et al. Clinical impact of elastic laminal invasion in colon cancer: Laminal invasion-positive stage II colon cancer is a high-risk equivalent to stage III. Dis Colon Rectum. 2014;57:830–8. https://doi.org/10.1097/DCR.0000000000000124.
20. Nakanishi Y, LeVea C, Dibaj S, et al. Reappraisal of serosal invasion in patients with T3 colorectal cancer by elastic stain. Arch Patho Lab Med. 2016;140:81–5. https://doi.org/10.5858/arpa.2014-0647-OA.
21. Sobin LH, Gospodarowicz MK, Wittekind C. *TNM Classification of Malignant Tumours*. 7th ed. Wiley-Blackwell: New York; 2009.
22. Tian D, Pei Y, Zheng Q, Zhan J, Li S, Wang X, et al. Effect of visceral pleural invasion on the prognosis of patients with lymph node negative non-small cell lung cancer. Thoracic Cancer. 2017;8:97–105. https://doi.org/10.1111/1759-7714.12412.
23. Yang H, Mei T. Prognostic significance of visceral pleural invasion in patients with surgically resected small-cell lung cancer: a population-based study. Jpn J Clin Oncol. 2022;52:1045–55. https://doi.org/10.1093/jjco/hyac062.
24. Lei G, Yang H, Hong T, et al. Elastic staining – a rejuvenated method to reassess prognosis and serosal invasion in patients with pT3N0M0 gastric cancer. Hum Pathol. 2017;65:79–84. https://doi.org/10.1016/j.humpath.2017.04.023.
25. Bi F, Li X, Zhang Y, et al. Prognostic value of elastic lamina staining in patients with stage III colon cancer. World J Surg Oncol. 2022;20:391–401. https://doi.org/10.1186/s12957-022-02865-y.
26. Jessup JM, Goldberg RM, Asare EA, et al. Colon and rectum. In: Amin M, editor., et al., AJCC Cancer Staging Manual. 8th ed. Chicag: Springer Nature; 2017. p. 251–74.
27. Shirouzu K. Clinicopathological studies on extramural local invasion and metastasis of advanced colorectal carcinomas. J Kurume Med Assoc. 1984;47:622–646. https://mol-medicalonline-jp.ejku.idm.oclc.org/library/journal/download?GoodsID=dm4kurum/1984/004706/002&name=0622-0646j&UserID=54.79.77.42&base=jamas_pdf (In Japanese, Abstract in English).
28. Shirouzu K, Isomoto H, Kakegawa T, et al. A prospective clinicopathologic study of venous invasion in colorectal cancer. Am J Surg. 1991;162:216–22. https://doi.org/10.1016/0002-9610(91)90073-m.
29. Shirouzu K, Isomoto H, Morodomi T, et al. Carcinomatous lymphatic permeation. Prognostic significance in patients with rectal carcinoma-a long term prospective study.Cancer.1995;75:4–10. https://doi.org/10.1002/1097-0142(19950101)75:1<4::aid-cncr2820750103>3.0.co;2-q.
30. Shirouzu K, Isomoto H, Kakegawa T. Prognostic evaluation of perineural invasion in rectal cancer. Am J Surg. 1993;165:233–7. https://doi.org/10.1016/s0002-9610(05)80517-3.
31. Shirouzu K, Murakami H, Ogou S, et al. High-quality follow-up system for colorectal cancer - A computer registration and administration system - J Jpn Soc Coloproctol. 2006;59:869–873 (In Japanese, Abstract in English). https://www.jstage.jst.go.jp/article/jcoloproctology1967/59/10/59_10_869/_article/-char/ja/.
32. Shirouzu K, Ogata Y. Histopathologic tumor spread in very low rectal cancer treated with abdominoperineal resection. Dis Colon Rectum. 2009;52:1887–94. https://doi.org/10.1007/DCR.0b013e3181b1585a.
33. Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma: the 3d English Edition [Secondary Publication], Japanese Society for Cancer of the Colon and Rectum. J Anus Rectum Colon. 2019;3:175–195. https://doi.org/10.23922/jarc.2019-018.
34. Kotake K, Mizuguchi T, Moritani K, et al. Impact of D3 lymph node dissection on survival for patients with T3 and T4 colon cancer. Int J Colorectal Dis. 2014;29:847–52. https://doi.org/10.1007/s00384-014-1885-z.
35. Japanese Society for Cancer of the Colon and Rectum. Japanese classification of colorectal carcinoma. 1st English ed. Kanehira: Tokyo, Japan, 1997. http://www.jsccr.jp/kiyaku/index_kako_en.html.
36. Morodomi T, Isomoto H, Shirouzu K, et al. An index for estimating the probability of lymph node metastasis in rectal cancers. Lymph node metastasis and the histopathology of actively invasive regions of cancer. Cancer;1989;63(3):539–543. https://doi.org/10.1002/1097-0142(19890201)63:3<539::aid-cncr2820630323>3.0.co;2-s.
37. Mitrovic B, Schaeffer DF, Riddell RH, et al. Tumor budding in colorectal carcinoma: time to take notice. Mod Pathol. 2012;25:1315–25. https://doi.org/10.1038/modpathol.2012.94.
38 Grigore AD, Jolly MK, Jia D, et al. Tumor budding: The name is EMT. Partial EMT J Clin Med. 2016;5(5):51. https://doi.org/10.3390/jcm5050051.
39. Stewart CJR, Hillery S, Platell C, et al. Assessment of serosal invasion and criteria for the classification of pathological (p) T4 staging in colorectal carcinoma: confusions, controversies and criticisms. Cancers. 2011;3:164–81. https://doi.org/10.3390/cancers3010164.
40. Zwanenburg ES, Wisselink DD, Klaver CEL, et al. The measured distance between tumor cells and the peritoneal surface predicts the risk of peritoneal metastases and offers an objective means to differentiate between pT3 and pT4a colon cancer. Mod Pathol. 2022;35:1991–2001. https://doi.org/10.1038/s41379-022-01154-z.
41. Kojima M, Shimazaki H, Iwaya K, et al. Practical utility and objectivity: Does evaluation of peritoneal elastic invasion in colorectal cancer overcome these contrary problems? Letters to the editor. Am J Sug Pathol. 2014;38:145. https://doi.org/10.1097/PAS.0000000000000121.
42. Grin A, Messenger DE, Cook M, et al. Peritoneal elastic lamina invasion: limitations in its use as a prognostic marker in stage II colorectal cancer. Human Pathol. 2013;44:2696–705. https://doi.org/10.1016/j.humpath.2013.07.013.

43. Frankel W, Jin M. Serosal surfaces, mucin pools, and deposits, Oh my: challenges in staging colorectal carcinoma. Mod Pathol. 2015;28:S95–108. https://doi.org/10.1038/modpathol.2014.128.
44. Panarelli NC, Schreiner AM, Brandt SM, et al. Histologic features and cytologic techniques that aid pathologic stage assessment of colonic adenocarcinoma. Am J Surg Pathol. 2013;37:1252–8. https://doi.org/10.1097/PAS.0b013e3182960e7c.
45. Zeng Z, Cohen AM, Hajdu S, et al. Serosal cytologic study to determine free mesothelial penetration of intraperitoneal colon cancer. Cancer. 1992;70:737–40. https://doi.org/10.1002/1097-0142(19920815)70:4%3c737::aid-cncr2820700404%3e3.0.co;2-s.
46. Hohenberger W, Weber K, Matzel K, et al. Standardized surgery for colonic cancer: complete mesocolic excision and central ligation–technical notes and outcome. Colorectal Dis. 2009;11:354–64. https://doi.org/10.1111/j.1463-1318.2008.01735.x.
47. Heald RJ, Moran BJ. Embryology and anatomy of the rectum. Semin Surg Oncol. 1998;15:66–71. https://doi.org/10.1002/(SICI)1098-2388(199809)15:2<66::AID-SSU2>3.0.CO;2-3.
48. El-Aziz AMA, Ali HHM, Tabak SAEL-H, et al. Peritoneal elastic lamina changes and D2–40 expression in colorectal carcinoma: a histopathological and immunochemical study. J Clin Diagn Res. 2019;13: EC01-EC05. https://doi.org/10.7860/JCDR/2019/42185.13113.
49. Macchi L, Bao QR, Albertoni L, et al. Prognostic significance of additional histologic features for subclassification of pathological T3 colon cancer. Int J Clin Oncol. 2022;27:1428–38. https://doi.org/10.1007/s10147-022-02192-y.

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