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Elastic Lamina Invasion in pStage III Colorectal Cancer: It is a useful feature resolving the diagnostic discrepancies related to serosal invasion and is also a significant prognostic indicator

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Original Paper

Elastic Lamina Invasion in pStage III Colorectal Cancer: It is a useful feature resolving the diagnostic discrepancies related to serosal invasion and is also a significant prognostic indicator.

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Running head: elastic lamina invasion

Abstract

***Background*:** The significance of elastic lamina invasion (ELI) detected by Elastica van Gieson staining remains unclear in colorectal cancer (CRC). ***Objective*:** The aim is twofold: firstly, to elucidate the diagnostic discrepancies concerning the macroscopic serosal invasion defined as sT4a from the perspective of ELI, and secondly, to examine the prognosis of pT3 cases subclassified pathologically based on ELI. ***Methods*:** We extracted 515 pStage III CRCs from our database. According to the ELI status, pT3 was classified as pT3a without ELI and as pT3b with ELI. pT4a was based on the TNM classification system. ***Results*:** The majority (75%) of sT4a cases were pT3b, while 11% and 13% were pT3a and pT4a, respectively, indicating discrepancies with underdiagnosis. The 5-year recurrence-free survival (RFS) was significantly lower for pT3b than for pT3a (61.9% vs. 86.4%, $P<0.0001$), but equivalent to that for pT4a (61.9% vs. 61.4%, $P=0.9810$). Significant difference was observed concerning peritoneal recurrence ($P=0.035$, pT3a;5.9%, pT3b;13.2%, pT4a;27.3%). The efficacy of adjuvant chemotherapy was not significant in patients with pT3b or pT4a tumors ($P=0.521$, $P=0.704$, respectively). A multivariate Cox's regression analysis

demonstrated that ELI was an independent strong prognostic factor for postoperative recurrence (HR;2.13, *P*=0.0008), CRC-specific death (HR;2.49, *P*=0.0004) and Overall death (HR;1.59, *P*=0.0004). ***Conclusions*:** The identification of ELI can resolve the diagnostic discrepancies concerning serosal invasion. ELI is a strong prognostic indicator, and both pT3b and pT4a tumors are associated with a poor prognosis. Intensive adjuvant chemotherapy is recommended for patients with pT3b and pT4a tumors.

Introduction

While colorectal cancer (CRC) is the most prevalent disease, the significance of elastic lamina invasion (ELI) detected by Elastica van Gieson (EVG) staining remains ambiguous, as ELI is not widely recognized. One of the reasons is that the surgical specimens are commonly diagnosed pathologically using hematoxylin-eosin (HE) staining alone. The use of HE staining alone often results in the underdiagnosis of sT4a (surgical positive serosal invasion) as pT3 (pathological negative serosal invasion), i.e., invasion into subserosa or non-peritonealized pericolic/perirectal tissue. The sT4a is rarely diagnosed as a pathological pT4a associated with a significant risk of peritoneal recurrence [1]. Considerable disagreement exists in the diagnosis of serosal invasion. However, the EVG staining method reveals that pT3 tumors frequently invade the elastic

lamina (EL), which extends just beneath the serosal layer. In addition, some researchers have indicated that pT3 tumor accompanied by ELI is regarded as an occult pT4a tumor. [1-3]. Furthermore, the ELI was found to be associated with a poor prognostic factor in patients with pStage II/III CRC [4-10], as well as lung and gastric cancer [11-13]. In accordance with the 7th edition of the TNM staging system, the ELI is defined as positive pleural invasion [14]. Nevertheless, the 8th edition of the TNM staging system has not yet provided a definition of the ELI in CRC [15]. This may be due to the fact that the clinicopathological significance of the ELI is as yet unclear. The author (KS) published a research article in 1984 in which a summary of the ELI was introduced [16]. In addition, the clinical importance of ELI in pT3/pStage II was demonstrated by the author (KS) in 2024 [17]. The present study reevaluated the clinicopathological significance of ELI in pT3/pStage III, thereby enhancing the comprehension of ELI and demonstrating the prognostic differences in patients with ELI-based pT3 subclassification. Moreover, the discrepancy between the macroscopic and pathological diagnoses concerning serosal invasion will be discussed from the view point of ELI.

Methods

Study design and selection of patients

Clinicopathological findings were prospectively entered into a computerized database,

which was constructed by the author (KS) in 1982 [18-22]. A retrospective analysis of the prospective registration records from January 1982 to December 2013 was conducted. A total of 3,364 patients diagnosed with CRC underwent surgical intervention. For the present study, a total of 515 pStage III CRCs (IIIb: 378 cases, IIIC: 137 cases) underwent curative resection (R0) were extracted from the database. The location of all tumors was proximally situated relative to the peritoneal reflection, and they were accompanied by the serosal membrane. There were 152 patients in the right colon, 278 in the left colon and 85 in the intra-abdominal rectum. A total of 465 cases of pT3 tumors and 50 cases of pT4a tumors were analyzed. In the present study, both sT4b and pT4b cases were excluded from the analysis. Furthermore, patients with multiple primary cancers, those who had undergone neoadjuvant chemotherapy, and those who had died in the hospital were excluded from the study. Patients lost to follow-up were excluded.

Surgical procedures

A total of 453 patients underwent open surgery with extended D3 lymph node (LN) *en-bloc* dissection [23], in accordance with the Japanese clinical practice guidelines [24], while 62 patients underwent non-extended D2 lymph node dissection. The decision to employ these surgical procedures was based on the patient's preoperative general condition. Laparoscopic surgery was not performed on all patients.

Handling of surgical specimens

The handling of surgical specimens has been previously documented [18-20, 22]. Firstly, the T factor was determined by means of a close examination of the serosal surface of resected fresh specimens. Subsequently, mesenteric LNs were retrieved, while pericolic/perirectal LNs located near the tumor were not picked up in order to avoid damage to the serosa and adipose tissue surrounding the tumor. Following a week of formalin fixation, the whole tumor mass was sliced longitudinally into step-shaped blocks with a thickness of 5–6 millimeters. Segments perpendicular to long axis were obtained to ascertain the extent of serosal invasion for some tumors. Subsequently, all materials were embedded in paraffin following alcohol dehydration. Thin sections were sliced with a thickness of 4-5 μm from the paraffin-embedded materials. Since 1982, all thin sections were routinely stained with both HE and EVG methods.

Definitions of pathological features

The author (KS) participated in the pathological diagnosis and meticulously documented the findings on the designated paper [18-20, 22]. The definitions of various pathological findings were based on the Japanese classification of colorectal carcinoma [24]. Histological differentiation was classified into the following three categories: well, moderately, and poorly differentiated adenocarcinoma, based on the glandular

structures. Mucinous adenocarcinoma was defined as cancer cells with abundant mucin production. Venous invasion (VI) was defined as tumor within veins with an unequivocal elastic fiber confirmed by the method of EVG staining [18]. Lymphatic invasion (LY) was defined as floating cancer cells within an endothelial-lined lumen without definitive elastic fiber. [19]. Perineural invasion (PNI) was characterized by the presence of cancer cells within the perineural space of extramural nerve bundles [20]. Tumor deposit (TD) was defined as isolated cancerous nodule without any lymph node structures found in extramural adipose tissue. Tumor budding (BD) was defined as isolated undifferentiated cancer cells at the invasive front of the tumor. The term "budding" was coined by the author (KS) in the 1980s. The clinical significance of BD was reported by his colleague (Morodomi) in 1989 [26]. Subsequently the term was introduced to the world by Western researchers [27, 28]. The grade of VI, LY, and BD was categorized into the following grades: negative, slight, moderate, and severe. With regard to the PNI and TD, the categorization was dichotomized as negative and positive, respectively, based on the presence or absence of the cancer cells (Tables 2-3).

Serosal invasion of resected fresh specimens and pT3 subclassification based on the definition of ELI

The uppermost row of figures shows the serosal surfaces of the resected fresh

specimens of tumors in the ascending colon, intra-abdominal rectum, rectosigmoid colon and sigmoid colon, as shown in Figs. 1A-D, respectively. The macroscopic findings of the serosa are sT3 in Fig. 1A, and unequivocal sT4a in Figs. 1B-D. HE and EVG stained micrographs of the same case are shown in a vertical row. The pathological diagnoses determined by HE staining alone are pT3, pT3, pT3, and pT4a, as shown in Figs. 1E-H, respectively. Consequently, the pT subclassification was defined by the status of ELI using EVG staining as follows: pT3u was defined as EL (-) in which the elastic lamina is either absent or cannot be identified, as shown in Fig. 1I. pT3a was defined as ELI (-), as shown in Fig. 1J, whereas pT3b was defined as ELI (+), as shown in Fig. 1K. The definition of pT4a has been established as a tumor characterized by the presence of cancer cells on the serosal surface or within the abdominal cavity, as shown in Fig. 1L, according to the TNM classification system [15].

Adjuvant chemotherapy after surgery

Adjuvant chemotherapy was administered to 312 patients (60.6%) based on oral 5-fluorouracil (5Fu)-based regimens. Of these patients, 157 (50.3%) were randomly assigned to a double-blind controlled trial as part of various projects conducted by several nationwide oncology groups. The remaining patients, who were considered to be at a high risk of recurrence, received oral 5-Fu-based regimens.

Postoperative follow-up surveillance

The outpatient visit date was recorded as the confirmed date of survival. The standard program encompassed serum tumor marker, chest radiography, and abdominal ultrasound at three-month intervals for the initial three years, followed by six-month intervals for the subsequent two years, and then annually. If recurrence was suspected, a definitive diagnosis was established using computed tomography (CT) and/or magnetic resonance imaging (MRI). Distant metastases were defined as tumors that developed in the liver, lung, brain, or other organs via hematogenous spread. Local recurrence was defined as the reappearance of tumor within either the abdominal or pelvic cavity. The other recurrences were classified as peritoneal dissemination or metastasis to intra- or extra-abdominal lymph nodes. The follow-up surveys were initiated in 1983 and were conducted on an annual basis [18-22]. The surveillance was concluded at the end of September 2016.

Statistical analysis

The statistical analyses were executed using the open-source software program `R` (version 4.6.0) [29]. For the Cox`s regression statistical analysis, the code numbers assigned to character-type variables were as follows; the number 0 for low-risk categories and the number 1 for high-risk categories. For instance, the number 0 was assigned to the

category of negative/slight BD, while the number 1 was assigned to the category of moderate/severe BD. Similarly, the number 0 was assigned to the category of negative PNI, while the number 1 was assigned to the category of positive PNI. The same applies to the remaining variables. With regard to continuous-type variables, optimal cutoff values for postoperative recurrence were calculated using the '*surv-cutpoin*t' function of 'survminer' R package. The cutoff values of body mass index (BMI), age, carcinoembryonic antigen (CEA), tumor size and intraoperative blood loss were determined to be 25.3 kg/m^2, 50 years, 6.6 ng/ml, 68 mm and 350 ml, respectively. The code numbers were assigned to each category dichotomized based on a risk of recurrence, as described above. For example, the code number 0 was assigned to Age≥50, and the number 1, to Age<50. Similarly, the number 0 was assigned to CEA<25.3, while the number 1, to CEA≥25.3. The same applies to the others. Univariate and multivariate Cox`s regression analyses were then performed to identify independent prognostic factors. Pearson's chi-square ($\chi^2$) tests assessed the associations between the risk of recurrence and categorical variables. Survival curves were estimated using the Kaplan-Meier method. The R function *`coxph()`* was used to calculate the *p*-value, hazard ratios (HR), and 95% confidence intervals (CI), and $P < 0.05$ was considered statistically significant for all the statistical tests.

Definition of survival

Recurrence-free survival (RFS) was defined as the time from surgery to the first recurrence. CRC-specific survival was defined as the time from surgery to CRC-related death. Overall survival (OS) was defined as the time from surgery to death from any cause. The survival curves were created using the R *functions `survfit()`* and *`ggsurvplot()`* and the HRs were calculated using the R *functio*n *`coxph`*.

Results

Tumor location and identification of elastic lamina (EL)

The tumor location was as follows; 28 cases in the cecum (C), 68 cases in the ascending colon (A), 56 cases in the transverse colon (T), 13 cases in the descending colon (D), 158 cases in the sigmoid colon (S), 107 cases of in the rectosigmoid colon (Rs), and 85 cases in the intra-abdominal rectum (Ra). A total of 4,521 block sections (mean 8.8, standard deviation [SD] 3.6; range 4-33, median 8.0) were obtained from the main tumor mass of the resected specimens. EL was identified in 95.1% (490/515), and was unidentified in 4.9% (25/515, Table 1). Although the data table was omitted, the presence of EL was clearly identified in 96.2% of S cancers and 96.3% of Rs cancers (152/158, 103/107, respectively). However, EL was not identified in 4.4% of A cancers and 5.9% in Ra cancers (3/68 and 5/85, respectively). The identification of EL was

difficult in approximately 7% of C, T, and D cancers (2/28, 4/56, and 1/13, respectively). No significant association was observed between tumor location and EL identification (P=0.9128).

Association between serosal invasion and elastic lamina invasion (ELI)

In the case of Fig. 1A, the EL was absent or unidentified because the tumor was located at the posterior wall of the ascending colon. Consequently, the macroscopic and pathological diagnoses of the serosal invasion were consistent with pT3 (Figs. 1E and 1I). In the case of Fig. 1D, there was no discrepancy between the diagnoses even when using HE staining alone (Fig. 1H). Also, ELI was observed in all pT4a tumors, as shown in Fig. 1L. However, in the cases of Figs. 1B-C, the pathological diagnoses using HE staining alone were underestimated as pT3 (Figs 1F-G) and not compatible with sT4a. Among the cases of sT4a, those diagnosed as pT3 by HE staining were further categorized into two groups based on the status of ELI detected by EVG staining. In other words, the sT4a cases could be dichotomized into two categories, ELI (−); pT3a and ELI (+); pT3b (Figs. 1J-K). As expected, cases in which cancer cells are exposed to the abdominal cavity were classified into the ELI (+); pT4a category (Fig. 1L).

Association between surgical T-factor and pT sub-classification

Macroscopic serosal invasion of sT3 was 143 cases as sown in Table 1. Of these, 65%

(93/143) were diagnosed as pT3a and 22.4% (32/143) as pT3b. However, among the 351 cases of sT4a, 75.2% (264/351) were pT3b, and only 13.4% (47/351) were pT4a. The pathological diagnosis was found to be inconsistent with the macroscopic diagnosis, revealing a significant discrepancy between the two diagnoses, confirming the findings shown in Fig. 1.

Association between pT sub-classification and independent variables

Among the 515 cases, pT3a was detected in 165 cases (32.0%), pT3b in 300 cases (58.3%), and pT4a in 50 cases (9.7%), as shown in Table 2. Significant association was observed between the pT sub-classification and pathological factors such as histologic differentiation, lymphatic invasion, venous invasion, tumor budding, tumor deposit, and perineural invasion with *p*-values ranging from 0.018 to less than 0.001. The pT sub-classification was also found to be significantly associated with other factors, such as BMI, CEA, tumor location, and tumor size with *p*-values ranging from 0.018 to less than 0.001. Postoperative recurrence rates were also higher for pT3b and pT4a tumors (37.7% and 38.0%, respectively) than for pT3a tumors (14.5%, $P<0.001$).

Causes of death after surgery

Of the 515 patients, 135 (26.2%) died from the primary CRC. Fifty-one patients died from secondary primary malignancies including hepatoma, gastric cancer, lung cancer,

and others. The primary causes of death for 136 patients were identified as follows: senility/dementia, cardiovascular, cerebrovascular, or pulmonary diseases. The remaining 193 patients were recurrence-free at the final follow-up date. Eligible surviving and censored patients were 380 cases. They were followed up for a mean of 129 months (range, 2–366, SD 76, median 123 months). Of these patients, 40 (10.5%) were followed up for less than three years, 39 (10.3%) for three to five years, 97 (25.5%) for five to 10 years, 168 (44.2%) for 10 to 20 years, and 36 (9.5%) for more than 20 years. The follow-up rate over 5 years was 79.2%. Sixteen patients (3.1%) were lost to follow-up and were excluded from the study.

Assessment of postoperative recurrence

Postoperative recurrence was observed in 156 (30.3%) of the 515 patients (Table 3a). A significant correlation was identified between recurrence and the pT category (P<0.0001, χ^2 test). The recurrence rates for pT3b and pT4a were comparable, at 37.3% and 38.0%, respectively. Cox`s regression analysis revealed significantly greater HRs for pT3b and for pT4a compared to that for pT3a (HR=3.0, 95%CI=1.9-4.7, P<0.0001, and HR=3.0, 95%CI=1.7-5.5, P=0.0003, respectively). There was no difference in HR between the pT3b and pT4a groups. A total of 241 lesions were found to contain various recurrence sites, with the liver and lung being the most affected organs, followed by the

peritoneum, local sites, and others (Table 3b). The associations between the site of recurrence and the pT category were not analyzed due to the variety of the sites. Distant metastasis was identified at a high rate in patients in each ELI-based pT category, but a significant correlation was not found (P=0.094; Table 3c). However, peritoneal recurrence occurred at a significantly higher rate in patients with pT3b and pT4a (13.2% and 27.3%, respectively) compared to those with pT3a (5.9%, P=0.035).

Survival and postoperative adjuvant chemotherapy

As shown in Fig. 2A, the 5-year RFS was lower for patients with pT3b than for those with pT3a (61.9% vs. 86.4%, respectively; HR=3.0, 95%CI=1.94-4.68, P<0.0001) but similar to that for patients with pT4a (61.9% vs. 61.4%, respectively; HR=1.0, 95%CI=0.62-1.64, P=0.9810). As shown in Fig. 2B, patients with pT3b exhibited a lower 5-year OS compared to those with pT3a (76.4% vs. 56.1%, respectively; HR=1.6, 95%CI=1.25-2.09, P=0.0003). However, the outcomes for patients with pT3b were similar to those with pT4a (56.1% vs. 50.0%, respectively; HR=1.1, 95%CI=0.76-1.49, P=0.725). The CRC-specific survival outcomes were as follows; pT3a vs. pT3b: 89.7% vs. 65.6%, respectively (P<0.0001), and pT3b vs. pT4a: 65.6% vs. 61.9%, respectively; (P=0.5505). The survival rates were comparable to those of the RFS. Therefore, please note that the CRC-specific survival curve was regrettably omitted from this paper.

With regard to the efficacy of postoperative adjuvant chemotherapy, the prognosis for pT3a was favorable regardless of whether chemotherapy was administered (Fig. 3A). Conversely, the prognosis for either pT3b or pT4a was extremely poor, despite undergoing the chemotherapy (Figs. 3A-B).

Univariate and multivariate analyses

Univariate analysis revealed that the following factors were significant risk factors for postoperative recurrence: CEA, intraoperative blood loss, histologic differentiation, lymphatic invasion, venous invasion, tumor budding, tumor deposit, perineural invasion and ELI (Table 4a). Following multivariate analysis, tumor budding and ELI were identified as strong independent risk factors (HR=2.37 95%CI=1.59-3.52, $P<0.0001$ and HR=2.13, 95%CI=1.37-3.33, $P=0.0008$, respectively). These factors were also strong independent predictors for CRC-specific death (HR=2.83, 95%CI=1.83-4.39, $P<0.0001$ and HR=2.49, 95%CI= 1.50-4.12, $P=0.0004$, respectively, Table 4b). Furthermore, tumor budding and ELI were strong independent risk factors for Overall death (HR=1.56, 95%CI=1.21-1.99, $P=0.0005$ and HR=1.59, 95%CI=1.23-2.06, $P=0.0004$, respectively, Table 4c).

Discussion

The reliability of the present study is substantiated by the consistent pathological

diagnoses by the author (KS) and a high rate of long-term follow-up over five years (79.2%). Although there are several reports on the pathological ELI in CRC, the association with not only serosal invasion but also its prognostic significance remains unclear, as ELI is not widely recognized. The EL is distributed directly beneath the serosa of the peritoneum, visceral organs, and intra-abdominal intestinal tracts. Colorectal cancers frequently invade the EL, which is occasionally breached or disrupted by tumor invasion or desmoplastic reaction. However, the EL can be traced by identifying the fragments of elastic fibers destroyed by tumor invasion. Furthermore, the identification of EL is influenced by several factors such as anatomical tumor location, tumor size, and circumferential distribution of the tumor. For instance, in cases of descending or ascending colon cancer (Fig. 1A) and intra-abdominal rectal cancer, the identification of EL may present a challenge through longitudinal sectioning of the tumor, due to the absence of coverage of the serous membrane at the posterior wall of the retroperitoneal side [32]. In such cases, sectioning perpendicular to the longitudinal axis of the bowel increases the probability of including the serosa, thereby enabling the assessment of the EL. Consequently, EL was identified at a low rate of only 4.9% (25/515) and was shown as pT3u in Table 1. Furthermore, the oncological outcomes for pT3u were found to be equivalent to those for pT3a in the present study. Moreover, due to the limited number of

pT3u tumors, the pT3u was included in the pT3a category (n=165, Table 2). Consequently, the oncological outcomes of pT3u were regrettably omitted from the analysis. Similar results were reported by some authors in stage III CRC [9, 10]. Lu et al. also reported that pT3u may be consistent with pT3a, because no differences in lymphatic invasion, perineural invasion, or tumor budding were observed between the two categories [30]. This issue is also more likely to occur when there is a small number of sectioning materials, as the serosa might not be adequately included. In the present study, a mean of 8.8 block sections were stained with both HE and EVG methods. Lu et al. examined EL with an average of 3.2 block sections using EVG staining and reported that the proportion of unidentified EL was 18.5% [30]. Liang et al.'s examination of a single EVG section per case revealed that the EL could not be identified in 58% of cases [6]. These unidentified rates were higher than our results shown in Table1. To ensure precise identification of the EL, approximately four to five block sections are required.

With regard to the ELI, the frequency of pT3b tumors has been reported with a range from 16.7% to 52.6% [4-8, 31], which is similar to our results of 58.3% (300/515, Tables 1-2).

As the invasion progresses, a variety of features are typically observed, including desmoplastic reaction, isolated small glandular foci, budding cells, and lympho-vascular

invasion on the tumor invasion front. EL is gradually lifted towards the intestinal lumen (Fig. 1J), resulting in an indentation and puckering of the serosal surface in resected fresh specimens as shown in Fig. 1B. When the invasion extends beyond the EL, which may be accompanied by disruption of the elastic fiber, the EL is observed to be markedly elevated into the intestinal lumen. The serosal surface often exhibits whitish stripes or streaks, resulting in the formation of flattened indentations or deep depressions of the serosa [2, 3, 33], as shown in Figs. 1C-D. These findings are confidently diagnosed as sT4a macroscopically by experienced surgeons. If cancer cells are not exposed in the abdominal cavity, the pathological examination using HE staining alone frequently results in an underdiagnosis of simple pT3 in the majority of sT4a cases, as shown in Figs. 1F-G. A substantial discrepancy appears to exist between macroscopic and pathological diagnoses. As shown in Table 1, the rate of diagnostic agreement related to serosal invasion is extremely low (13.4%, 47/351). However, the use of EVG staining enables an accurate diagnosis of pT3a or pT3b rather than a simple pT3 (Figs, 1J-K). In some cases where cancer cells are exposed in the abdominal cavity, a definitive diagnosis of pT4a can be made even without the use of EVG staining (Fig. 1L), which diagnosis is compatible with sT4a. ELI appears to play a significant role related to serosal invasion. The aforementioned issues have not yet been recognized as a serious problem on a global

scale by surgeons and pathologists. As indicated in the earlier report published in 2014 [17], pathological diagnosis without consideration of ELI appears to contribute to diagnostic discrepancies related to the serosal invasion. The diagnostic difficulty is attributable to the ambiguity of diagnosis as determined by HE staining alone, as noted by Lu et al. [30]. Furthermore, variations in the interpretation of the clinical and pathological definitions related to the serosal invasion may be present.

Additionally, as shown in Tables 2-3, both pT3b and pT4a tumors invading EL strongly associated with high-grade pathological features such as lympho-vascular invasion, tumor budding, perineural invasion and others, and postoperative recurrence. Kojima et al. also reported that the tumor area beyond the EL is characterized by extensive tumor budding and desmoplastic reaction [4]. In the event of a desmoplastic reaction occurring on the tumor invasion front, there may be a moment when cancer cells were exfoliated into the abdominal cavity [3]. Some researchers have reported that serosal cytologic smears demonstrated the presence of cancer cells in 19-26% of pT3 tumors [34, 35]. These pT3 tumors appear to correspond to the pT3b tumors classified in this study. In other words, pT3b tumor may be an occult pT4a tumor [1-3]. The present study demonstrated that the prognosis for pT3b is analogous to that for pT4a (Table 3, Fig. 2). Liang et al. proposed that in the presence of ELI, the tumor should be considered serosal invasion positive and

staged as pT4a [9]. We concur with this concept entirely. Puppa et al. also reported that the cap of fibrosis, which is caused by a fibro-inflammatory reaction on the cancer invasive front near the serosa, is likely to replace areas of earlier peritoneal invasion [1]. In the case of lung or gastric cancer, the oncological significance of the ELI has been reported to be closely associated with a negative prognosis [11-13]. Several studies have also indicated that ELI is a poor prognostic indicator for pT3 CRC [4-10], while others have reported that it is not a prognostic factor for pStage II CRC [31]. This difference is likely due to the small number of cases. Furthermore, the incorporation of pStage II/III CRCs in some studies may introduce a bias due to the absence of uniformity [4, 5, 7, 9, 30]. The present study focused exclusively on pStage III CRCs in order to avoid any potential bias caused by differences in pStages [6, 31].

As previously documented in other studies [4, 5, 7], the liver, lung, peritoneum, and local site were identified as the top four sites of recurrence (Table 3b). The studies also indicated that the distant metastasis was observed at a higher frequency in the pT3b and pT4a groups compared to the pT3a group [4, 5]. Another study also demonstrated that distant metastasis rates were significantly different depending on the ELI status [36]. However, the present study found no association between distant metastasis and the ELI-based pT category (P=0.094, Table 3c). Conversely, pT4a appears to be susceptible

to peritoneal dissemination, as cancer cells are exposed to the serosal surface and/or exfoliated into the abdominal cavity. Consequently, pT4a has been recognized as a prognostic indicator for peritoneal recurrence [1, 37, 38]. However, the association between ELI and peritoneal dissemination has not yet been examined sufficiently. In consistent with the findings of other studies [4, 8], the present study demonstrated that peritoneal recurrence exhibited a stronger association with pT3b and pT4a than with pT3a (P=0.035, Table 3c). The hypothesis that pT3b might be an occult pT4a is noteworthy and is easily understandable [1-3].

As shown in Table 2, pT3a is classified as a low-grade malignant tumor, and the survival outcomes (Figs. 2A-B) are favorable, as observed in other studies [6, 30]. However, pT3b appears to be a higher-grade malignant tumor than pT3a, with the survival outcomes that are comparable to those of pT4a (Figs. 2A-B), as demonstrated in other studies [4-8]. It is reasonable to consider pT3b to be a heterogeneous type of tumor within the pT3 category. However, in contrast to the findings of our study, there are a few reports suggesting that there is no difference in DFS or OS between pT3a and pT3b [31, 39].

With regard to the surgical independent variables, the extended D3 LN en-bloc dissection [23], which is analogous to complete mesocolic or total mesorectal excision

[40, 41], has been suggested to be an independent factor for OS (Table 4c). This is likely due to the fact that the majority of patients (77.4 %, 48/62) who underwent D2 LN dissection (Table 2) died from serious diseases, including cardio/cerebrovascular disease, renal failure, pulmonary disease, and secondary malignancies in addition to CRC-specific death. Furthermore, multivariate analysis demonstrated that the intraoperative blood loss (IOBL) was an independent prognostic factor for each postoperative recurrence or CRC-specific death (Tables 4a-b). A systematic literature review and meta-analysis reported by Simillis et al. [42] demonstrated that increased IOBL was associated with worse OS above a range of 200–500 ml (HR; 1.15, $p < 0.001$), and worse RFS above 200-400 ml (HR; 1.33, $p = 0.01$). They also noted an association between increased blood loss and small bowel obstruction, which may be indicative of peritoneal dissemination resulting from colorectal cancer recurrence. Interestingly, the present study also revealed that the cutoff point for IOBL for postoperative recurrence was 350 ml. Therefore, peritoneal dissemination may have been caused not only by oncological malignancy but also by surgical manipulation and increased IOBL.

The survival outcomes for pT3a were favorable irrespective of the administration of adjuvant chemotherapy (Fig. 3A), but those for pT3b were extremely poor and

comparable to those for pT4a, even with adjuvant chemotherapy (Figs. 3A-B). Therefore, pT3b and pT4a should be considered the same entity from an oncological perspective. For patients with pT3b or pT4a disease, the use of more intensive intravenous adjuvant chemotherapy should be reconsidered.

Multivariate analysis revealed an important impact of the ELI on postoperative recurrence, CRC-specific death, and overall death in patients with pStage III disease (Tables 4a-c). As demonstrated in other studies, the ELI appears to be an independent predictor for postoperative survival and recurrence [4, 5]. The ELI has been demonstrated to be a superior prognostic indicator in comparison to conventional prognostic factors, including venous [18], lymphatic [19], and perineural invasion [20]. In patients with pStage III disease, ELI was indicated to be an effective pathological feature for identifying high-risk patients. More importantly, use of EVG staining is required to identify ELI and can resolve any discrepancies between macroscopic and pathological diagnoses related to serosal invasion.

Conclusions

The identification of ELI using EVG staining can resolve the diagnostic discrepancies related to serosal invasion. In consideration of the status of ELI, the T-classification can be subdivided into pT3a, pT3b, and pT4a. ELI is a significant prognostic indicator, and

pT3b tumor should be considered pT4a tumor, and both tumors are associated with a poor prognosis. Intensive adjuvant chemotherapy is recommended for patients with pT3b and pT4a tumors.

Abbreviations

CRC: colorectal cancer, BMI: body mass index, CEA: carcinoembryonic antigen, EL: elastic lamina, ELI: elastic lamina invasion, VI: venous invasion, Ly: lymphatic invasion, PNI: perineural invasion, BD: tumor budding, TD: tumor deposit, HE: hematoxylin-eosin, EVG: Elastica van Gieson, RFS: recurrence-free survival, OS: overall survival, LN: lymph node, 5-Fu: 5-fluorouracil, IOBL: intraoperative blood loss, CI: confidence interval, HR: hazard ratio, SD: standard deviation.

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.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available (because another project by our team is ongoing using the same dataset), but are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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

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LEGEND for FIGURES

FIGURE 1. *Serosal invasion and pT3 subclassification based on the definition of ELI*

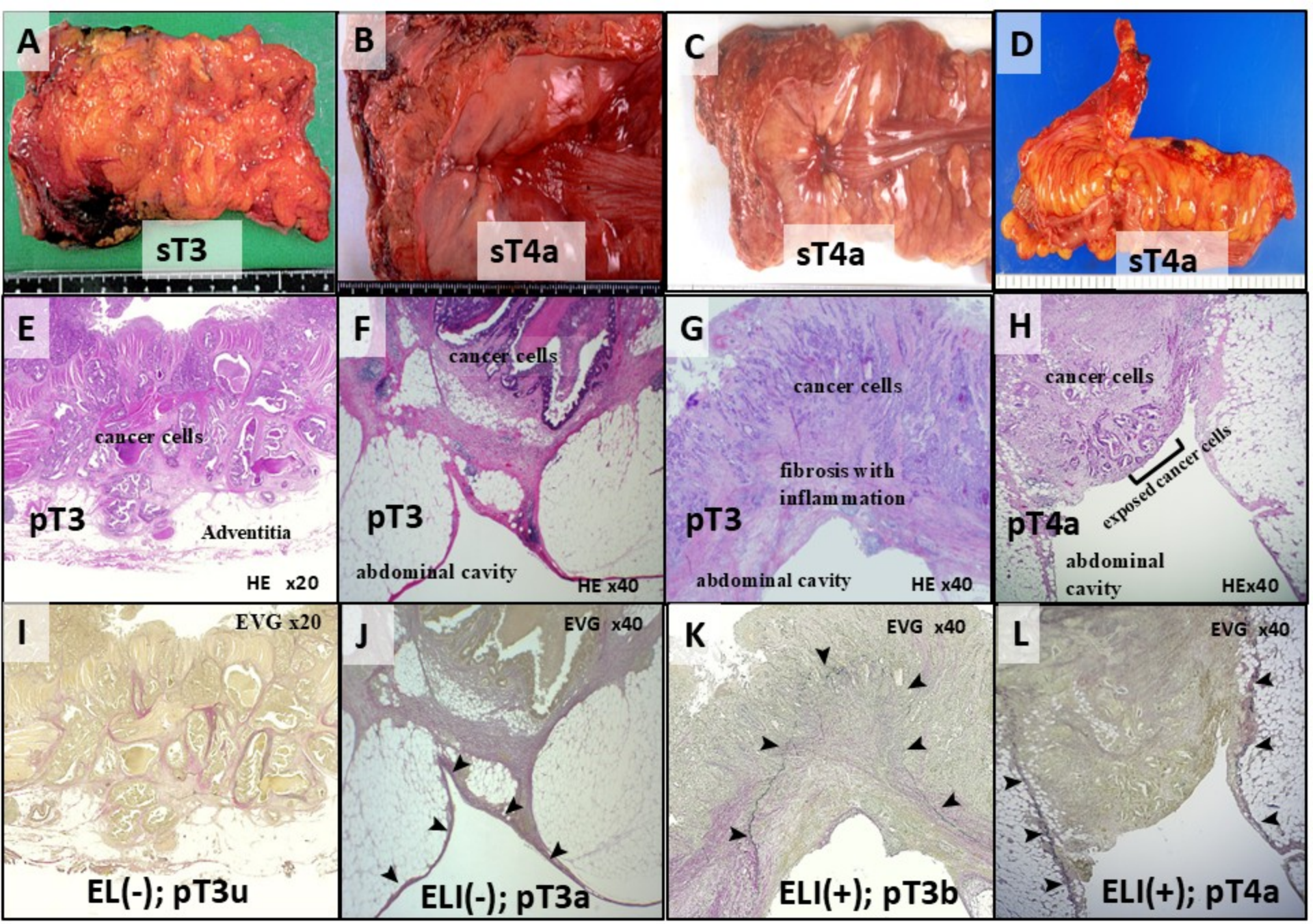
The top row of the figures shows the surgical diagnoses of the serosal surfaces of resected fresh specimens, as follows. **A**) sT3, **B**) sT4a, **C**) sT4a, **D**) sT4a. The middle and bottom rows show hematoxylin-eosin (HE) and Elastica van Gieson (EVG) stained micrographs, respectively. Arrow heads: elastic lamina (EL); EL(−): unidentified elastic lamina; ELI(−): negative elastic lamina invasion; ELI(+): positive elastic lamina invasion, **I)** EL(-); pT3u. **J)** ELI(−); pT3a. **K)** ELI(+); pT3b. **L)** ELI(+); pT4a.

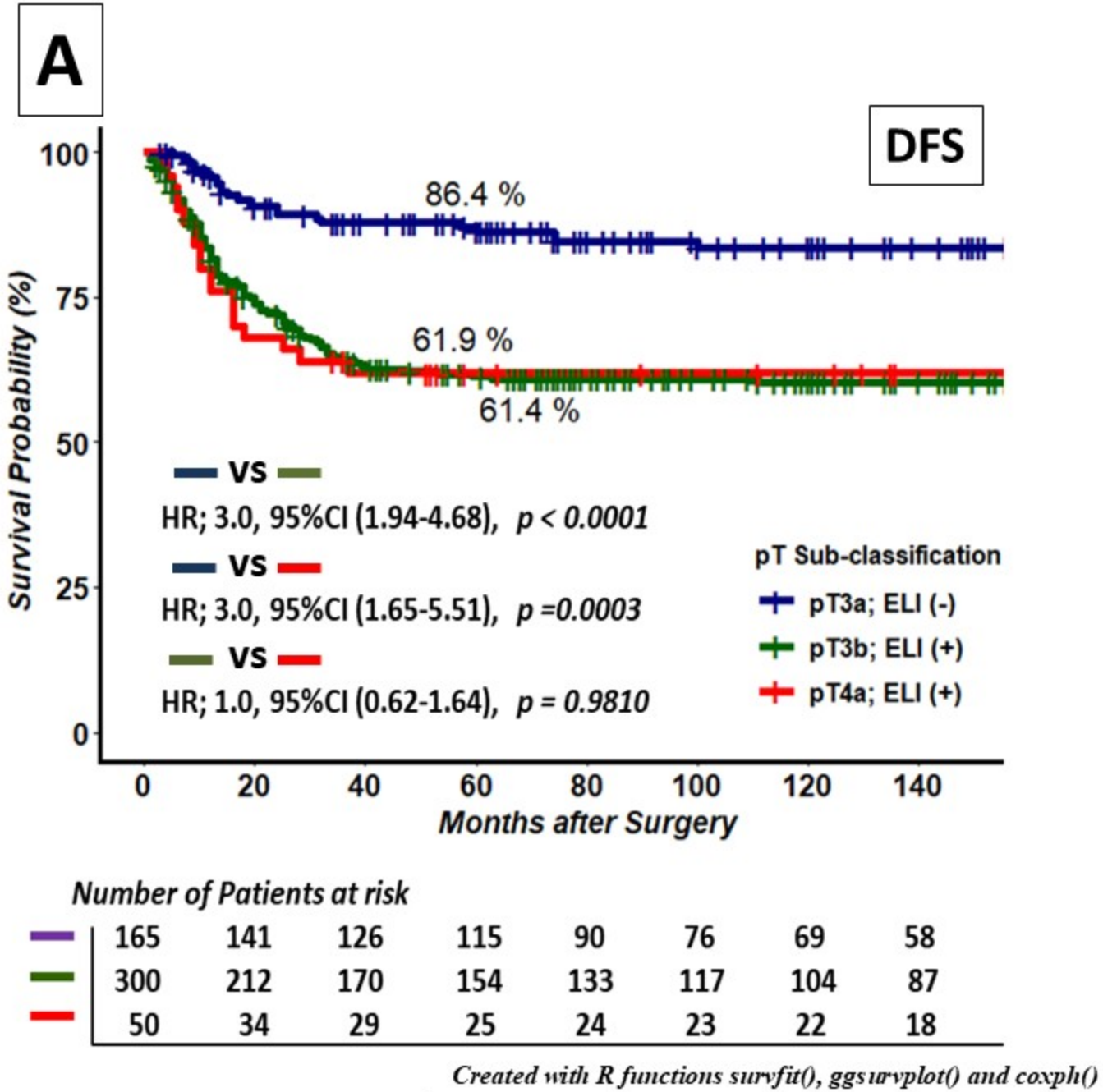
FIGURE 2. *Recurrence-free and overall survivals based on pT subclassification*

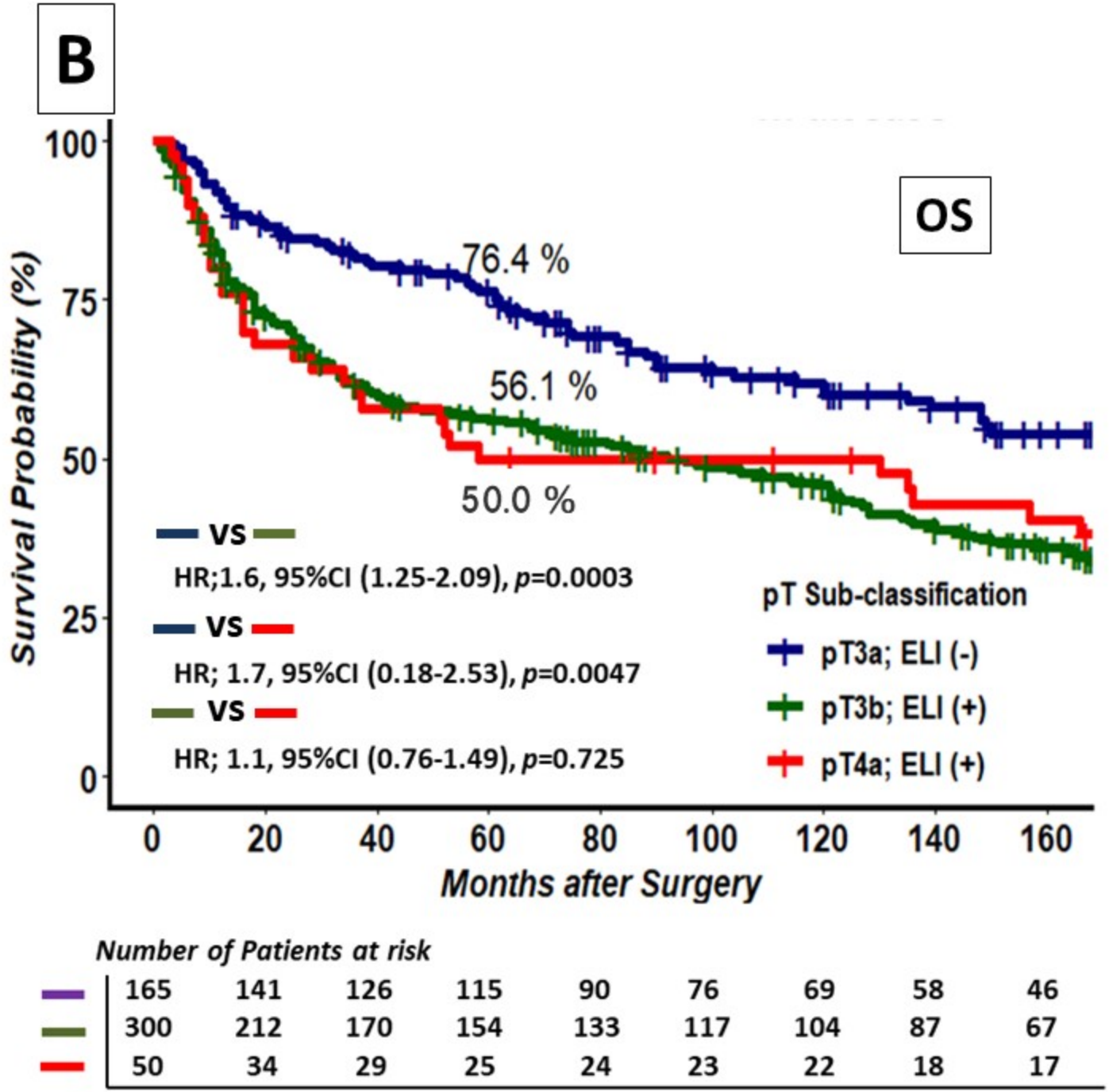
A) Recurrence-free survival (RFS), **B)** Overall survival (OS), **A) and B);** The RFS and OS rates of pT3b are significantly lower than those of pT3a but do not differ from those of pT4a. Survival curves were created with R functions *'survft()'* and *'ggsurvplot()'*. HR (hazard ratio), CI (confidence interval) and *p*-values were calculated using R function *'coxph()'*.

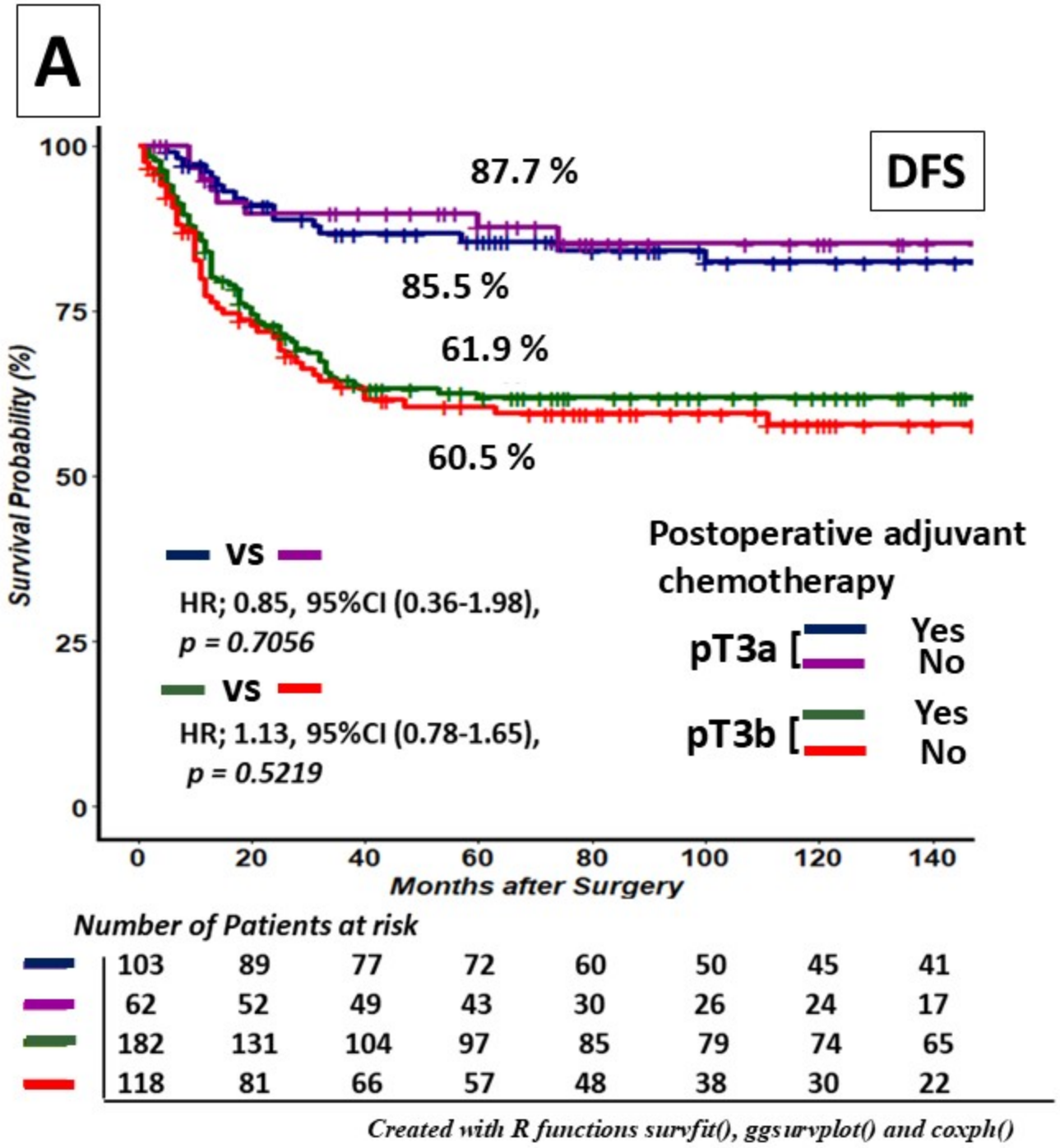
FIGURE 3. *Chemotherapy-based Recurrence-free survival*

Survival curves were created with R functions *'survft()'* and *'ggsurvplot()'*. HR (hazard ratio), CI (confidence interval) and *p*-values were calculated using R function *'coxph()'*. **A)** Chemotherapy-based recurrence-free survivals (RFS) for pT3a and pT3b, and **B**) Chemotherapy-based RFS for pT4a. In each pT3a, pT3b, and pT4a tumor, there is no significant difference in RFS based on the administration of chemotherapy.


A
sT3
B
sT4a
C
sT4a
D
sT4a
E
cancer cells
pT3
Adventitia
HE x20
F
cancer cells
pT3
abdominal cavity
HE x40
G
cancer cells
fibrosis with inflammation
pT3
abdominal cavity
HE x40
H
cancer cells
exposed cancer cells
pT4a
abdominal cavity
HEx40
I
EVG x20
EL(-); pT3u
J
EVG x40
ELI(-); pT3a
K
EVG x40
ELI(+); pT3b
L
EVG x40
ELI(+); pT4a


A
DFS
100
75
50
25
0
Survival Probability (%)
86.4 %
61.9 %
61.4 %
VS
HR; 3.0, 95%CI (1.94-4.68), p < 0.0001
VS
HR; 3.0, 95%CI (1.65-5.51), p =0.0003
VS
HR; 1.0, 95%CI (0.62-1.64), p = 0.9810
pT Sub-classification
pT3a; ELI (-)
pT3b; ELI (+)
pT4a; ELI (+)
0
20
40
60
80
100
120
140
Months after Surgery
Number of Patients at risk
165 141 126 115 90 76 69 58
300 212 170 154 133 117 104 87
50 34 29 25 24 23 22 18
Created with R functions survfit(), ggsurvplot() and coxph()


B
OS
Survival Probability (%)
100
75
50
25
0
76.4 %
56.1 %
50.0 %
VS
HR;1.6, 95%CI (1.25-2.09), p=0.0003
VS
HR; 1.7, 95%CI (0.18-2.53), p=0.0047
VS
HR; 1.1, 95%CI (0.76-1.49), p=0.725
pT Sub-classification
pT3a; ELI (-)
pT3b; ELI (+)
pT4a; ELI (+)
0 20 40 60 80 100 120 140 160
Months after Surgery
Number of Patients at risk
165 141 126 115 90 76 69 58 46
300 212 170 154 133 117 104 87 67
50 34 29 25 24 23 22 18 17


A
DFS
87.7 %
85.5 %
61.9 %
60.5 %
Survival Probability (%)
100
75
50
25
0
vs
HR; 0.85, 95%CI (0.36-1.98),
p = 0.7056
vs
HR; 1.13, 95%CI (0.78-1.65),
p = 0.5219
Postoperative adjuvant chemotherapy
pT3a [Yes No
pT3b [Yes No
0 20 40 60 80 100 120 140
Months after Surgery
Number of Patients at risk
103 89 77 72 60 50 45 41
62 52 49 43 30 26 24 17
182 131 104 97 85 79 74 65
118 81 66 57 48 38 30 22
Created with R functions survfit(), ggsurvplot() and coxph()

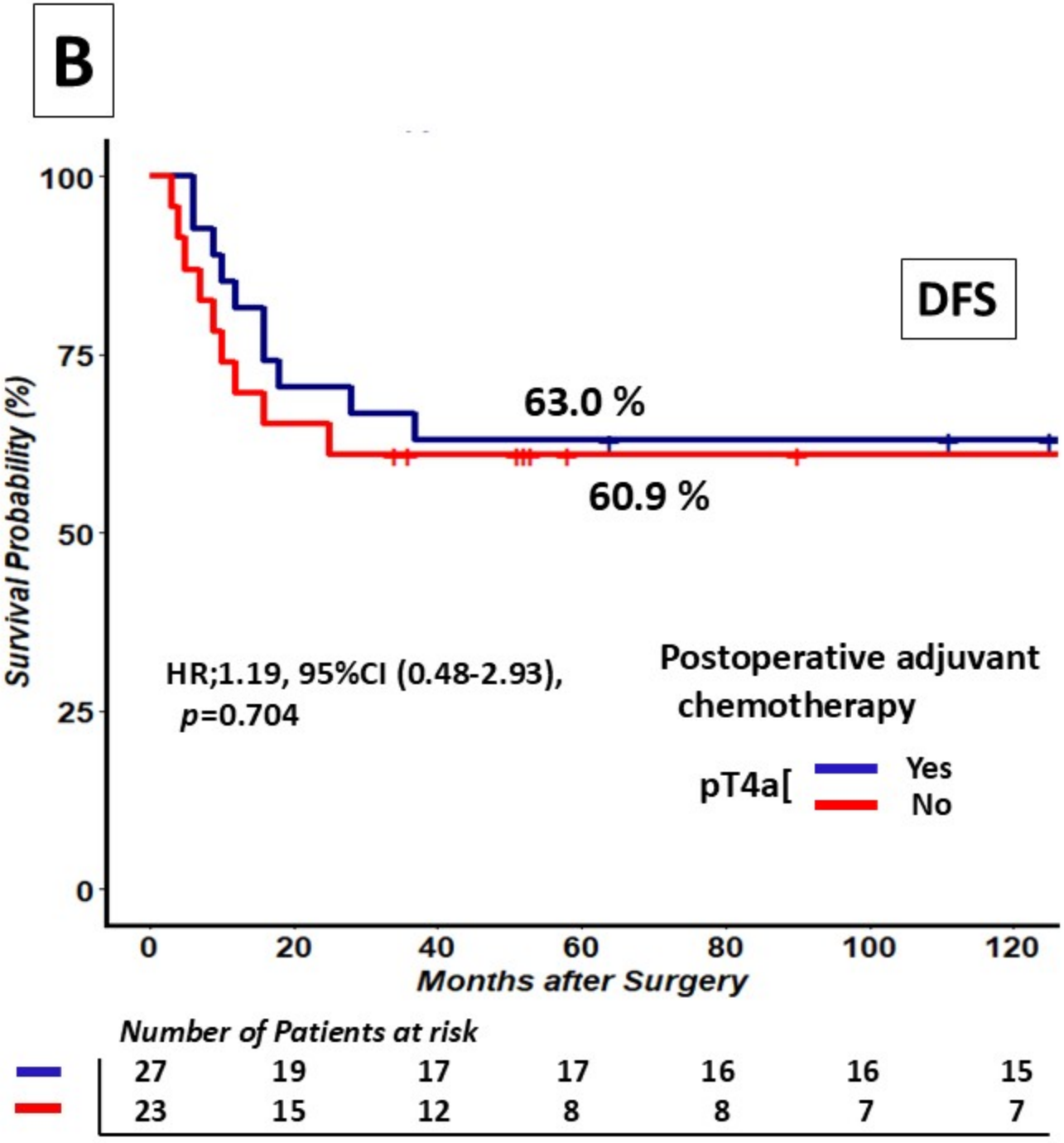

B
DFS
100
75
50
25
0
Survival Probability (%)
63.0 %
60.9 %
HR;1.19, 95%CI (0.48-2.93),
p=0.704
Postoperative adjuvant chemotherapy
pT4a[
Yes
No
0
20
40
60
80
100
120
Months after Surgery
Number of Patients at risk
27 19 17 17 16 16 15
23 15 12 8 8 7 7

Table 1. Association between surgical T-factor and pT sub-classification

pT sub-classification	sT1	sT2	sT3	sT4a
pT3u [n=25]	1	5 (26.3)	17 (11.9)	2 (0.6)
pT3a [n=140]	0	9 (47.4)	93 (**65.0**)	38 (10.8)
pT3b [n=300]	1	3 (15.8)	32 (**22.4**)	264 (**75.2**)
pT4a [n=50]	0	2 (10.5)	1 (0.7)	47 (**13.4**)
Total [515]	2	19	143	351

The symbol `s` is used to denote a surgical diagnosis. Of the sT3 cases, 65% were diagnosed as pT3a and 22.4% as pT3b. Among the sT4a cases, 75.2% were pT3b, and only 13.4% was concordant with the pathologic diagnosis. The rate of diagnostic agreement related to serosal invasion is extremely low. ( ):%

Table 2. Association between pT sub-classification and independent variables

Independent variables		pT3a (n =165)	pT3b (n = 300)	pT4a (n=50)	*P*-value
Age (years)	< 50	11 (6.7)	37 (12.3)	5 (10.0)	0.157
	≥ 50	154 (93.3)	263 (87.7)	45 (90.0)	
Gender	female	72 (43.6)	113 (37.7)	21 (42.0)	0.433
	male	93 (56.4)	187 (62.3)	29 (58.0)	
BMI (kg/m^2)	< 25.3	133 (80.6)	265 (88.3)	49 (98.0)	**0.003**
	≥ 25.3	32 (19.4)	35 (11.7)	1 (2.0)	
CEA (ng/ml)	< 6.6	134 (81.2)	178 (59.3)	35 (70.0)	**<0.001**
	≥ 6.7	31 (18.8)	122 (40.7)	15 (30.0)	
Tumor location	colon	127 (77.0)	258 (86.0)	45 (90.0)	**0.018**
	rectum	38 (23.0)	42 (14.0)	5 (10.0)	
Tumor size (mm)	< 68	142 (86.1)	224 (74.7)	33 (66.0)	**0.002**
	≥ 68	23 (13.9)	76 (25.3)	17 (34.0)	
Intraoperative blood loss (ml)	< 350	128 (77.6)	214 (71.3)	35 (70.0)	0.301
	≥ 350	37 (22.4)	86 (28.7)	15 (30.0)	
Lymph node dissection	D2	24 (14.5)	32 (10.7)	6 (12.0)	0.469
	D3	141 (85.5)	268 (89.3)	44 (88.0)	
Histologic differentiation	well	72 (43.6)	90 (30.0)	10 (20.0)	**0.001**
	others	93 (56.4)	210 (70.0)	40 (80.0)	
Lymphatic invasion	negative/slight	121(73.3)	180 (60.0)	29 (58.0)	**0.018**
	moderate/severe	44 (26.7)	120 (40.0)	21 (42.0)	
Venous invasion	negative/slight	142 (86.1)	218 (72.7)	37 (74.0)	**0.004**
	moderate/severe	23 (13.9)	82 (27.3)	13 (26.0)	
Tumor budding	negative/slight	109 (66.1)	142 (47.3)	19 (38.0)	**<0.001**
	moderate/severe	56 (33.9)	158 (52.7)	31 (62.0)	
Tumor deposit	negative	131 (79.4)	202 (67.3)	33 (66.0)	**0.016**
	positive	34 (20.6)	98 (32.7)	17 (34.0)	
Perineural invasion	negative	130 (78.8)	184 (61.3)	26 (52.0)	**<0.001**
	positive	35 (21.2)	116 (38.7)	24 (48.0)	
TNM pStage	IIIB	150 (90.9)	194 (64.7)	3 (68.0)	**<0.001**
	IIIC	15 (9.1)	106 (35.3)	16 (32.0)	
Postoperative adjuvant chemotherapy	yes	103 (62.4)	182 (60.7)	27 (54.0)	0.114
	no	62 (37.6)	118 (39.3)	23 (46.0)	
Postoperative recurrence	no	141 (85.5)	187 (62.3)	31 (62.0)	**<0.001**
	yes	24 (14.5)	113 (37.7)	19 (38.0)	

well: well differentiated adenocarcinoma, others: moderately or poorly differentiated adenocarcinoma or mucinous adenocarcinoma, BMI: body mass index, CEA: carcinoembryonic antigen, D2: standard lymph node (LN) dissection, D3: extended LN dissection, pT3a: pT3 without elastic lamina invasion (ELI): including cases where the elastic lamina is absent or unidentifiable, pT3b: pT3 with ELI, pT4a: serosal invasion with cancer cells exposed in the abdominal cavity, A significant association was observed between the pT subclassification and, primarily, pathological variables. ELI appears to be associated with tumor malignancy. *Created with R function* '*CreateTableOne()*'.

Table 3. pT subclassification and postoperative recurrence

a. Recurrence in each pT Category

pT	Recurrence				Cox's Regression Analysis	
	Presence	Absence	Total	*P*-value	HR (95%CI)	*P*-value
pT3a	25 (15.2)	140 (84.8)	165	<0.0001	1 (reference)	-
pT3b	112 (37.3)	188 (62.7)	300		3.0 (1.9-4.7)	<0.0001
pT4a	19 (38.0)	31 (62.0)	50		3.0 (1.7-.5.5)	0.0003
Total	156 (30.3)	359 (69.7)	515			

HR: Hazard Ratio; Recurrence and pT category were significantly correlated ($p<0.0001$, χ^2 test). The rate of recurrence did not differ between pT3b and pT4a. HRs were significantly higher for pT3b and pT4a compared to that for pT3a. ( ):%

b. Recurrence Sites, including Multiple Sites of Recurrence

pT	Liver	Lung	Peritoneum	Local	Bone/Kidney Brain/Skin	Others	Total
pT3a	14 (41.2)	10 (29.4)	2 (5.9)	2 (5.9)	3 (8.8)	3 (8.8)	34
pT3b	65 (38.2)	44 (25.9)	23 (13.2)	16 (9.4)	9 (5.3)	17 (10.0)	174
pT4a	11 (33.3)	6 (18.2)	9 (27.3)	1 (3.0)	1 (3.0)	5 (15.2)	33
Total	90 (37.3)	60 (24.9)	34 (14.1)	19 (7.9)	14 (5.8)	24 (10.0)	241

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	*P*-value	Presence	Absence	Total	*P*-value
pT3a	27 (79.4)	7 (20.6)	34	0.094	2 (5.9)	32 (94.1)	34	0.035
pT3b	118 (67.8)	56 (32.2)	174		23 (13.2)	151 (86.8)	174	
pT4a	18 (54.5)	15 (45.5)	33		9 (27.3)	24 (72..7)	33	
Total	164 (68.0)	77 (32.0)	241		34 (14.1)	207 (85.9)	241	

Distant metastasis was found at a high rate in each pT category without correlation ($p=0.094$, χ^2 test). Peritoneal recurrence was significantly associated with both pT3b and pT4a compared to pT3a ($p=0.035$, χ^2 test). ( ):%

Table 4. Univariate and multivariate Cox's regression analyses of independent variables for pStage III disease

a. ***Analysis for postoperative recurrence***

Independent variables		Univariate analysis HR (95%CI)	*P*-value	Multivariate analysis HR (95%CI)	*P*-value
Age (years)	≥50 <50	1 1.56 (0.99-2.45)	0.0521	–	–
Gender	Female Male	1 1.10 (0.80-1.52)	0.5558	–	–
BMI (kg/m^2)	<25.3 ≥25.3	1 0.76 (0.46-1.25)	0.2767	–	–
CEA (ng/ml)	<6.6 ≥6.7	1 1.49 (1.08-2.05)	**0.0149**	1 1.21 (0.87-1.69)	0.2516
Tumor location	Colon Rectum	1 1.31 (0.88-1.96)	0.1799	–	–
Tumor size (mm)	<68 ≥68	1 1.25 (0.87-1.79)	0.2231	–	–
Intraoperative blood loss (ml)	<350 ≥350	1 1.85 (1.33-2.56)	**0.0002**	1 1.46 (1.03-2.08)	**0.0323**
Lymph node dissection	D2 D3	1 0.95 (0.57-1.57)	0.8289	–	–
Histologic differentiation	well others	1 1.84 (1.27-2.67)	**0.0014**	1 1.14 (0.77-1.69)	0.5228
Lymphatic invasion	0/1 2/3	1 1.99 (1.45-2.72)	**<0.0001**	1 1.11 (0.79-1.56)	0.5557
Venous invasion	0/1 2/3	1 2.61 (1.89-3.61)	**<0.0001**	1 1.40 (0.97-2.01)	0.0705
Tumor budding	0/1 2/3	1 3.60 (2.53-5.13)	**<0.0001**	1 2.37 (1.59-3.52)	**<0.0001**
Tumor deposit	negative positive	1 2.31 (1.68-3.17)	**<0.0001**	1 1.33 (0.93-1.88)	0.1161
Perineural invasion	negative positive	1 2.35 (1.71-3.21)	**<0.0001**	1 1.68 (1.21-2.32)	**0.0019**
ELI	negative positive	1 3.02 (1.95-4.67)	**<0.0001**	1 2.13 (1.37-3.33)	**0.0008**
Postoperative adjuvant chemotherapy	no yes	1.15 (0.82-1.62) 1	0.4227	–	–

ELI: elastic lamina invasion; well: well differentiated adenocarcinoma, others: moderately or poorly differentiated adenocarcinoma or mucinous adenocarcinoma, BMI: body mass index, CEA: carcinoembryonic antigen, D2: standard lymph node (LN) dissection, D3: extended LN dissection, 0/1: negative/slight, 2/3: moderate/severe, HR: hazard ratio; CI: confidence interval; Multivariate analysis identified tumor budding and ELI as strong independent risk factors for postoperative recurrence.

b. *Analysis for CRC-specific death*

Independent variables		Univariate analysis HR (95%CI)	*P*-value	Multivariate analysis HR (95%CI)	*P*-value
Age (years)	≥50 <50	1 1.66 (1.03-2.66)	**0.0371**	1 1.18 (0.73-1.92)	0.5055
Gender	Female Male	1 1.30 (0.91-1.85)	0.1527	–	–
BMI (kg/m^2)	<25.3 ≥25.3	1 0.71 (0.41-1.24)	0.2337	–	–
CEA (ng/ml)	<6.6 ≥6.7	1 1.36 (0.96-1.93)	0.0813	–	–
Tumor location	Colon Rectum	1 1.25 (0.81-1.93)	0.3148	–	–
Tumor size (mm)	<68 ≥68	1 1.37 (0.94-1.99)	0.1051	–	–
Intraoperative blood loss (ml)	<350 ≥350	1 2.07 (1.46-2.92)	**<0.0001**	1 1.58 (1.09-2.28)	**0.0139**
Lymph node dissection	D2 D3	1 0.89 (0.51-1.55)	0.6831	–	–
Histologic differentiation	well others	1 1.84 (1.23-2.74)	**0.0029**	1 1.10 (0.72-1.69)	0.6547
Lymphatic invasion	0/1 2/3	1 2.0 (1.43-2.80)	**<0.0001**	1 1.03 (0.71-1.49)	0.8704
Venous invasion	0/1 2/3	1 2.59 (1.83-3.66)	**<0.0001**	1 1.28 (0.87-1.89)	0.2152
Tumor budding	0/1 2/3	1 4.22 (2.84-6.27)	**<0.0001**	1 2.83 (1.83-4.39)	**< 0.0001**
Tumor deposit	negative positive	1 2.44 (1.74-3.43)	**<0.0001**	1 1.39 (0.95-2.02)	0.0892
Perineural invasion	negative positive	1 2.44 (1.74-3.42)	**<0.0001**	1 1.72 (1.21-2.44)	**0.0025**
ELI	negative positive	1 3.54 (2.16-5.82)	**<0.0001**	1 2.49 (1.50-4.12)	**0.0004**
Postoperative adjuvant chemotherapy	no yes	1.27 (0.91-1.79) 1	0.1614	–	–

CRC: colorectal cancer; ELI: elastic lamina invasion; 0/1: negative/slight, 2/3: moderate/severe, Please refer to the footnotes in Table 1a for the details of other abbreviations. Multivariate analysis identified tumor budding and ELI as strong independent risk factors for CRC-specific death.

c. *Analysis for Overall death*

Independent variables		Univariate analysis HR (95%CI)	*P*-value	Multivariate analysis HR (95%CI)	*P*-value
Age (years)	≥50 <50	1 0.56 (0.37-0.84)	**0.0058**	1 0.48 (0.32-0.74)	**0.0009**
Gender	Female Male	1 1.23 (0.96-1.57)	0.1786	–	–
BMI (kg/m^2)	<25.3 ≥25.3	1 0.96 (0.67-1.37)	0.8374	–	–
CEA (ng/ml)	<6.6 ≥6.7	1 1.21 (0.95-1.54)	0.1120	–	–
Tumor location	Colon Rectum	1 0.97 (0.72-1.30)	0.8276	–	–
Tumor size (mm)	<68 ≥68	1 1.06 (0.83-1.37)	0.6327	–	–
Intraoperative blood loss (ml)	<350 ≥350	1 1.31 (1.03-1.66)	**0.0258**	1 1.23 (0.96-1.56)	0.0966
Lymph node dissection	D2 D3	1 1.43 (1.05-1.94)	**0.0219**	1 1.51 (1.11-2.07)	**0.0087**
Histologic differentiation	well others	1 1.17 (0.93-1.48)	0.1751	–	–
Lymphatic invasion	0/1 2/3	1 1.26 (1.01-1.57)	**0.0481**	1 0.99 (0.77-1.27)	0.9235
Venous invasion	0/1 2/3	1 1.23 (0.95-1.59)	0.1111	–	–
Tumor budding	0/1 2/3	1 1.70 (1.36-2.12)	**<0.0001**	1 1.56 (1.21-1.99)	**0.0005**
Tumor deposit	negative positive	1 1.49 (1.17-1.89)	**0.0011**	1 1.30 (1.01-1.67)	**0.0399**
Perineural invasion	negative positive	1 1.32 (1.05-1.66)	**0.0194**	1 1.17 (0.92-1.49)	0.1948
ELI	negative positive	1 1.63 (1.27-2.10)	**0.0001**	1 1.59 (1.23-2.06)	**0.0004**
Postoperative adjuvant chemotherapy	no yes	1.23 (0.97-1.71) 1	0.063	–	–

ELI; elastic lamina invasion; 0/1: negative/slight, 2/3: moderate/severe, Please refer to the footnotes in Table 1a for the details of abbreviations. Multivariate analysis identified ELI and tumor budding as strong independent risk factors for Overall death.