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Original contribution

Staging of colorectal cancers based on elastic lamina invasion☆

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Summary Peritoneal involvement in colorectal cancer (CRC) has prognostic significance and is an important parameter in pathologic tumor staging. Restaging of tumors based on peritoneal elastic lamina invasion (ELI) has prognostic significance in CRCs classified as pathologic stage 3 tumors without regional lymph node metastasis (pT3N0). However, limited data on the significance of ELI in patients with node-positive disease are available. We applied elastic stain to one block per case for 141 consecutive patients with pT3N1M0 CRCs. The elastic lamina was identified in only 62 cases (44%), of which 39 (27.6%) displayed ELI. The ELI+ group was associated with a significantly worse (0.;$P < .001$) 5-year disease-free survival (5-year DFS, 48.7%) and 5-year overall survival (5-year OS, 61.4%) compared with the ELI− (5-year DFS, 73.9%; OS, 95.7%) and no elastic lamina (5-year DFS, 79.5%; OS, 85.7%) groups. Comparison of outcomes in cases with pT3N1M0 with peritoneal ELI and pT4aN1M0 tumors (based on the original pathologic assessment without the use of elastic staining) showed no significant differences in the 5-year DFS ($P = .47$) and OS ($P = .65$). These findings suggest that ELI is a significant prognostic marker and that elastic staining should be considered for routine use in pT3 CRCs in a node-positive setting. Upstaging of pT3 tumors with ELI should be considered in the future iterations of the American Joint Committee on Cancer/Union for International Cancer Control tumor-node-metastasis staging system for CRC.

1. Introduction

Pathologic tumor node metastasis staging remains the basis for prognostic assessment in colorectal cancer (CRC) and is critically important in guiding treatment decisions [1]. Consequently, refinement of the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) staging system to provide an accurate prognostic stratification

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is an area of ongoing interest. In the AJCC/UICC eighth edition, the pathologic tumor (pT) stage is based on the depth of tumor invasion in the colon wall [2]. In large population-based studies, most (>50%) CRCs are reported to be of pathologic tumor stage 3 (pT3), which indicates the invasion of the subserosal adipose tissue without peritoneal involvement [3,4]. Peritoneal surface invasion defines stage pT4a disease, which has been associated with decreased overall survival (OS) [3,4]. However, the population-based Surveillance, Epidemiology, and End Results studies, which include data from more than 130 000 patients, report peritoneal invasion in less than 10% of CRCs, which is far lower than the rate reported in smaller studies (59%) focused on a thorough assessment of the peritoneum [5]. A study of 412 cases used careful gross sampling of the tumors, evaluation of multiple sections through blocks, and expanded histologic criteria to identify peritoneal involvement [6]. The colon has a submesothelial elastic lamina (EL), which can be visualized using elastic stains. Five previous studies including ours [7] have identified that EL invasion (ELI) in CRC is associated with adverse prognosis in pT3 tumors [8-11]. The prognostic outcomes of our approach of elastic staining in a single tissue block were comparable to that of prior studies that evaluated multiple blocks, despite the failure to identify the EL in a large number of them.

Although our previous study was limited to patients with pT3N0M0 disease, the earlier studies had a limited number of patients with node-positive disease. The present study aims to determine the prognostic significance of ELI in node-positive CRCs by applying elastic stain to a single tissue block from a cohort of consecutive patients with pT3N1M0 CRCs.

2. Materials and methods

2.1. Patient characteristics

After a retrospective search of the pathology archives, we identified consecutive patients with pT3N1M0 and pT4aN1M0 CRCs at surgical resection between 2003 and 2007 at the Taipei Veterans General Hospital (Taipei, Taiwan). Patients with synchronous or metachronous CRCs, those with other primary cancers besides CRCs, those with distant metastases, and those who received neoadjuvant chemotherapy or radiation therapy were excluded from the study. Patient characteristics such as age at resection, sex, site of the tumor, number of lymph nodes sampled, and tumor grade were documented by reviewing the pathology reports. The status of perineural invasion, lymphovascular invasion, signet ring cell differentiation, mucinous differentiation, and lymphocytic reaction was also recorded. The study protocol was

Table 1 Clinicopathological characteristics of T3N1M0 CRCs based on elastin lamina invasion

	EL not identified	Negative for ELI	Positive for ELI	Total	P [a]
Case no.	79	23	39	141	
Age (y), mean (range)	63.2 (28-86)	68.1 (40-90)	70.2 (47-87)	65.9 (28-90)	
Sex					.0061
Male	48	14	12	74	
Female	31	9	27	67	
Site of primary					.180
Cecum + A-colon	14	5	11	30	
T-colon	12	0	1	13	
D-colon + S-colon	31	11	18	60	
Rectum	22	7	9	38	
No. lymph nodes examined, mean (range)	20.1 (6-57)	17.3 (5-31)	18.7 (7-43)	19.3 (5-57)	
Tumor size (cm), mean (range)	5.1 (1-10)	5.2 (2-13)	4.8 (1.5-11)	5.0 (1-13)	
Tumor differentiation					.259
Well to moderate	74	20	33	127	
Poorly to undifferentiated	5	3	6	14	
Mucinous component present, n (%)	24 (30.4)	7 (30.4)	11 (28.2)	42 (29.8)	.97
Signet ring cells present, n (%)	1 (1.3)	1 (4.3)	1 (2.6)	3 (2.1)	.564
Perineural invasion present, n (%)	7 (8.9)	1 (4.3)	6 (15.4)	14 (9.9)	.362
Serosal retraction or fibrosis present, n (%)	26 (33.0)	7 (30.4)	32 (82.1)	65 (46.1)	<.001
Invasive tumor growth pattern, n (%)	72 (91.1)	15 (65.2)	35 (89.7)	122 (86.5)	.0046
Lymphocytic reaction present, n (%)	25 (31.6)	9 (39.1)	11 (28.2)	45 (31.9)	.67
Lymphovascular invasion present, n (%)	11 (13.9)	3 (13.0)	8 (20.5)	22 (15.6)	.61
Develop secondary malignancy, n (%)	3 (3.8)	3 (13.0)	3 (7.7)	9 (6.4)	.207

Abbreviations: A-colon, ascending colon; D-colon, descending colon; T-colon, transverse colon.

[a] P value was calculated using the χ^2 test for categorical variables and using the Fisher exact test (2-sided) if more than 20% of cells had an expected count of less than 5.

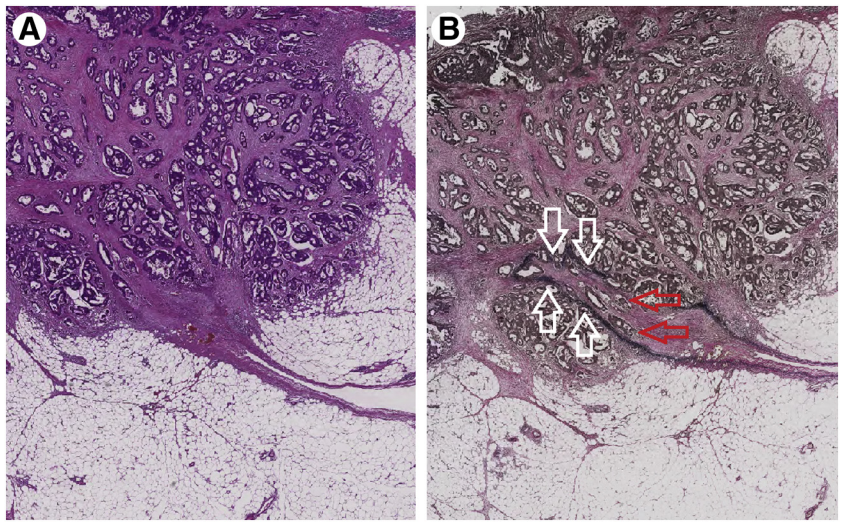

Fig. 1 Histologic features of peritoneal ELI in CRC. Shown here are induction of inflammatory reaction and serosal retraction by tumor cells growing near the serosal surface and (A; H&E, original magnification ×10) and tumor (red arrows) penetrating through the EL (B, white arrows; elastic stain, ×10).

approved by the institutional review board of the Taipei Veterans General Hospital.

2.2. Histologic examination

The original hematoxylin and eosin (H&E)–stained slides of all cases were regained from the pathology files and reviewed by one specialist gastrointestinal pathologist (W. Y. L.) who was blinded to the clinical follow-up data and the original pathology evaluation. The single section in which the tumor was nearest to the peritoneal surface was chosen for each case, and the elastic staining (Van Gieson method) was performed. We chose a single block from each case for elastic staining because of time and cost restraints. The same reviewer assessed the slides for the EL of the serosa and depth of tumor invasion. Based on the ELI status, the cases were categorized as ELI+, ELI−, or EL not identified. However, in some cases where severe CRC-induced inflammation obscured the peritoneal EL, an imaginary line was drawn between the remaining EL on the 2 sides of the tumor, and cases with obvious tumor invasion beyond the imaginary line were categorized as ELI+.

2.3. Clinical follow-up and survival analysis

The patients' clinical records were reviewed from the time of surgery until January 31, 2014. Disease-free survival (DFS), tumor recurrence, and OS were recorded for all the pT3N1M0 and pT4N1M0 cases. The pT3N1M0 cases were then subgrouped based on their elastic staining (ELI+, ELI−, or EL not identified).

2.4. Statistical analysis

Patient demographics and pathologic characteristics of the CRCs in the 3 groups were evaluated. Categorical variables were compared using the χ^2 test, and the Fisher exact test (2-sided) was applied if more than 20% of cells had an expected count of less than 5. Continuous variables were evaluated using the Student *t* test. Survival was analyzed using the Kaplan-Meier method, and differences in survival were analyzed using the log-rank test (Stata Software, College Station, TX). A *P* value of <.05 was considered statistically significant.

Table 2 Prognosis in T3N1M0 CRCs based on elastin lamina invasion

	No EL identified	Negative for ELI	Positive for ELI	Total	*P* [a]
Case no.	79	23	39	141	
Recurrence or metastasis	17 (21.5%)	6 (26.1%)	20 (51.3%)	43 (30.5%)	.002
Death from disease	7 (4.9%)	1 (2.4%)	17 (43.6%)	25 (17.7%)	.001
5-y DFS	63 (79.5%)	17 (73.9%)	19 (48.7%)	99 (70.2%)	.0008
5-y OS	68 (85.7%)	22 (95.7%)	24 (61.4%)	114 (80.8%)	.0002

[a] *P* value was calculated using the χ^2 test for categorical variables and the log-rank test for differences in survival.

Table 3 Comparison of prognosis in patients with T3N1M0 positive for ELI and T4aN1M0 CRCs

	T3N1M0 positive for ELI	T4aN1M0	Total	P [a]
Case no.	39	31	70	
Recurrence or metastasis	20 (51.3%)	12 (38.7%)	32 (45.7%)	.34
Death from disease	17 (43.6%)	11 (35.5%)	28 (40.0%)	.62
5-y DFS	19 (48.7%)	18 (58.9%)	37 (52.8%)	.47
5-y OS	24 (61.4%)	23 (73.2%)	47 (67.1%)	.65

[a] P value was calculated using the χ^2 test for categorical variables and the log-rank test for differences in survival.

3. Results

3.1. Patient demographics, pathologic characteristics of tumors, and elastic staining results

The characteristics and clinicopathological features of patients in the ELI+, ELI−, and EL not identified groups are summarized in Table 1. A total of 141 patients (74 men and 67 women) with T3N1M0 CRCs were included in this study. The mean age of the patients was 65.9 years (range, 28-90 years). Fig. 1 shows representative samples of EL assessments. The EL was identified in 62 cases (44.0%), but not in the remaining 79 cases (56.0%). Among the cases where it was identified, although 39 (27.6%) showed invasion (ELI+), 23 cases (16.3%) showed no invasion (ELI−). Patient characteristics including age, incidence of a second primary malignancy, tumor size and grade, location, mucinous differentiation, signet ring cell differentiation, perineural invasion, lymphocytic reaction, lymphovascular invasion, and number of lymph nodes sampled showed no association with ELI. However, invasive tumor growth patterns and serosal retraction or fibrosis were significantly associated (P = .005 and P < .001, respectively) with the ELI+ group compared with the other groups (Table 1). We additionally evaluated 31 patients with pT4N1M0 CRCs, which were diagnosed by H&E criteria alone (continuous invasion of the primary tumor to the surface of the visceral peritoneum without adjacent organ or structure invasion or adhesion), for the incidence of recurrence, death from disease, 5-year DFS, and 5-year OS. These characteristics were compared between patients in the T3N1M0/ELI+ group and the T4N1M0 group (see Section 3.2).

3.2. Association between ELI and prognosis

Table 2 summarizes the survival outcomes in the ELI−, ELI+, and EL not identified groups of T3N1M0 CRCs. Table 3 shows a comparison between patients in the T3N1M0/ELI+ and T4N1M0 groups. In the T3N1M0 group, ELI positivity was associated with a lower 5-year DFS (48.7%) and 5-year OS (61.4%) compared with the ELI− (5-year DFS, 73.9%; OS, 95.7%) and EL not identified (5-year DFS, 79.5%; OS, 85.7%) groups. Fig. 2 shows that the ELI+ group was associated with a significantly lower DFS (P = .0007) and OS (P = .0002) compared with the ELI − and EL not identified groups. There was no significant difference in the DFS (P = .6318) or OS (P = .8413) between the ELI− and the EL not identified groups. Similarly, no significant difference was noted in the 5-year DFS (P = .4746) or 5-year OS (P = .6464) between the T3N1M0/ELI+ and T4aN1M0 groups (Table 3).

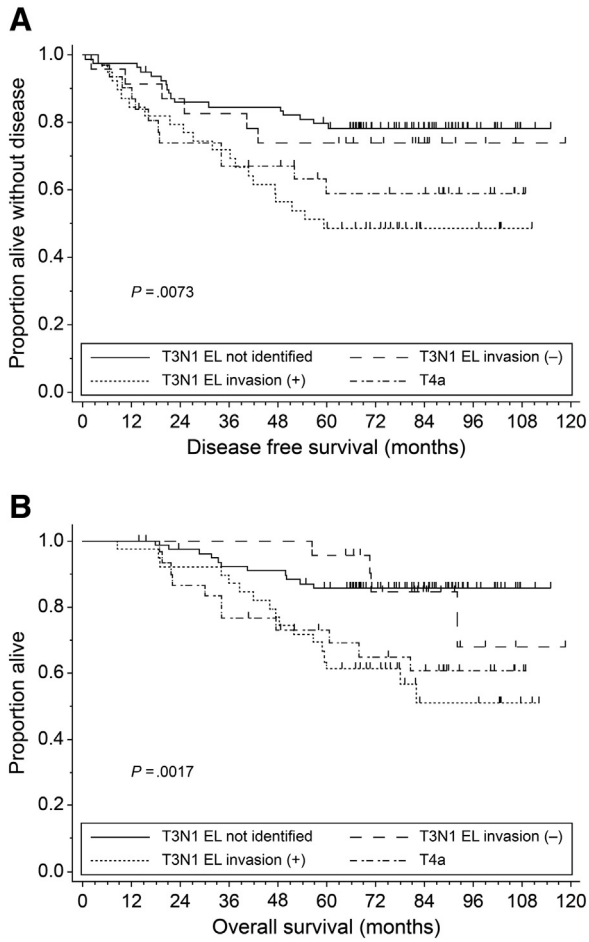

Fig. 2 Effect of ELI on survival outcomes. Shown are the DFS (A) and OS (B) curves for patients with pT3N1M0 negative for ELI (ELI−), with no EL identified, or positive for ELI (ELI+) and pT4aN1M0 CRCs.

Table 4 T staging with and without the use of ELI as a criterion

	T1	T2	T3 [a]	T4a [a]	T4b
2010-2012	186 (9.0%)	275 (13.2%)	1338 (64.5%)	168 (8.1%)	108 (5.2%)
2014-2015	77 (10%)	106 (13.7%)	386 (49.9%)	162 (21.0%)	42 (5.4%)

[a] *P* value was calculated using the χ^2 test and was <.001.

Table 5 ELI status in T3 and T4a CRCs

T3		T4a		
EVG(−)	EVG(+)	ELI(+) only	H&E only	H&E + ELI(+)
230 (60%)	156 (40%)	104 (64.2%)	12 (7.4%)	46 (28.4%)

Table 6 Studies on ELl involvement in T3 CRCs

	Cases no.	Stage	ELI+ (%)	*P* value of survival between ELI− ;and ELI+	*P* value of survival between ELI+ ;and T4a CRCs	Type of stain
Shinto et al [9]	325	II + III + IV	52	.0023	NS	EVG
Kojima et al [8]	564	II + III + IV	44	<.01	NS	EVG
Liang et al [7]	244	II	25	<.001	NS	EVG
Grin et al [20]	217	II	17	.517	.026	ET or MP
Yokota et al [10]	393	II + III	36	.02	NS	EVG
Nakanishi et al [11]	139	II + III	23	<.001	<.001	EVG
Lu et al [18]	225	II + III	35	Not provided	Not provided	EVG
Liang et al, 2018	141	III	28	<.001	NS	EVG

Abbreviations: ELI, elastic lamina invasion; EVG: Elastica van Gieson staining; ;ET, elastic trichrome staining; MP, Movat pentachrome stain; NS, not significant.

4. Discussion

Colon cancer with serosal surface invasion is defined as T4a in the eighth TMN staging system of CRC and has a poor prognosis. However, the microscopic evaluation of the extent of serosal invasion is challenging because the tenuous mesothelial cells denuded from the serosal surface and the waving contour of the pericolonic adipose tissue with slits that invaginate toward the muscularis propria make it hard to distinguish tumor cell infiltration from tumor-related secondary tissue reactions including inflammation and stromal desmoplasia. The reported rate of peritoneal involvement in stage II CRC ranged from 5% to 43% [11-18] and was about 59% in a previous study with extensive peritoneal sampling [6]. These variations are potentially due to the different methods used for tissue sampling and the varied criteria for defining serosal invasion [19].

Although the detection of ELI by elastic stain seems to be very useful in T3 CRC, there are some problems in its application. These include difficulty in identifying the EL, poor staining quality, and misinterpretation of the findings. Sampling the representative site is crucial for a good diagnosis. In our experience, a site with serosal retraction, marked fibrosis, and inflammation is likely to have ELI. Therefore, in some cases, performing elastic staining in multiple slides is helpful. Finally, it is possible that different histochemical stains will help increase the sensitivity of detection.

Elastic fibers of the vessels are good internal controls for the elastic stain, but misinterpreting them as ELI should be avoided.

In our department, since late 2013, we have applied a new reporting form that includes the elastic staining results. Shown below is an example of a report:

- Invasion depth
  - Penetrates the muscle layers up to the subserosal soft tissue (T3)
  - Perforates visceral peritoneum (T4a: by H&E/ELI)
- Elastica van Gieson (EVG) stain result
  - EL demonstrated by EVG stain: yes/no
  - EL invasion: yes/no

If ELI is observed, the tumor is regarded as positive for serosal invasion and is staged as T4a CRC. Table 4 summarizes the staging results of CRCs in our department at the Taipei Veterans General Hospital, between 2010 and 2015, with and without the use of ELI as a criterion. Table 5 compares the ELI status in stage T3 or T4a CRCs from 2014 to 2015. Although no significant difference was observed in the proportions of T1, T2, and T4b cases, a significant increase (from 8.1% to 21.0%) was noted in the detection of T4a cases (0.;$P < .001$).

Approximately 64% of the T4a cases were diagnosed based on ELI positivity only.

In most previous series, when compared with T3 CRCs/ELI−, the T3 CRCs/ELI+ had significantly worse survival, which was comparable to the survival rate of T4a CRCs. Table 6 lists some studies on the involvement of the EL in T3 CRCs. Grin et al [20] applied a different staining method and showed a lower incidence of ELI positivity when compared with most other studies. Unlike Nakanishi et al [11], who included both nodal-negative and nodal-positive CRCs for analysis, we only compared the nodal-positive T3 and T4a CRCs in this study.

The traditional convention of determining the pathologic T stage based on the H&E morphology is preferred because ancillary studies are more challenging due to limitations in the availability and assignment of resources in different laboratories. However, the methods for diagnosis have changed. For instance, the staging of lung adenocarcinoma has integrated elastic stain for evaluation of pleural invasion since the previous edition (seventh edition) of the AJCC tumor node metastasis staging system [2]. Recent studies have shown that elastic staining for CRCs can differentiate between pT3 tumors with and without ELI, which is a marker of poor prognosis [8,9]. These studies, therefore, suggest that the ELI status should be considered as a criterion for CRC staging in the future.

In conclusion, we show in 2 series that pT3 CRCs with ELI have a poorer prognosis than those without ELI. We also found that the prognosis for pT3/ELI+ was comparable to that of pT4a in this study. Therefore, we suggest that all pT3 CRCs should be evaluated by elastic stain for their ELI status, and those with ELI should be upstaged to pT4a.

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