의학석사 학위논문

T3N0 대장직장암에서 림프혈관침습이 보조항암요법 결정에 미치는 중요성과 효용성 및 대체인자

The importance and utility of lymphovascular invasion in T3N0 colorectal cancer on adjuvant chemotherapy decision and replaceable factors

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이 논문을 의학석사학위 논문으로 제출함

2022 년 2 월

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영문요약(Abstract)

Background: Unlike in advanced colorectal cancer stages like stage III, adjuvant chemotherapy application in T3N0 colorectal cancer is consistently on debates. Lymphovascular invasion (LVI), known as a major prognostic factor in colorectal cancer, is also as one of the high-risk features that guide adjuvant chemotherapy. This study aimed to evaluate the prognostic importance and reliability of LVI as an adjuvant chemotherapy indicator in patients with T3N0 colorectal cancer and analyze the impact of adjuvant chemotherapy on oncologic outcomes in these patients. **Material and Methods:** We included 1634 patients with pathologically proven T3N0 colorectal cancer who underwent curative radical resection at Asan Medical Center, Seoul, Korea between January 2012 and December 2016. For review cohort of 242 patients, pathologic slide review including dual immunohistochemistry with D2-40 and CD 31 to identify lymphatic and vascular invasion, was performed. Changes on the reviewed LVI status after dual immunohistochemical stain (rLVI) were evaluated. The rLVI included small blood and lymphatic vessel invasion and large vessel invasion. Revised LVI (ReLVI) excluded large vessel invasion after rLVI was distributed. The association between high-risk features including perineural invasion, preoperative obstruction, resection margin involvement, and redistributed LVI that indicated adjuvant chemotherapy, as well as adjuvant chemotherapy with recurrence free survival (RFS) and overall survival (OS) for the overall and review cohort were analyzed. **Results:** In the overall cohort, 772 patients received adjuvant chemotherapy. Among chemotherapy group, 179 patients (23.2%) had LVI as the only high-risk feature. LVI and perineural invasion (PNI) were found in 384 (23.5%) and 272 patients (16.5%), respectively, in the overall cohort. The 5-year RFS was 92% and 5-year OS was 91.4%. High-risk features such as preoperative obstruction, PNI, and positive margins were significantly associated with RFS and OS. Adjuvant chemotherapy and LVI were not associated with RFS (p=0.593) or OS (p=0.218). In the review cohort, the diagnosis of LVI was changed in 82 (33.2%) and that of PNI was changed in 25.2% of the patients. LVI and large vessel invasion were not associated with RFS and OS. The rLVI only showed relationship with RFS in the univariate study while reLVI

which excludes large vessel invasion did not. **Conclusions:** In this cohort of patients with T3N0 colorectal cancer, the prognostic importance of LVI is not defined and may cause diagnostic inaccuracy and low recurrence rate prediction. There might be a need to reconsider LVI as a guiding sign for adjuvant chemotherapy and consensus on detailed definition.

Key Words: colorectal cancer, lymphovascular invasion, adjuvant chemotherapy, recurrence, survival.

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Introduction

The colorectal cancer, which gained the third place in incidence and second in mortality worldwide in 2020 [1], has set guidelines for treatment and surveillance accumulated over a long period of time and experience. Some portions of the colorectal cancer treatment have concrete consensus, but some other factors have been on controversy. The benefits of adjuvant chemotherapy after resection in stage III colorectal cancer have been well recognized [2-4]. However, whether adjuvant chemotherapy is beneficial or not in the lymph node-negative cancer, particularly pathologic T3N0 cancer, is still debatable [5-8]. Although the chemotherapy has been proven to affect tumor recurrence and survival [9], adjuvant chemotherapy in pT3N0 colorectal cancer does not have enough beneficial evidence to overcome the complications of chemotherapy [10-14].

To make a decision of indicating adjuvant chemotherapy to patients with pT3N0 colorectal cancer, we usually adapt high-risk features for prognosis. These high-risk features include: preoperative obstruction, perineural invasion (PNI), lymphovascular invasion (LVI), <12 lymph nodes retrieved, poor differentiation, and resection margin involvement. If a patient clinically or pathologically has the high-risk features, it is believed that the features implement worse prognosis such as more recurrence and greater mortality, encouraging adjuvant chemotherapy application to ameliorate the recurrence and mortality risks.

Among abovementioned high-risk features, LVI and PNI are the well-known prognostic factors for oncologic outcomes [15-18]. However, diagnosis of LVI and PNI depends on observer and frequently has been the hot spot for debates. Traditional Hematoxylin and Eosin (H&E) staining has been routinely used to identify LVI, which has been denoted as vascular invasion regardless of the types of vessels involved, either lymphatic vessel or blood vessels. However, additional staining methods including elastic tissue staining and podoplanin, and other immunohistochemical (IHC) staining have been developed to distinguish between lymphatics and blood vessels, resulting in changes in diagnosis [19-21]. This change in diagnosis methods led to interobserver variability that has resulted in diversion of diagnosis in over 20% of cases [22].

Although CAP(College of American Pathologists) established definition of LVI to reduce vagueness, diagnosis of LVI is still a challenge for pathologists [23]. The guideline identified small vessel invasion including lymphatic and blood vessel invasion without muscle layer as LVI, distinct from large vessel invasion and insisted separate reports for them. The pathologic report of LVI definition in Asan medical center (AMC), Seoul, South Korea has been following the CAP guideline since 2017. However, LVI usually encompassed the large vessel invasion and other small vessel invasion including lymphatic vessel invasion altogether, not specifically separating them before 2017.

Whether before or after 2016, physicians have applied the adjuvant chemotherapy to the pT3N0 patients with high-risk features described above, including LVI, PNI, positive margin, etc. on the pathologic reports. Because LVI report system changed, we wanted to know if LVI before 2017 and revised LVI can show differences and importance in prognosis in the pT3N0 patients. Also, by thorough intensive slide review including dual immunohistochemistry staining, we tried to check the variability of the pathologic high-risk features such as LVI and PNI, to verify the utility of them as an adjuvant chemotherapy indicator.

Materials and methods

Patients and treatment, surveillance

Due to change in report system from 2017 in AMC, we collected the data of colorectal cancer patients from January 2012 to December 2016 who underwent curative surgical resection in AMC, Seoul, South Korea in retrospective manner. It is approved by institutional review board and waived informed consent (IRB No. 2017-0955). They followed routine preoperative stage assessment including CT, endoscopy, and serum CEA values before resection, and additional metastasis work up including chest CT and Positron Emission Tomography (PET) CT if needed. 8786 patients were included for primary colorectal adenocarcinoma, first. Stage IV patients (n=1479), local excision (n=272), and inaccurate staging patients (n=18) were excluded. Out of 7017 patients after the exclusion, 1409 patients were excluded due to concurrent other malignancy (n=208), synchronous or metachronous colorectal

cancer (n=169), patients who received preoperative chemotherapy (n=996), and Familial adenomatous polyposis (FAP) and Lynch syndrome (n=36). From the 5608 patients of stage I-III colorectal cancer, through pathologic reports, after exclusion of extended resection (n=77), pathologic T4 (n=249) and T1-2(n=1839), and patients with metastatic LNs (n=1809), total of 1634 pathologically confirmed T3N0 patients were included finally (Figure 1).

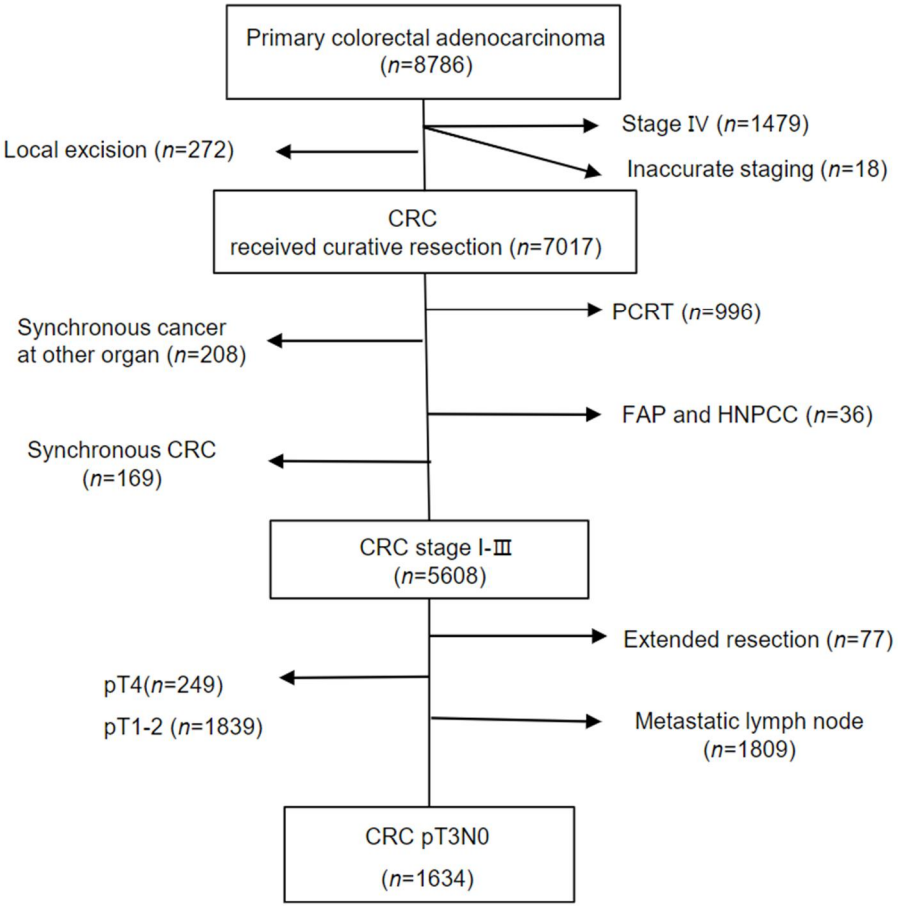


Figure 1. Inclusion and Exclusion criteria for the overall cohort

We collected clinical characteristics of them including age, sex, preoperative obstruction, medical co-morbidity, and preoperative CEA values, and pathologic information such as LVI, PNI, the number of lymph nodes harvested, MSI (Microsatellite Instability), and margin involvement.

Adjuvant chemotherapy was recommended for pT3N0 colorectal cancer patients with one of the following high-risk features; presence of preoperative obstruction which contains both endoscopic obstruction and clinically total obstruction, inadequate LNs examination less than 12, presence of LVI,

PNI, poorly histologic differentiation, and resection margin involvement by tumor. The microsatellite instability (MSI) status of the tumor was evaluated.

The adjuvant chemotherapy regimen included oral 5-FU(Fluorouracil)/Leucovorin (425mg/m²/20mg/m² per dose for 5 days), FOLFOX (5-FU/Leucovorin/Oxaliplatin 2800mg/m²/400mg/m²/85mg/m² per dose), oral Capecitabine (1250mg/ m² twice a day for maximum 14 days), and XELOX(Capecitabin/Oxaliplatin 1000mg/ m² twice a day for maximum 14 days/ 130mg/ m² on day 1).

Surveillance

Patients underwent a standardized postoperative follow-up, including physical examination, serum CEA, laboratory test with complete blood cell count, liver function test, and/or chest radiographs every 6 months during 5 years after surgery. Patients also underwent abdomino-pelvic CT scanning every 6 months. Colonoscopy was performed within 1 year after the operation, then once every 2 or 3 years. If patients had preoperative obstruction, colonoscopy was performed within 6 months after operation.

Recurrence was diagnosed based on imaging modality and confirmed pathologically by biopsy if possible. When pathologic confirmation was not possible, diagnosis of recurrence was made combining more than 2 imaging modality report or serial change on same imaging method. Local recurrence was defined as the presence of a suspicious lesion around the primary tumor operative field (the site of anastomosis, the bed of the primary resection, etc.). Distant metastasis was defined as recurrence at the place other than local recurrence, for instance, lung or liver. The RFS was defined as the interval between the date of resection of the primary tumor and the date of diagnosis of recurrence.

Pathologic examination

The original pathologic reports followed routine diagnosis protocol, using H&E staining that included including PNI, LVI, MSI-status, the number of retrieved lymph nodes, and resection margin

status. These pathologic reports declared the LVI disregarding lymphatic or small vessel invasion and large vessel invasion. Original H&E stain could identify LVI and Large vessel invasion (LaVI) in some range by identifying specific features such as protruding tongue sign or Orphan arteriole sign (Figure 2).

2-a 2-b

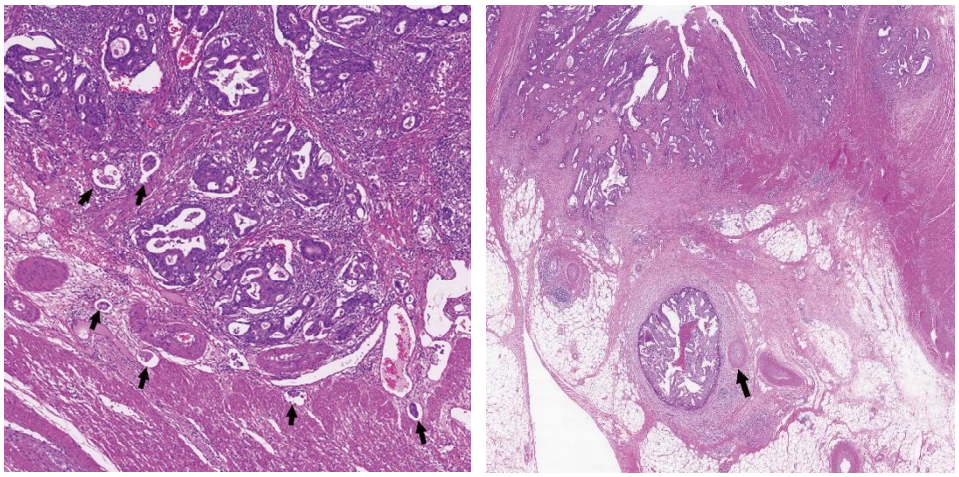
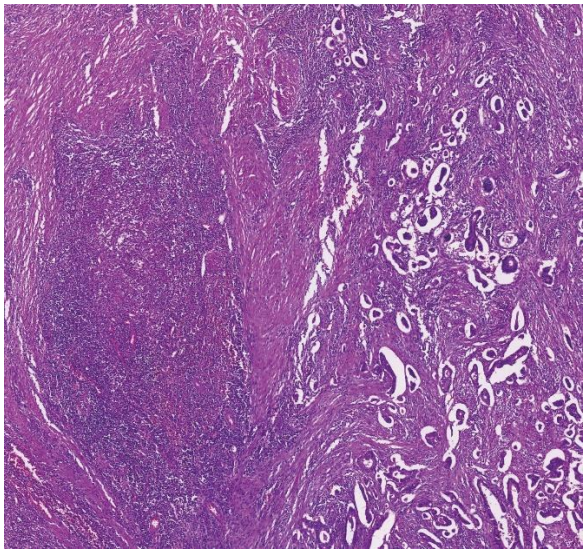


Figure 2. Original H&E staining slides for lymphovascular invasion; 2-a. LVI feature- tumor cell clusters floating in clearly delineated microvascular space(arrow); 2-b. LaVI feature- Protruding tongue sign and Orphan arteriole sign(arrow)

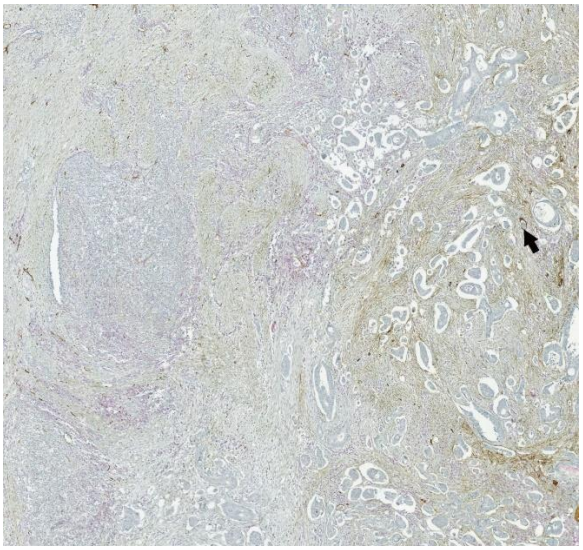
For MSI- status, the cases were evaluated by the microsatellite markers (BAT 25, BAT 26, D17S250, D2S123, and D5S346) and distributed to three groups. First, MSI-H group is a high-frequency group which have more than 2 unstable markers and MSI-L group stands for low frequency group with 1 unstable marker. Finally, Microsatellite stability (MSS) means no unstable markers [24]. The resection margin involvement, especially circumferential resection margin, was judged by gross abutment and invasion within 1mm microscopically.

From the overall cohort, we identified patients receiving surgical resection during 2014 as the "review cohort". This review cohort underwent a detailed pathologic review that included PNI and its location, mucin, tumor grade, budding, resection margin status, and LVI. PNI which checked with dedicated review was defined as reviewed PNI (rPNI). If reviewed diagnosis was equivocal, in addition to routine H&E staining, dual staining (D2-40 and CD31) was performed for the differential diagnosis of lymphatic vs. small blood vessel invasion (Figure 3).

3-a



3-b



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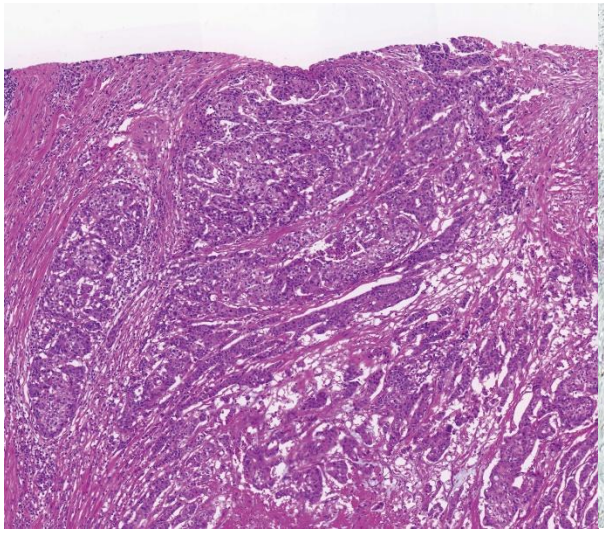


3-d

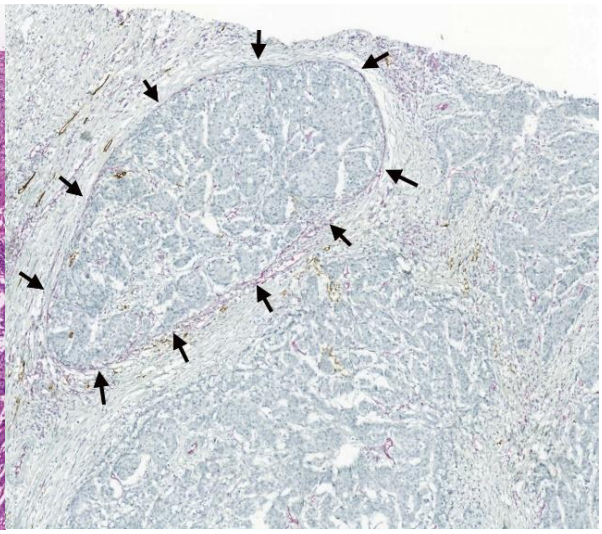


Figure 3. Comparison between original H&E stain and dual immunohistochemistry staining. 3-a&b. Lymphatic invasion showing few true LI (arrow) after dual IHC; 3-c&d. Small vessel invasion that could only be identified by IHC CD31 (arrow)

D2-40 was used to detect lymphatic invasion, while CD31, an endothelial cell marker, was used to detect vascular invasion [25]. LVI was then described according to CAP's guidelines, and was separately defined as lymphatic invasion, small vessel invasion, or large vessel invasion. The reviewed LVI (rLVI) included all the lymphatic invasion, small blood vessel invasion, and large vessel invasion. The small vessel invasion was defined as a tumor deposit in or over the thin-walled lumen such as

lymphatics or capillaries, and the large vessel invasion was a tumor invasion in or beyond the endothelium structure with smooth muscle layer. The location of invasion was also evaluated as intramural, extramural, intra-tumoral, and peritumoral. In the review cohort, LVI was divided into large vessel invasion and revised LVI (reLVI, small blood vessel invasion or lymphatic invasion). Two pathologists reviewed the slides until a consensus was reached.

Statistical analysis

The primary endpoint was recurrence after the resection. RFS was the interval between the operation date and the diagnosis date of relapse including both local recurrence and distant recurrence. Also, OS was drawn from the period between the surgery and the date of any cause of death.

For continuous variables, the mean value with standard deviation (SD) was calculated and compared by the Student's t-test. Patients were categorized into 2 groups as adjuvant chemotherapy and no adjuvant chemotherapy groups to evaluate association of risk factors with RFS and OS according to receipt of adjuvant chemotherapy. In comparison between chemotherapy and no chemotherapy group, independent-samples Student's t-test was used in continuous variables, and Chi-square test or Fishers' exact test was used for categorical ones. To evaluate the degree of discrepancy in LVI and PNI between results obtained before (original report) and after (review report) the slide reviews with dual IHC, we used Chi-square test. The association between the clinicopathological risk factors and recurrence was evaluated using a logistic regression test. Furthermore, risk factors according to recurrence types such as lymph nodes, peritoneal seeding, or distant metastasis were also analyzed.

Survival curves of OS and RFS were plotted using the Kaplan-Meier method and compared using the log-rank test. To assess the prognostic impact of the risk factors on RFS and OS, univariate Cox regression test was performed first for each risk factor. Among those factors, if the p-value was less than 0.1 in the univariate test, the relevant factors were included in multivariate Cox-regression test, in order. These tests described above were performed equally in the overall cohort and the review

cohort. The results were considered statistically significant if the p-value was less than 0.05. Data analysis was drawn with SPSS software (version 18.0; IBM Statistics, Armonk, NY, USA)

Results

Clinicopathological characteristics of patients with pT3N0 colorectal cancer

The clinicopathological features of the overall and review cohort are showed in Table 1. Colon cancer (1166, 71.4%) was more than rectal cancer. For colon cancer, right hemicolectomy was done the most as it sums up to 543 cases, followed by 445 anterior resection cases, 107 left hemicolectomy operations, 65 for lower anterior resection, and 4 for Hartmann's operation. Among 468(28.6%) patients with rectal cancer, 459 received sphincter-saving operations such as anterior resection, lower anterior resection, ultra-low anterior resection, and Hartmann's operation (44, 341, 67, and 5 cases, respectively).

Table 1 Clinicopathologic features of the patients

	The overall cohort (n=1634)	**The review cohort (n=242)**
Age average(range)	63(24~94)	62(25~90)
Sex		
Male	971 (59.4%)	136(56.2%)
Female	663 (40.6%)	106 (43.9%)
Location		
Colon	1166 (71.4%)	172 (71.1%)
Rectum	468 (28.6%)	70 (28.9%)
LNs acquired average(range)	27(4~86)	28(8~79)
LNs acquired <12	17 (1.0%)	5 (2.1%)
LVI	384 (23.5%)	57 (23.6%)
PNI	272 (16.6%)	32 (13.2%)
Differentiation		
WD	141 (8.6%)	25 (10.3%)

MD	1379 (84.4%)	199 (82.2%)
PD	50 (3.1%)	6 (2.5%)
MUC/SRC	3 (0.2%)	1 (0.4%)
Unknown	61 (3.7%)	11 (4.5%)
Preoperative obstruction	437 (26.7%)	82 (33.9%)
Positive margin	22 (1.3%)	6 (2.5%)
MSI status		
MSS	1292 (79.1%)	199 (82.2%)
MSI-L	62 (3.8%)	0 (0%)
MSI-H	169 (10.3%)	27 (11.2%)
Unknown	111 (6.8%)	16 (6.6%)
Adjuvant chemotherapy		
Yes	772 (47.2%)	127 (52.5%)
No	853 (52.2%)	111 (45.9%)
Unknown	9 (0.6%)	4 (1.7%)

LNs, Lymph nodes ; LVI, lymphovascular invasion; PNI, perineural invasion; WD, well differentiated; MD, Moderately differentiated; PD, Poorly differentiated; MUC/SRC, Mucinous or signet ring cell tumor; MSI, Microsatellite instability; MSS, Microsatellite stable; MSI-L, MSI-Low frequency; MSI-H, MSI-High frequency

In overall cohort, the mean age was 63 ± 12 (SD, 24 - 94) years old. 971 patients (59.4%) were males. LVI and PNI were observed in 384 (23.5%) and 272 (16.6%) patients, respectively. The average number of examined LNs found with standard deviation was 27 ± 11 (4 - 86). 17 cases were reported with the LNs acquired less than 12. For tumor differentiation, moderate differentiation was the most common differentiation grade (n=1379, 84.4%), and poorly differentiated tumor was found in 50 cases. Among the 437 (26.7%) patients with preoperative obstruction found, 351 had endoscopic and 86 showed complete symptomatic obstruction. Total of 22 (1.3%) had a resection margin involvement, in which circumferential margin involvement was observed in 11 patients.

For the review cohort, 242 patients who received surgical resection in 2014 were included. There was no significant difference on average age, sex ratio, tumor location with the overall cohort and other risk factors except preoperative obstruction between overall and review cohort. In the review cohorts, they had higher rates of preoperative obstruction (26.7% vs. 33.9%, p=0.028) without specific reasons documented.

772 patients (47.2%) received adjuvant chemotherapy. The chemotherapy regimens varied; 279 of 5-FU/Leucovorin, 196 of FOLFOX, 150 of capecitabine, 22 UFT-E/Leucovorin, and others. 527 patients (68.3%) of them had at least 1 risk factor (Table 2) and 351 (45.5%) patients took chemotherapy when they had only one risk factor. 280 people (36.3%) in the chemotherapy group had LVI, while preoperative obstruction was found in 236 patients (30.6%). Adjuvant chemotherapy was applied in 179 patients when they had the LVI as a single risk factor. PNI was observed in 176 patients in the adjuvant chemotherapy group.

Table 2. Clinicopathological features of the patients according to adjuvant chemotherapy

	Adjuvant chemotherapy (n=772)	**No adjuvant chemotherapy (n=853)**	**P-value**
Age average(range)	59(27~86)	67(24~94)	<0.001
Sex			0.311
Male	468(60.6%)	496(58.1%)	
Female	304(39.4%)	357(41.9%)	
Location			<0.001
Colon	492(63.7%)	669(78.4%)	
Rectum	280(36.3%)	184(21.6%)	
LNs acquired average(range)	28(4~83)	27(6~86)	0.155
LNs acquired <12	6 (0.8%)	11 (1.3%)	0.311
LVI	280(36.3%)	104(12.2%)	<0.001
PNI	176(22.8%)	95(11.1%)	<0.001
Differentiation			0.116
PD	29(3.8%)	20(2.3%)	
MSI-H	73(9.5%)	95(11.1%)	0.218

Preoperative obstruction	236(30.6%)	199(23.3%)	0.001
Positive margin	12(1.6%)	10(1.2%)	0.506
CRM involve	7(0.9%)	4(0.5%)	
No. of risk factor			<0.001
No risk	245 (31.7%)	528(61.9%)	
Single	351 (45.5%)	238(27.9%)	
More than 2	176 (22.8%)	87(10.2%)	

LNs, Lymph nodes ; LVI, lymphovascular invasion; PNI, perineural invasion; PD, Poorly differentiated; MSI, Microsatellite instability; MSI-H, MSI-High frequency; CRM, Circumferential resection margin

Compared to the chemotherapy group, no chemotherapy group patients were significantly older (mean age, 59 vs. 67, $p<0.001$), and consisted of more colon cancer than rectal cancer. LVI and PNI were markedly less in no chemotherapy group. 325 patients (38.1%) had more than 1 risk factors in the no adjuvant chemotherapy group, and this was significantly lower than 527 cases (68.3%) of the adjuvant chemotherapy group ($p<0.001$).

Results of review including dual IHC of review cohort

In the review cohort, total of 109 patients had large, small blood vessel, or lymphatic invasion, which we defined as rLVI. 82 patients (33.9%) were converted diagnosis of LVI after reviewing. 15 patients who were diagnosed to have LVI had no invasion after the review, and 67 patients out of 185 patients who initially did not have LVI were found to have it (Table 3).

Table 3. Lymphovascular invasion report change after review

	LVI before review, n (%)		**Total n(%)**
	LVI-	**LVI+**	

rLVI (SVI+LVI+LaVI) after review (%)	LVI –	118(48.8)	15(6.2)	133(55.0)
	LVI +	67(27.7)	42(17.4)	109(45.0)
Total n(%)		185(76.4)	57(23.6)	242(100)

LVI, lymphovascular invasion; SVI, Small vessel invasion; LI, Lymphatic invasion; LaVI, Large vessel invasion; rLVI, LVI after review

Location of small or lymphatic invasion, and large vessel invasion were evaluated. 75 patients with small or lymphatic vessel invasion, comprised of 59 intramural invasion (25 intratumoral and 34 peritumoral invasion) and 16 extramural invasions (2 intratumoral and 14 peritumoral invasion) (Figure 3-a). The large vessel invasion was observed in 47 patients, in which, there were 13 patients for intramural invasion (3 intratumoral and 11 peritumoral invasion), 31 for extramural (2 intratumoral and 29 peritumoral invasion), and 2 for distant invasion (Figure 3-b). Using D2-40 staining, 74 patients were identified as lymphatic invasion, distinct from blood vessel invasion among the rLVI group. In those with lymphatic invasions, 57 cases were intramural invasion and 17 were extramural invasion.

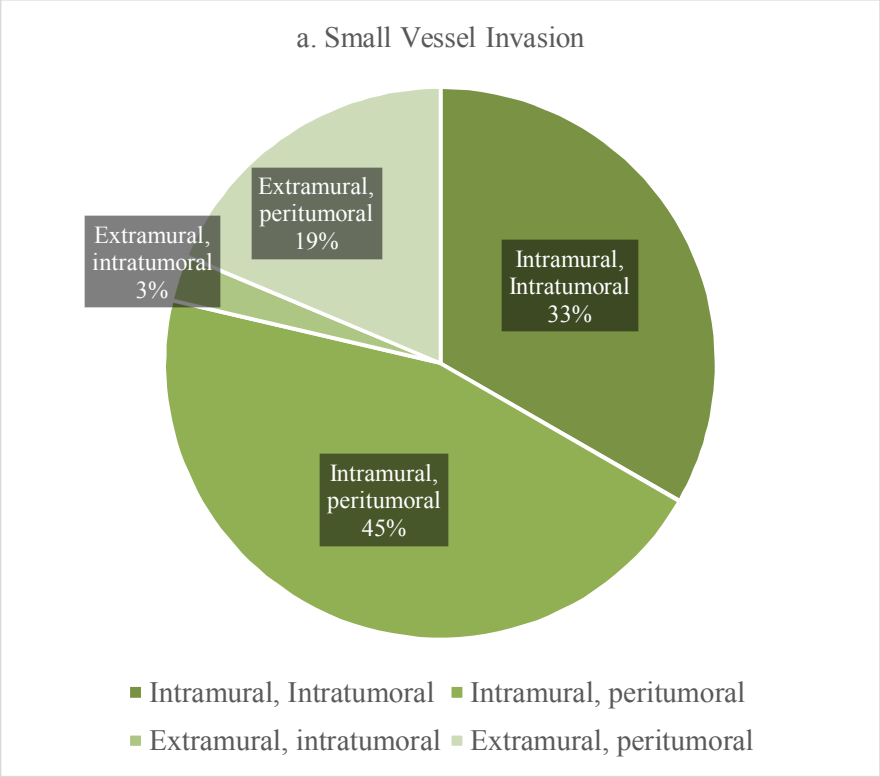
PNI status was also changed via meticulous review of slide (Table 4). 61 out of 242 (25.2%) patients met different diagnosis. The review made more detection of PNI up to 56 patients from 210 initially PNI-negative patients.

Table 4. Perineural invasion report change after review

		PNI before review, n (%)		**Total n(%)**
		PNI-	**PNI+**	
PNI_change n(%)	PNI – after review	154(63.6)	5(2.1)	159(65.7)
	PNI + after review	56(23.1)	27(11.2)	83(34.3)

Total n(%)	210(86.8)	32(13.2)	240(100)

PNI, perineural invasion



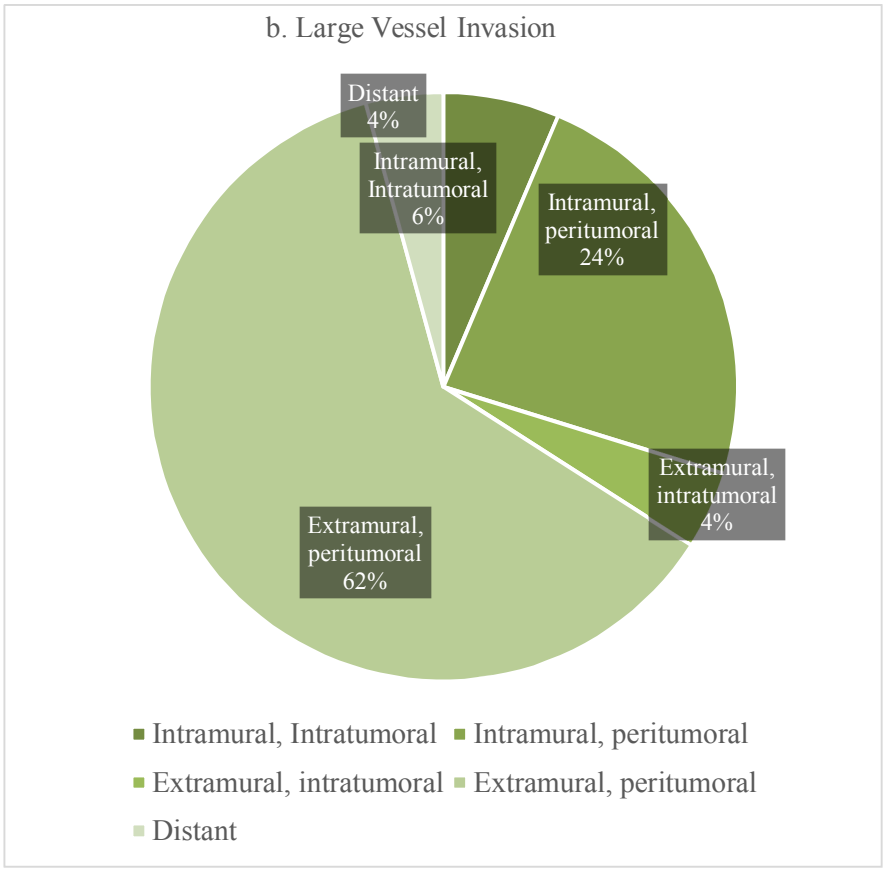


Figure 4. Small and Large vessel invasion locations

Recurrence and associated factors

In the overall cohort, 116 patients (7.1%) experienced the recurrence. The most common recurrence site was the lung (n=43, 37.1%), followed by liver (n=39, 33.6%), peritoneal seeding (n=16, 13.8%), and lymph nodes (n=11, 9.5%); local recurrence was also seen (n=10, 8.6%). Age, preoperative obstruction, PNI, and positive margin were associated with recurrence in the univariate analysis (all $p<0.05$). In the multivariate analysis, preoperative obstruction (HR, 1.575; 95% CI, 1.051-2.359), PNI (HR, 2.693; 95% CI 1.753-4.139), and positive margin (HR, 3.979; 95% CI, 1.381-11.468) were confirmed as risk factors for recurrence (all $p<0.05$). Adjuvant chemotherapy was not associated with recurrence (Table 5, HR 0.972, p=0.824). In the review cohort, we evaluated whether discrepancies between the rLVI and rPNI diagnosis were risk factors for recurrences and found no statistically significant results in multivariate analysis (eTable1).

Risk factors associated with recurrence were analyzed according to location of recurrence. For

LN recurrence and liver metastasis, there was no significant risk factor in the multivariate study. Preoperative obstruction was associated with peritoneal seeding (multivariate analysis, HR 2.856; 95% CI, 1.063-7.675, p=0.038). For lung metastasis, obstruction and PNI were associated factors (HR 2.038. 95% CI, 1.095-3.792, p=0.025 for the obstruction and HR 3.477, 95% CI, 1.855-6.518, p<0.01 for PNI). Local recurrence was related with only <12 LNs harvest in univariate study (HR 10.736, 95% CI, 1.284-89.792, p=0.028) but it was not confirmed in multivariate analysis.

RFS, OS, and risk factors according to the indication of adjuvant chemotherapy

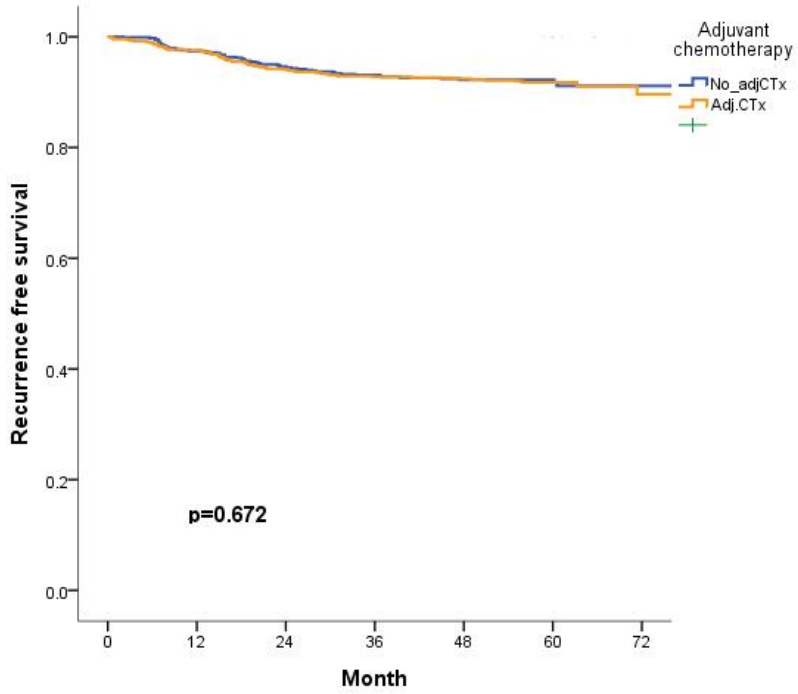
The 5-year RFS and the OS rate were 92% in the overall cohort and 91.4%, respectively. Adjuvant chemotherapy administration was not associated with RFS (Figure 5).

Table 5. Risk factors associated with recurrence in the overall cohort with pT3N0 colorectal cancer

		Univariate analysis		Multivariate analysis		
Variables	**No. (%)**	**HR**	**P-value**	**HR**	**95% CI**	**P-value**
Age		1.017	0.044	1.015	0.998-1.032	0.077
Adj. CTx.	772(47.2)	0.972	0.824			
LNs<12	17(1.0)	0.783	0.813			
Preoperative Obstruction	437(26.7)	1.648	0.014	1.575	1.051-2.359	0.028
PD	50(3.1)	0.996	0.945			
LVI	384(23.5)	1.452	0.078	1.177	0.759-1.825	0.466
PNI	272(16.6)	2.861	<0.001	2.693	1.753-4.139	<0.001
Positive margin	22(1.3)	4.054	0.007	3.979	1.381-11.468	0.011

Adj. CTx., Adjuvant chemotherapy; LVI, lymphovascular invasion; PNI, perineural invasion; PD, Poorly differentiated; HR, Hazard ratio; CI, confidence interval

5-a. Overall cohort



5-b. Review cohort

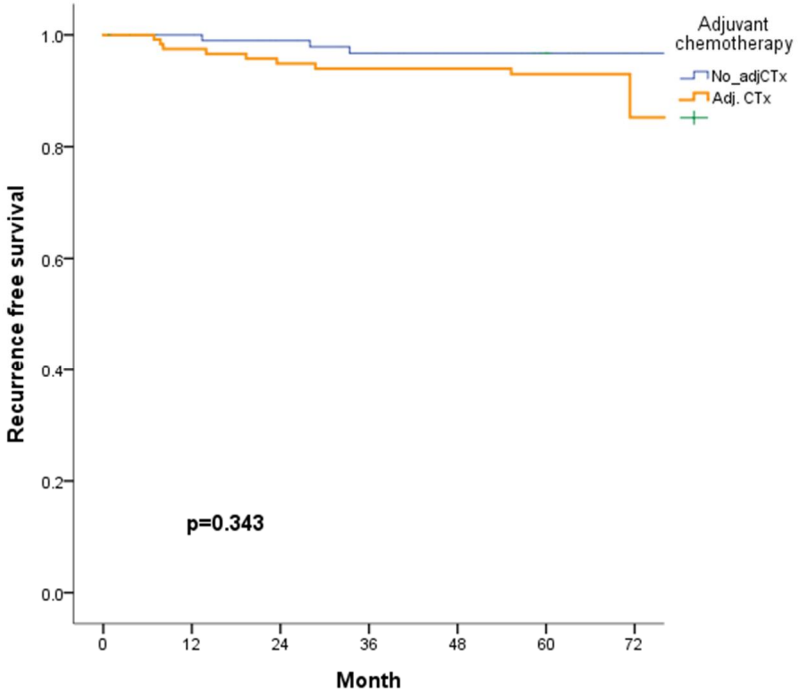
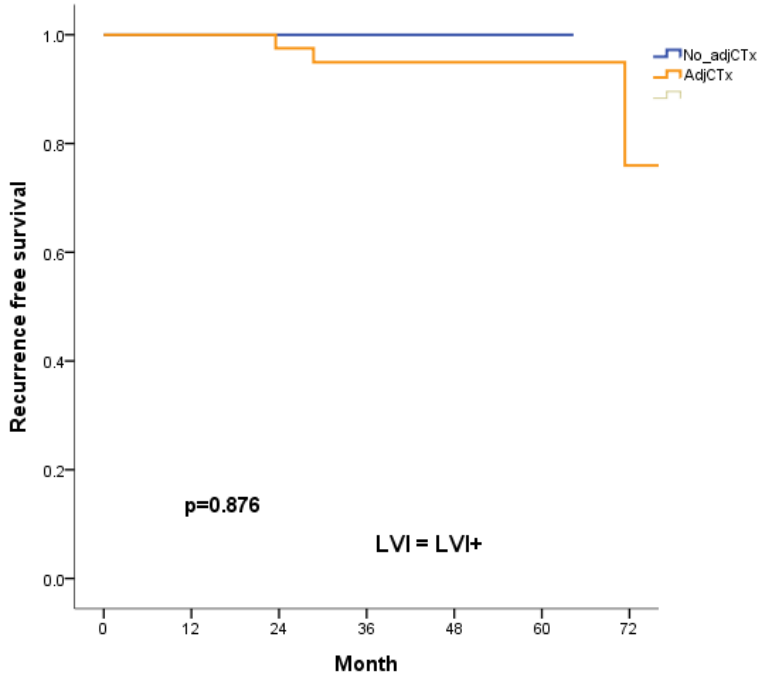


Figure 5. Recurrence free survival according to adjuvant chemotherapy

RFS was compared according to receipt of adjuvant chemotherapy in patients with each "high-

risk features" (Figure 6-9). Adjuvant chemotherapy was not associated with RFS in patients categorized by high-risk feature.

6-a. Overall cohort



6-b. Review cohort

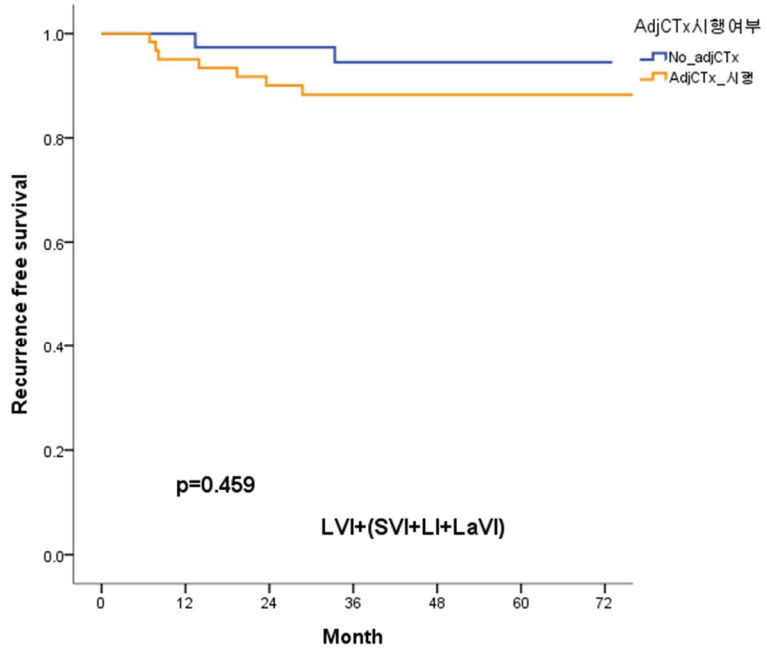
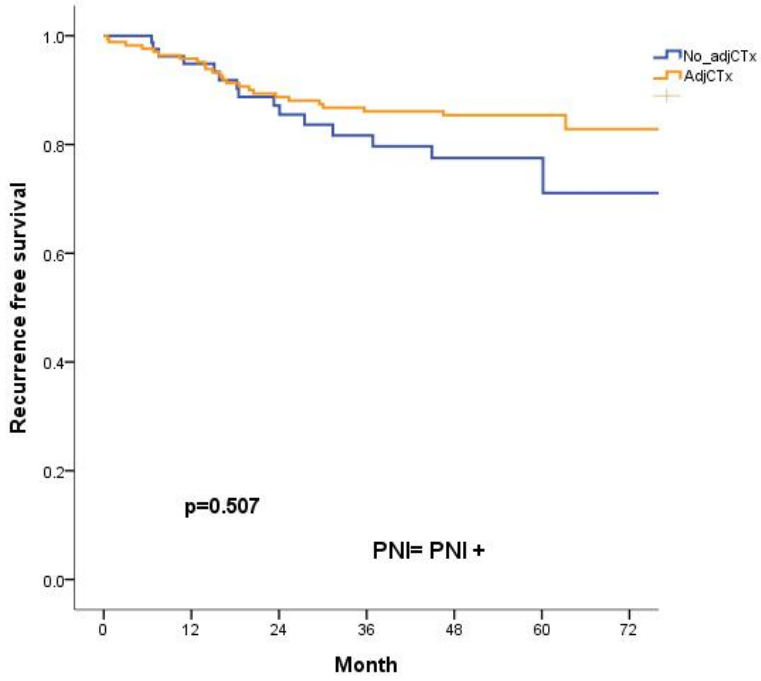


Figure 6. Recurrence free survival according to adjuvant chemotherapy appliance in lymphovascular invasion positive patients

7-a. Overall cohort



7-b. Review cohort

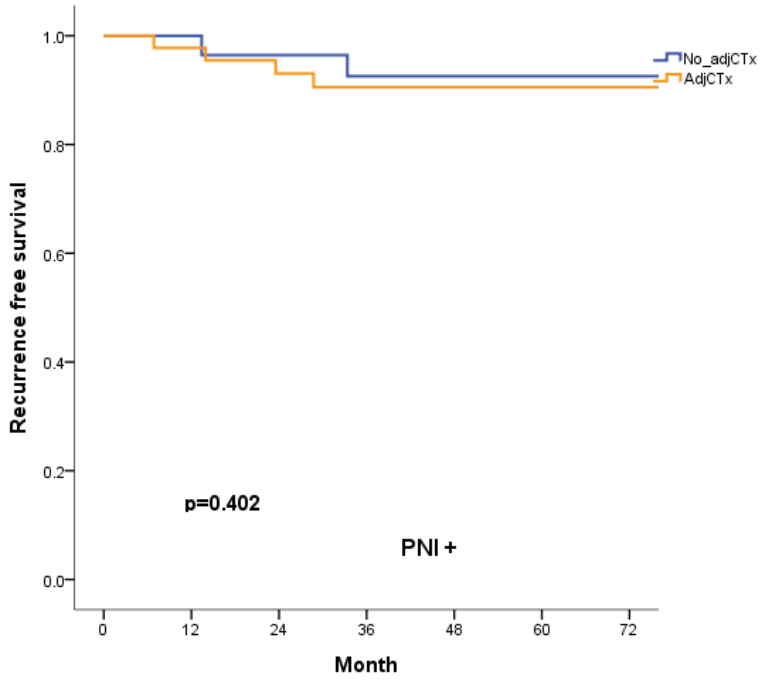
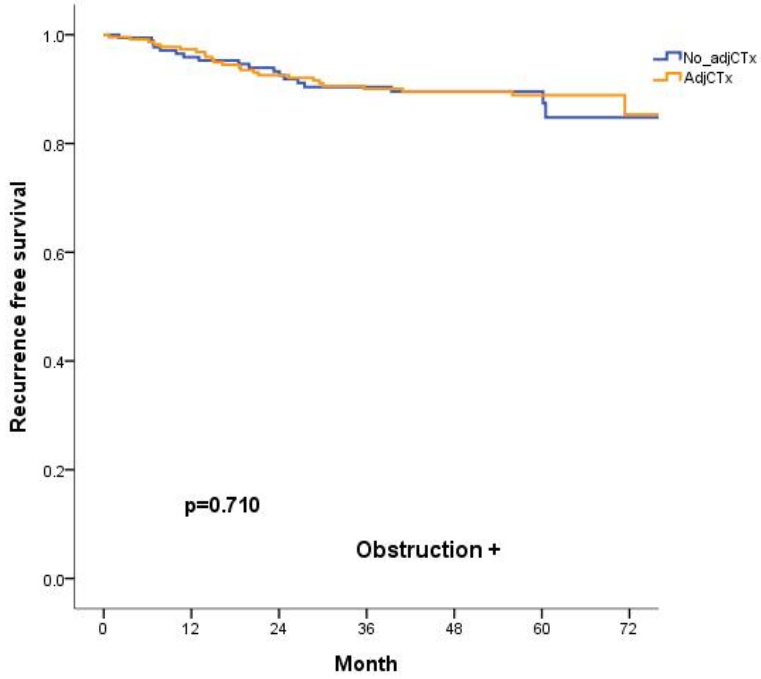


Figure 7. Recurrence free survival according to adjuvant chemotherapy appliance in Perineural invasion positive patients

8-a. Overall cohort



8-b. Review cohort

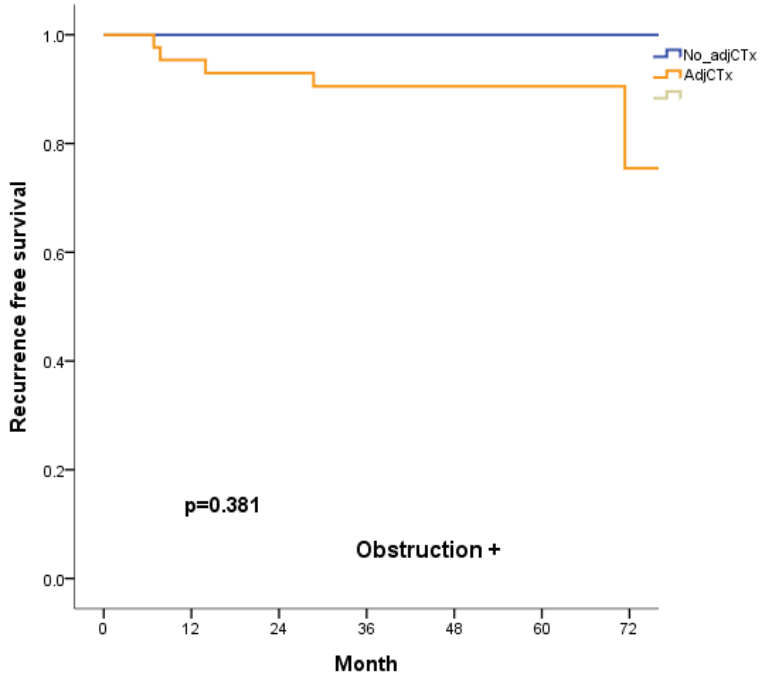


Figure 8. Recurrence free survival according to adjuvant chemotherapy appliance in Preoperative obstructive patients

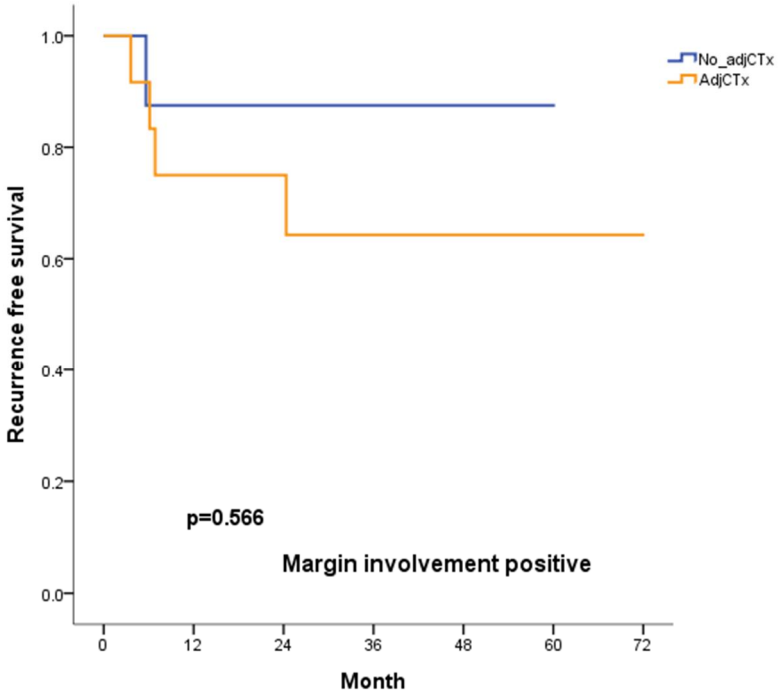
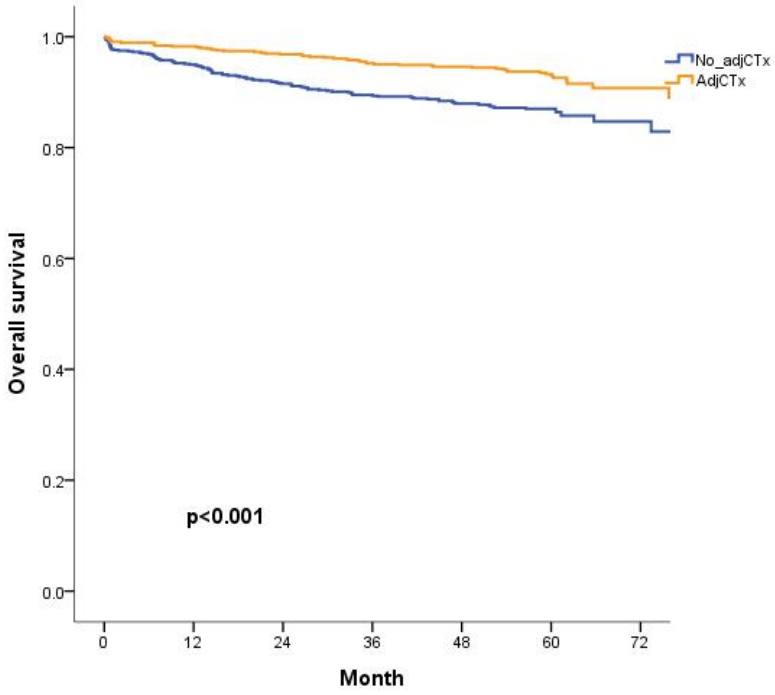


Figure 9. Recurrence free survival according to adjuvant chemotherapy appliance in positive resection margin patients

Adjuvant chemotherapy group expressed better OS curve with statistical significance ($p<0.001$, Figure 10)

10-a. Overall cohort



10-b. review cohort

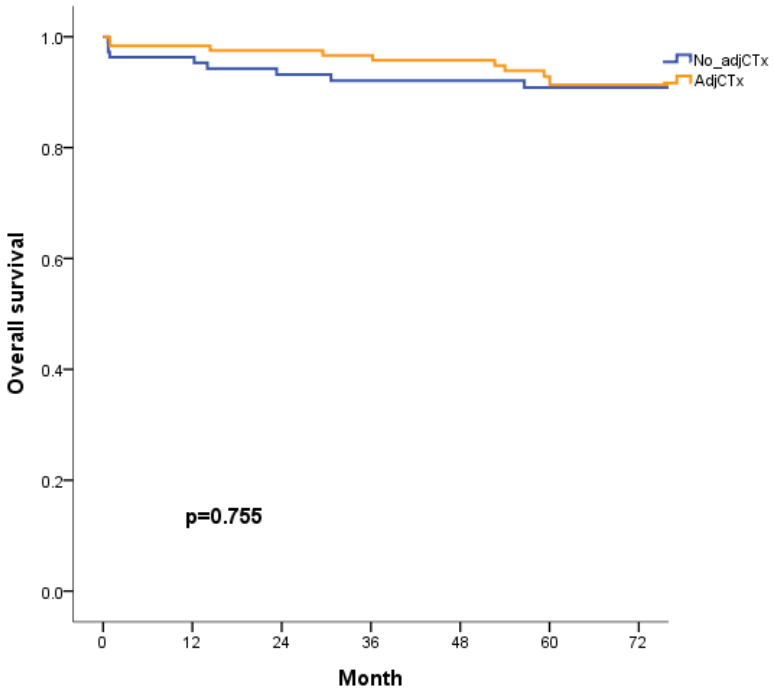


Figure 10. Overall survival according to adjuvant chemotherapy

Risk factors associated with recurrence free survival and overall survival

Among the high-risk features, preoperative obstruction, PNI, and positive margin status were statistically significant risk factors of RFS in univariate analysis (all $p<0.05$). Preoperative obstruction, PNI, and margin involvement were confirmed as associated factors with RFS in multivariate analysis (HR 1.602, CI 1.096-2.343; 2.737, 1.827-4.099; and 5.399, 2.176-13.399, respectively, all $p<0.05$). Adjuvant chemotherapy was confirmed that it did not improve RFS in the overall cohort ($p=0.593$, Table 6). When the number of risk factors were categorized, although single risk factor did not have significance, having more than 2 risk factors showed hazard ratio of 3.306 ($p<0.001$) in univariate study.

In the review cohort. 12 patients (5.0%) experienced recurrence. Reviewed LVI, encompassing small vessel invasion (SVI), lymphatic invasion (LI), and large vessel invasion (LaVI), was only associated factor with RFS ($p=0.043$) while revised LVI, including only LI and SVI, did not have statistical meaning with RFS ($p=0.149$) and LaVI did not show significance neither ($p=0.203$).

The rPNI diagnosed after the slide review and locations of each invasion factors were studied, and none of the factors showed differences in RFS. Following the location of invasion, the extents of the invasion were analyzed with RFS. Extramural small vessel invasion, intramural lymphatic invasion, and extramural PNI were related with poorer RFS in the univariate study (HR 10.186, 5.262, and 7.626, respectively) but none of them kept the significance in the multivariate study.

Risk factors associated with OS were also tested. Adjuvant chemotherapy administration improved the overall survival in the univariate study ($p<0.01$), but not in multivariate study in the overall cohort ($p=0.218$, Table 8). The number of LNs examined < 12, preoperative obstruction, PD feature, PNI, and positive margin status were related to the poorer OS in the univariate study ($p<0.05$). In the multivariate study, preoperative obstruction, PD grade, PNI, and margin involvement were still associated with worse OS (HR 1.580; CI 1.133-2.203, 1.099; 1.027-1.176, 2.136; 1.499-3.043, and 3.1; 1.139-8.439, $p=0.007$, 0.006, <0.001, and 0.027, respectively). LVI did not show association with OS ($p=0.341$). Adjuvant chemotherapy application on the patients with high risks described above had beneficial effect on OS ($p<0.001$)

For the review cohort. the 5-year OS rate was 93% (death=17). Preoperative obstruction was the risk factor related with worse OS rate in both univariate and multivariate study (Table 9). PD feature and margin invasion did not have significance for OS. After the review, rPNI showed association with OS in the univariate study (p=0.022), but it did not reach the statistical significance in the multivariate study (p=0.051, CI 0.994-6.694). reLVI and LaVI were not associated with OS in the review cohort (p=0.200 and 0.752, respectively).

Table 6. Factors associated with recurrence-free survival in the overall cohorts (n=1634)

		Univariate analysis		Multivariate analysis		
Variables	**No.(%)**	**HR**	**P-value**	**HR**	**95% CI**	**P-value**
Age		1.026	0.003	1.023	1.007-1.041	0.006
Adj. CTx.	772(47.2)	0.932	0.593			
LNs<12	17(1.0)	0.860	0.990			
Preoperativeobstruction	437(26.7)	1.666	0.008	1.602	1.096-2.343	0.015
PD	50(3.1)	0.999	0.987			
LVI	384(23.5)	1.449	0.067	1.157	0.762-1.756	0.494
PNI	272(16.6)	2.380	<0.001	2.737	1.827-4.099	<0.001
Positive margin	22(1.3)	4.705	0.001	5.399	2.176-13.399	<0.001

Adj. CTx., Adjuvant chemotherapy; LVI, lymphovascular invasion; PNI, perineural invasion; PD, Poorly differentiated; HR, Hazard ratio; CI, confidence interval

Table 7. Factors associated with recurrence-free survival in the review cohorts (n=242)

		Recurrence				
		Univariate analysis		Multivariate analysis		
Variables	**No.(%)**	**HR**	**P-value**	**HR**	**95% CI**	**P-value**
Age		1.017	0.503			
Adj. CTx.	127(52.5)	1.052	0.814			
LNs<12	5(2.1)	0.048	0.759			
Preoperative obstruction	82(33.9)	1.519	0.477			
PD	6(2.5)	0.668	0.635			
Positive margin	6(2.5)	3.279	0.257			
rLVI(LI+SVI+LaVI)	109(45.0)	3.859	0.043			
reLVI(LI+SVI)	75(31.0)	2.303	0.149			
LaVI	47(19.4)	2.186	0.203			
rPNI	83(34.3)	1.958	0.245			

Adj. CTx., Adjuvant chemotherapy; LVI, lymphovascular invasion; PD, Poorly differentiated; HR, Hazard ratio; SVI; Small vessel invasion; LI, Lymphatic invasion; rPNI: PNI changed after the review; LaVI, Large vessel invasion; rLVI, LVI after review; reLVI, revised LVI according to CAP guideline; CI, confidence interval

Table 8. Factors associated with Overall survival in the overall cohorts (n=1634)

		Death				
		Univariate analysis		Multivariate analysis		
Variables	**No.(%)**	**HR**	**P-value**	**HR**	**95% CI**	**P-value**
Age		1.096	<0.001	1.086	1.066-1.106	<0.001
Adj. CTx.	772(47.2)	0.518	<0.001	0.808	0.576-1.134	0.218
LNs<12	17(1.0)	2.892	0.036	2.095	0.772-5.881	0.146
Preoperative obstruction	437(26.7)	1.673	0.002	1.580	1.133-2.203	0.007
PD	50(3.1)	1.091	0.011	1.099	1.027-1.176	0.006
LVI	384(23.5)	1.191	0.341			
PNI	272(16.6)	2.254	<0.001	2.136	1.499-3.043	<0.001
Positive margin	22(1.3)	2.723	0.048	3.1	1.139-8.439	0.027

Adj. CTx., Adjuvant chemotherapy; LVI, lymphovascular invasion; PNI, perineural invasion; PD, Poorly differentiated; HR, Hazard ratio; CI, confidence interval

Table 9. Factors associated with Overall survival in the review cohorts (n=242)

		Death				
		Univariate analysis		Multivariate analysis		
Variables	**No.(%)**	**HR**	**P-value**	**HR**	**95% CI**	**P-value**
Age		1.074	0.002	1.074	1.026-1.123	0.002
Adj. CTx.	127(52.5)	0.749	0.467			
LNs<12	5(2.1)	0.049	0.714			
Preoperative obstruction	82(33.9)	2.953	0.023	3.412	1.327-8.772	0.011
PD	6(2.5)	1.023	0.846			
Positive margin	6(2.5)	2.332	0.411			
rLVI(LI+SVI+LVI)	109(45.0)	1.520	0.377			
reLVI(LI+SVI)	75(31.0)	1.837	0.200	1.491	0.569-3.906	0.417
LaVI	47(19.4)	0.819	0.752	0.879	0.246-3.148	0.843
rPNI	83(34.3)	3.015	0.022	2.579	0.994-6.694	0.051

Adj. CTx., Adjuvant chemotherapy; LVI, lymphovascular invasion; PD, Poorly differentiated; HR, Hazard ratio; SVI; Small vessel invasion; LI, Lymphatic invasion; rPNI: PNI changed after the review; LaVI, Large vessel invasion; rLVI, LVI after review; reLVI, revised LVI according to CAP guideline; CI, confidence interval

Discussion

Adjuvant chemotherapy was not related to better outcome in both recurrence event and RFS statistically in our study and although it showed better outcome in OS univariate study, there was no statistical significance in multivariate study. However, OS improvement by adjuvant chemotherapy in patients with statistically meaningful high-risk features was observed, reinforcing adequate application for specific patients. We could not find out association of LVI with RFS and OS in our analysis for the overall cohort. In our review cohort, the diagnosis of LVI, changed substantially over 30%, through the slide review. The new LVI (rLVI) included SVI, LI, and LaVI, so additionally Revised LVI (reLVI) without LaVI was separately examined and it did not show association with RFS or OS.

It is well defined that adjuvant chemotherapy was favorable for stage III colorectal cancer [10, 26]. However, effect of adjuvant chemotherapy in terms of RFS/OS improvement in pT3N0 colorectal cancer is still controversial and decision for adjuvant chemotherapy administration for T3N0 colorectal cancer is variable for the physicians and the patients. The American Society of Clinical Oncology (ASCO), the European Society of Medical Oncology (ESMO), and the National Comprehensive Cancer network (NCCN) suggest different guidelines for criteria of adjuvant chemotherapy treatment in pT3N0 colorectal cancer [2, 10, 27, 28]. They all take poorly differentiated grade of tumor, perforation, LVI, and PNI as the high-risk features. ESMO and NCCN agree on the number of lymph nodes acquired less than 12 and preoperative obstruction while ASCO sets the risk factor of the number of lymph nodes to be less than 13 and does not include the obstruction. In Asan Medical Center, the pathologic reports include all pathologic features among those risk factors above, and clinicians decide the chemotherapy according to the pathologic report and clinical events such as obstruction and perforation, taking account for all three guidelines mentioned before. This decision is usually multidisciplinary between the surgery department and oncology department, and interchangeable. Also, the patient factors including comorbidities, age, and free will affect the decision substantially.

Some studies reported higher survival outcome of the stage IIA colorectal cancer with adjuvant

chemotherapy [6, 9, 29]. They said the chemotherapy shows benefits in tumor recurrence and survival statistically [11, 30, 31]. QUASAR study, one of those studies, was published in 2007 and, had 3239 patients randomly assigned to chemotherapy group or no chemotherapy group. The study reported significant recurrence decrease up to 22% ($p=0.001$) and 18% of reduction in death ($p=0.008$). However, it also indicated that 5- year OS improvement was only 3-4%, leading others consider on the subtle benefits and disadvantages of chemotherapy. Achili et al. reported that the chemotherapy has a survival benefit in stage II colorectal cancer [6] and Kumar et al. insisted that adjuvant chemotherapy can have some benefits in T4 tumors among stage II disease [11]. However in other studies, chemotherapy does not seem to be beneficial option for colorectal cancer in RFS and OS, especially in early stage like Stage I and II cancer [8] and some trials showed no differences in oncologic outcomes after adjuvant chemotherapy, indicating that complications and harmful nature of chemotherapy can exceed the chemotherapy benefits [6, 10, 12, 32-34]. The Cancer Care Ontario program study in 2004 [33], a meta-analysis of 37 trials and 11 meta-analysis, analyzed stage II cancer randomly assigned to observation or adjuvant chemotherapy, did not advocate the chemotherapy in the study because even though it reported RFS improvement of 5% without p-value, there was no statistical value for OS improvement.

In our study, adjuvant chemotherapy did not show association with recurrence and no significant improvement in RFS. This insignificance can also be drawn from that the 5-year RFS rate of T3N0 cancer was 94.8%, too good to show distinctively better results with the chemotherapy, while Figuerdo et al. reported 5-year RFS usually less than 90% [33]. We also analyzed the chemotherapy effect when there is each significant risk factor in RFS, however, the effect of the chemotherapy was not observed in cases with high-risk features of PNI, LVI, preoperative obstruction, and margin positive patients.

On the other hand, adjuvant chemotherapy had benefits on OS in univariate analysis in this study. Also, for the patients with each risk factor which showed association with OS in univariate study, including PNI, resection margin involvement, lymph node less than 12, and poorly differentiated tumor, adjuvant chemotherapy had beneficial effect on OS statistically (all $p<0.05$), while LVI failed to show association with OS rate. Even though adjuvant chemotherapy by itself did not have statistical

significance in multivariate study, this finding of OS improvement by adjuvant chemotherapy when with certain high-risk features may implicate possible benefits and reason of postoperative chemotherapy in especially high-risk patients for improvement in OS.

Consequently, the role of high-risk features in chemotherapy decision is substantial. Schrag et al. reported that magnificent portion up to 27% in the stage II colorectal cancer in their study received adjuvant chemotherapy with uncertain benefits [35]. Likewise, in present study, 772 (47.2%) out of 1634 patients received chemotherapy. Comparing the 772 patients who had adjuvant chemotherapy after the surgery with no adjuvant chemotherapy group, 527 patients (68.3%) had at least 1 risk factor in the chemo group, and it were 325 patients (38.1%) in the non-chemo group ($p<0.001$). Also, having more than 2 risk factors was associated with worse RFS (HR 3.306, 95% CI, 2.210-5.154, $p<0.01$). It can be assumed that the presence of the risk factors affected the chemotherapy decision with expectation for better outcome. Interestingly, 351 patients (45.5%) were given the chemotherapy when with only one risk factor, and out of them, 179 cases (23.2%) were with only LVI. Although it seems that known high risk features affect the chemotherapy decision mostly, other conditions affect the choice, too. In this study, 87 patients, 10.2% of the no chemotherapy group were with more than 2 risk factors. In contrast, 245 patients, 31.7% of chemotherapy group did not have any risk factors. This means that other additional factors such as performance status of patients, age, co-morbidities, and refusal or patient's want could affect the decision hugely, not depending on the high-risk features recommendation only.

Based on the result of this study, Intensive adjuvant therapy would be considered for patients with certain high-risk features if they are related with poor oncologic outcomes. There are several studies which analyzed the prognostic value of the high-risk features and following effect of chemotherapy according to certain high-risk features. Achili et al. reported there is limited consensus on some risk factors specifically LVI, PNI, and MSI [6]. Rebuzzi et al. chose the insufficient lymph nodes less than 12 and differentiation grading as major significant risk factors and LVI as a minor prognostic factor due to its subtle difference in OS [7, 36].

Among those high-risk features, LVI, the most common risk factor in our study, is a frequent

subject on this issue. Many researchers reported prognostic significance of LVI in colorectal cancer [15, 18, 37, 38]. Skancke et al. said LVI in stage II colon cancer has significant effect on the overall survival, but not on the recurrence [18]. Other reports insisted its role in prediction of occult lymph node metastasis in node-negative colorectal tumors and therefore, additional staining should be considered to identify LVI better [16, 17]. In contrast, other physicians like Artac et al. reported no statistical value for association between LVI and RFS [30]. Zhang et al. analyzed the effect of adjuvant chemotherapy by each risk factor and there was no significant effect of adjuvant chemotherapy in LVI patients, especially in OS [39, 40]. Others including Lee et al. also could not find significance of LVI in stage IIA colon cancer [8, 41, 42]. Difference according to the tumor location existed, too. Hogan et al. reported LVI as a worse prognostic factor in only rectal cancer, not in colon cancer [43]. Present study could not find prognostic value of LVI in none of our multivariate analysis in the overall cohort, neither on recurrence nor on overall survival.

However, the utility of LVI is frequently on debates because of its subjectivity within diagnostic variability [19, 44-46]. Harris et al. reported the moderate variability of LVI diagnosis in their paper. Although the CAP(College of American Pathologists) guideline recommends pathologists to report separately LVI, as known as small vessel invasion in definition [23], from large vessel invasion, many studies reported LVI as lymphatic and blood vessel invasion altogether, resulting in wide disparity in diagnosis among the studies. Also, the CAP does not recommend IHC stain, but some added IHC staining for improvement in accuracy of the diagnosis due to limitations of conventional H&E stain, such as difficulty of identification of lymphatic invasion from blood vessel invasion [20, 21]. These different methods in diagnosis of LVI aggravate interobserver subjectiveness. Liang et al. reported the false positive rate of H&E stain used for LVI was up to 9.1% and the false negative rate as 12.6%, using additional podoplanin and CD34 staining. The dual stain extracted additional 53 vessel invasions which were hidden with previous H&E stain. They also subdivided the LVI into lymphatic invasion and blood vessel invasion and reported that blood vessel invasion showed association with distant metastasis while lymphatic vessel invasion lost its association with recurrence [22]. Kingston et al. compared the immunohistochemistry such as CD31and CD 34, and special staining like elastic Van Gieson, with

conventional H&E stain in their paper, and reported huge improvement in identifying vascular invasion. There were other studies which reported that only vascular invasion, not including lymphatic invasions, showed prognostic impact distinctively, emphasizing on additional staining system in diagnosis [47, 48].

The original pathologic reports in this study from 2012 to 2016, included large vessel invasion as a LVI and it was not identified separately, which means that LVI in the reports encompassed lymphatic vessel invasion, small vessel invasion, and large vessel invasion altogether. For the review of some slides with equivocal findings, additional staining was applied. We used dual IHC to diagnose LVI better and subdivided the LVI into lymphatic vessel and blood vessel invasion. Interestingly, over the 30% of the diagnosis were changed in the review cohort. Mostly the diversion was from negative to positive findings in LVI, either into lymphatic or blood vessel invasion. The rLVI, encompassing large vessel invasion with small vessel invasion was the only risk factor associated with RFS. In contrast, reLVI according to CAP guideline did not show significance with RFS and OS, neither. While Betge et al. reported blood vessel invasion as an independent predictor and lymphatic invasion was not [49], we could not find association with RFS and OS in large vessel invasion. The subdivision of location as intramural and extramural did not make significant difference in RFS and OS, neither.

On the other hand, PNI, the important risk factor in colorectal cancer, was proved as significant prognostic factor in recurrence and RFS in our overall cohort (HR 2.693 and 2.737). PNI was also associated with worse OS (HR 4.711, p=0.001). There are also many reports insisting prognostic importance of PNI in oncologic outcomes [50-52]. Knijn et al reported strong association of PNI with local recurrence and prognostic strength of it [51]. Yang et al. even stated that stage II colorectal cancer with PNI was similar with stage III patients, implicating the importance of postoperative chemotherapy in PNI positive patients [52]. Also, it changed less than the LVI did (25.2% vs. 33.9%) in our study after the review. The review did not require any additional stain, but only meticulous review was performed. This may indicate that PNI can be used as more stabilized high-risk feature for adjuvant chemotherapy between pathologists with original H&E stain if more specific consensus in diagnosis is made.

This study has some limitations. We could not have all our overall cohort pathologic slides

reviewed. We just randomly selected and reviewed the year of 2014, and this maybe have caused some selection bias, and this may have caused difference in risk factors association patterns with RFS between the review cohort and the overall cohort.

As we reviewed the slides, there were some cases in which the initial stage diagnosis was changed, however the number was very small. Also, the reasons of chemotherapy administration in individual patients were unclear in that 245 patients without any risk factor received the treatment. Likewise, we did not categorize the reasons of why 87 patients did not receive the treatment when they had more than 2 risk factors. The differences following chemotherapy regimen was not evaluated neither in this study.

Conclusion

In our study, adjuvant chemotherapy did not improve RFS in pT3N0 colorectal cancer, while it showed better outcome in OS in only univariate analysis. The adjuvant chemotherapy was given based on the existence of high-risk features such as preoperative obstruction, PNI, LVI, resection margin involvement, and the number of LNs examined less than 12. Among those indications for adjuvant chemotherapy, LVI was the most common high-risk feature in this study. However, LVI was not associated with worse RFS and OS in this study for the overall cohort. Furthermore, LVI showed diversion in diagnosis in over 30% of the cases after the review. These findings suggest consideration on the official reliability of LVI as a high-risk factor, in specifically pT3N0 patients, who are on the crossroads for chemotherapy. Also, although rLVI in the review cohort was the risk factor for RFS, reLVI which we use as LVI these days was not associated with RFS. More detailed consensus on staining system and classification in pathology diagnosis, and following studies are needed on LVI.

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국문요약(Abstract)

연구 배경 및 목적: III기나 IV기의 진행된 대장직장암과 달리 병리학적으로 진단된 T3N0의 림프절 전이가 없는 II기 대장암에서 근치적 절제술 후 보조항암요법을 시행하는 것은 논란이 되어 왔다. 이 연구는 항암치료의 적응증으로 쓰이는 고위험군 인자 중 주요위험군인 림프혈관침습의 종양학적 예후인자로서 가지는 중요도와 효용성, 그리고 그에 따른 보조항암치료의 효과를 평가하기 위한 연구이다.

연구재료와 연구 방법: 이 연구는 서울아산병원에서 2012년 1월부터 2016년 12월까지 근치적 절제술을 받고 병리학적으로 T3N0 로 진단된 1634명의 대장직장암 환자를 대상으로 했으며, 그 중 242명을 대상으로 D2-40, CD31 이중면역염색을 이용한 병리학적 리뷰를 시행하여 림프관 침습과 혈관 침습을 확인하였다. 전체군 및 리뷰군에서 신경주위 침습, 수술 전 폐색, 절단면 침습 등의 고위험군 인자들의 재발율 및 생존율에 대한 예후인자로서의 효용성을 분석했다.

연구결과: 전체군에서 772명이 보조항암치료를 받았고, 전체군의 5년 무재발 생존율과 5년 생존율은 각각 92%, 91.4% 였다. 보조항암치료는 재발율의 호전을 보여주지 못했으나 (p=0.593), 단변량 분석에서 생존율 향상과 관계성을 보여주었다. 그러나 다변량 분석에서는 관계성이 없었다 (p=0.218). 본 연구에서 무재발 생존율과 연관을 보인 고위험군 인자는 수술 전 폐색, 신경주위 침습, 절제면 침습이었고, 생존율과 연관을 보인 건 수술 전 폐색, 저분화 종양, 신경주위 침습, 절제면 침습이었다. 림프혈관 침습과 신경주위 침습은 각각 384명(23.5%), 272명(16.6%)에서 확인되었다. 슬라이드리뷰 후에 림프혈관 침습은 33.9%에서 변화를 보였으며, 신경주위 침습은 25.4%에서 변화를 보였다. 리뷰군에서 리뷰 후 대혈관침습까지 포함한 림프혈관침습이 단변량분석에서 유의함을 보였으나, 가이드라인에 따른 대혈관침습을 제외한 림프혈관 침습으로 대상군을 분석시에는 무재발 생존율이나 생존율과 통계적으로 연관이 없었다.

결론: 보조항암요법 진행여부 결정이 필요한 병리학적으로 진단된 T3N0 대장직장암에서 수술 전 폐색, 림프혈관 침습, 신경주위 침습 등을 포함한 항암결정에 필요한 고위험군 인자들이 있으나, 이 중 림프혈관 침습은 진단 시 주관성이 크고 염색법에 따라 변화가능성이 크며 본 연구에서는 종양학적 결과와 관련을 보이지 않았다. 따라서 이러한 림프혈관 침습을 보조적 항암치료를 결정

하는 데 써도 되는지 재확인과 세밀한 진단 기준에 대한 연구가 필요하다.

Supplementary tables

eTable 1. Risk factors associated with recurrence in the review cohort

		Recurrence				
		Univariate analysis		Multivariate analysis		
Variables	**No.(%)**	**HR**	**P-value**	**HR**	**95% CI**	**P-value**
Age		1.012	0.640			
Adj. CTx.	127(52.5)	1.042	0.849			
LNs<12	5(2.1)	-	-			
Preoperative obstruction	82(33.9)	1.438	0.546			
PD	6(2.5)	-	-			
Positive margin	6(2.5)	3.909	0.231			
rLVI (LI+SVI+LVI)	109(45.0)	3.735	0.053			
reLVI (LI+SVI)	75(31.0)	2.235	0.177			
Large VI	47(19.4)	2.1190	0.238			
rPNI	83(34.3)	1.946	0.263			

Adj. CTx., Adjuvant chemotherapy; LVI, lymphovascular invasion; PD, Poorly differentiated; HR, Hazard ratio; SVI, Small vessel invasion; LI, Lymphatic invasion; rPNI: PNI changed after the review; LaVI, Large vessel invasion; rLVI, LVI after review; reLVI, revised LVI according to CAP guideline; CI, confidence interval

eTable 2. Factors associated with recurrence-free survival in the review cohort according to tumor invasion locations

		Recurrence				
		Univariate analysis		Multivariate analysis		
Variables	**No.(%)**	**HR**	**P-value**	**HR**	**95% CI**	**P-value**
SmallVI location						
Intramural	116(47.9)	5.498	0.111	2.891	0.291-28.731	0.365
Extramural	37(15.3)	10.186	0.038	3.373	0.261-43.672	0.352
LargeVI location						
Intramural	28(11.6)	1.545	0.589			
Extramural	54(22.3)	1.273	0.726			
LI location						
Intramural	57(23.6)	5.262	0.008	3.114	0.765-12.668	0.113
Extramural	17(7.0)	2.391	0.436	1.767	0.177-17.629	0.628
PNI location						
Intramural	73(30.2)	1.070	0.924	0.804	0.195-3.313	0.762
Extramural	12(5.0)	7.626	0.004	5.000	1.176-21.267	0.029

LI, Lymphatic invasion; PNI , Perineural invasion; CI, confidence interval

eTable 3. Factors associated with overall survival in the review cohort according to tumor invasion locations

		Death				
		Univariate analysis		Multivariate analysis		
Variables	**No.(%)**	**HR**	**P-value**	**HR**	**95% CI**	**P-value**
SmallVI location						
Intramural	116(47.9)	2.635	0.141	2.245	0.601-8.395	0.229
Extramural	37(15.3)	43.412	0.042	1.959	0.384-9.987	0.418
LargeVI location						
Intramural	28(11.6)	0.867	0.850			
Extramural	54(22.3)	0.418	0.249			
LI location						
Intramural	57(23.6)	1.562	0.373			
Extramural	17(7.0)	-	-			
PNI location						
Intramural	73(30.2)	1.809	0.287	1.123	0.339-3.718	0.849
Extramural	12(5.0)	9.732	<0.001	4.360	0.945-20.122	0.059

LI, Lymphatic invasion; PNI , Perineural invasion; CI, confidence interval