

Anastomotic Leakage Contributes to the Risk for Systemic Recurrence in Stage II Colorectal Cancer

Hiroshi Katoh · Keishi Yamashita · Guoqin Wang ·
Takeo Sato · Takatoshi Nakamura ·
Masahiko Watanabe

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Abstract

Purpose In stage II colorectal cancer (CRC), high-risk patient selection is required, but no candidate markers have been elucidated. Our concern was whether anastomotic leakage (Lk) is a potential available clinicopathological factor for selecting high-risk stage II.

Methods Two hundred seven patients with stage II CRC who underwent curative resection were analyzed. Clinical variables were tested for their relationship to survival.

Results The 5-year disease-free survival rate (DFS) was 87.0%. The univariable prognostic analyses indicated that Lk ($P=0.003$) was the only significant factor. The multivariable prognostic analysis revealed that Lk remained to be potentially independent [hazard ratio (HR), 4.21, $P=0.021$], and the DFS was 58.3% in cases with Lk, while 88.7% in the counterpart. The multivariable logistic regression analysis revealed perioperative blood transfusion ($P=0.001$) was independently associated with Lk. Intriguingly, Lk was closely associated with hematogenic recurrence ($P=0.003$) rather than peritoneal or local recurrence. Although sustained increase of the serum C-reactive protein at 2 weeks after operation predicted poor prognosis, the multivariable analysis including the C-reactive protein level revealed that Lk still indicated the prognostic potential (HR, 3.70, $P=0.075$).

Conclusions The findings concluded that Lk may be a high risk for systemic recurrence in stage II CRC.

Keywords Colorectal cancer · Stage II · Prognosis · Anastomotic leakage

Introduction

Colorectal cancer (CRC) is the second most prevalent cancer,¹ and chemotherapy has dramatically improved prognostic outcome of CRC patients over the past decades.^{2,3} Nevertheless, CRC remains the fourth leading cause of cancer death worldwide with about 530,000 deaths every year.¹ Recently, as the prognostic outcome of stage III patients has been dramatically improved due to prevalent use of adjuvant chemotherapy and improvement of chemotherapy regimens,^{2,4} adjuvant chemotherapy is consented as standard therapy in stage III CRC. Similarly, application of adjuvant chemotherapy is under discussion for patients with high-risk stage II disease⁵ although no selecting marker has been clinically identified at present. In stage II patients, approximately 20% of the patients have yet suffered from recurrence in spite of potentially curative resection.⁶ Therefore, pre- or postoperative prognostic markers have been anticipated for selecting high-risk patients who may benefit from adjuvant

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H. Katoh · K. Yamashita · T. Sato · T. Nakamura ·
M. Watanabe (✉)

Department of Surgery, Kitasato University School of Medicine,
Kitasato 1-15-1, Minami-ku,
Sagamihara 252-0374 Kanagawa, Japan
e-mail: gekaw@med.kitasato-u.ac.jp

G. Wang
Department on Community-Based Perinatal and Emergency
Medicine, Kitasato Clinical Research Center,
Kitasato University School of Medicine,
Tokyo, Japan

chemotherapy after curative operation of stage II CRC. Several prognostic markers or predictors of chemosensitivity for stage II patients have been reported such as allelic imbalance,⁷ gene expression profiling by cDNA microarray,⁸ or microsatellite instability,⁹ respectively. However, such molecular markers have been unsuitable for routine application at present because they have not been finally validated yet and are still costly and time-consuming.

Anastomotic leakage (Lk) is thought to occur at a rate ranged from 3% to 18% and has been reported to be a risk factor for local recurrences in curatively operated CRC patients.^{10–12} In this meaning, at least patients with Lk may be potential candidate for adjuvant chemotherapy. However, these results were based upon curatively operated patients with CRC of several stages, and the impact of Lk on long-term survival remains controversial,^{10–14} especially in stage II CRC. Accordingly, clinicopathological factors including Lk were prognostically analyzed within stage II patients to evaluate whether Lk could be a clinically available parameter for predicting long-term prognosis.

Patients and Methods

Characteristics of Patients with Stage II CRC

A total of 1,101 patients having electively undergone surgical resection of primary CRC at the Kitasato University Hospital from January 1, 1990 to March 31, 2000, were reviewed. Patients with colorectal multiple cancer, malignant disease of other organ, familial adenomatous polyposis, or inflammatory bowel diseases, patients who underwent resections without anastomosis, and patients undergone emergency resection for perforation or one-stage resection for obstruction were excluded. Among the remaining 946 patients of sporadic CRC, 207 patients were diagnosed (21.9%) as stage II CRC disease and were operated on with curative intent. Preoperative chemotherapy or radiation therapy had not been performed in this cohort. Patients without obstruction received mechanical bowel preparation with polyethylene glycol electrolyte solution the day before surgery, and patients with obstruction and patients with rectal cancer received bedside orthograde colorectal lavage with lukewarm water. Prophylactic intravenous antibiotics were administered at the induction of anesthesia and 3 h after the beginning of operation. Patients were followed up until the recurrence of cancer or end point (April 30 2007). All patients were followed up at least every 3 months for the first year and every 6 months thereafter. Follow-up assessment involved a medical history-taking, physical examination, biologic tests, measurement of the serum CEA and CA19-9 levels, colonoscopy, chest radiography, abdominal ultrasonography (US), and chest/abdominal computed tomography

(CT). Serum CEA and CA19-9 were usually evaluated every visit, and abdominal US and CT were performed every 6 months. Chest CT and colonoscopy were examined every year. Recurrence was diagnosed on the basis of imaging and, if necessary, either cytologic analysis or biopsy was performed. Patient demographics, tumor characteristics, and postoperative course were recorded and analyzed. Perioperative transfusion was defined as allogeneic blood transfusion during surgery or in the first two postoperative days, as in previous press,¹⁵ and was performed at the discretion of the treating surgeons and anesthesiologists. The number of total dissected lymph nodes was also classified according to previous press.¹⁶ Pathological TNM classification was made according to the UICC (*Unio Internationalis Contra Cancrum*) staging system.

Patients who received adjuvant chemotherapy for more than 3 months were defined as adjuvant chemotherapy “Yes” group. Adjuvant chemotherapy was consisted of oral administration of 5-fluorouracil (5FU)-based regimens: 5FU, Tegafur/uracil (UFT), or Furtulon (5'-deoxy-5-fluorouridine) alone, or one of them plus PSK (protein-bound polysaccharide K). Although curative operation alone is a standard therapy in stage II CRC at present, oral adjuvant chemotherapy had been recommended to patients with stage II CRC during the term of this patient cohort if they fulfilled the following eligibility criteria: age of 20 to 75 years; the absence of prior chemo-immunotherapy or radiotherapy, and the absence of severe liver dysfunction, heart failure, renal dysfunction, or other severe systemic complications. Therefore, patients who received oral adjuvant chemotherapy reached 180 cases, and the remaining 27 patients declined or did not fulfill the above criteria.

Lk was defined as any clinical or radiological evidence of dehiscence of the anastomosis: the presence of peritonitis caused by anastomosis dehiscence, the presence of feculent discharge from the drainage tube, or the presence of abscess with demonstration of Lk. These were also confirmed by radiography from drainage tube, hydrosoluble enema, or CT-guided abscess drainage except the cases with obvious feculent discharge from the drainage tube (Supplemental Table 1). Anastomotic dehiscence, which was basically diagnosed by, later, routine imagings prior to closure of diverting ileostomy, was not included. We performed routine imagings only for patients with diverting ileostomy prior to ileostomy closure more than 3 months after primary operation. Four patients underwent diverting ileostomy, but no anastomotic dehiscence was detected in such routine diagnosis.

Statistical Analysis

The relationship between Lk and clinicopathological parameters were assessed by Pearson's chi-square test or

Fisher's exact test, as appropriate, and multivariate logistic regression analysis were performed to obtain an adjusted effect of each factor. The time of follow-up was calculated from the operation date for the primary lesion to the date of recurrence. Cumulative disease-free survival (DFS) of patients was estimated using the Kaplan–Meier method, and statistical significance of the difference of the survival rate between groups was tested using the log-rank test. For the Kaplan–Meier estimate of the survival curves, we truncated the data at a follow-up period of 5 years to avoid the number at risk to be too small. Those with a survival time of more than 5 years were reported to be 5 years, and events occurring after the end of the 5-year follow-up period were computed as censored data. Five-year cumulative DFS probability was estimated using the life table method with the interval length set at 1 month. Multivariable analysis was performed by employing the Cox proportional hazards model to examine the interaction between Lk and other clinicopathological variables and estimate the independent prognostic effect of Lk on survival by adjusting for confounding factors. For ordinal variable, when zero event was detected in the lowest exposure group, analyses was designed to be performed by grouping categories together, treating it as ordinal data to get an average effect, or by confounding sensitivity analyses excluding it from analysis. Within the present study population, there were 27 recurrences of stage II CRC which allows up to three variables to be included in a multivariable regression model. To avoid over-fitting, all potential confounding factors of Lk were reduced to one single composite characteristic by applying a propensity score.¹⁷ The conventional *P* value of 0.05 or less was used to determine the level of statistical significance. All reported *P* values are two-sided. Analyses were performed independently at our clinical research center using SPSS version 17.0 software (SPSS Inc., Chicago, IL).

Results

Patients' Characteristics and Their Association with Lk

The clinicopathological characteristics were shown in Table 1. One hundred twenty-seven males and 80 females were analyzed with age being 61.0 ± 11.1 years. Lk occurred in 12 (5.8%) cases, and, among them, only one patient had a particularly preoperative complication (diabetes mellitus). The diabetes of this patient was well-controlled by insulin from preoperation through postoperation. And, there was no patient with other factors for poor nourishment such as medication of steroids. Lk occurred in 22.2% of patients with perioperative blood transfusion and in 1.2% of those without perioperative blood transfusion. Lk was signifi-

cantly related to perioperative blood transfusion ($P < 0.001$, Fisher's exact test), followed by T4 factor (direct invasion into other organ; $P = 0.071$), the elevation of preoperative CEA ($P = 0.110$), and tumor position ($P = 0.129$). Preoperative obstruction was observed in only one patient with Lk (Table 1). There was also no significance in relationship between Lk and obstruction in the present study population. Lk occurred in five cases (3.8%) in colon cancer and seven in rectal cancer (9.2%). Among them, two patients required ileostomy (reoperation) for Lk in colon cancer and five in rectal cancer, and one patient (colon cancer) underwent ileostomy before curative resection (two-stage operation) for obstruction, one patient (rectal cancer) underwent diverting ileostomy, and the remaining three patients were conservatively observed with percutaneous drainage and finally cured. The multivariable logistic regression analysis of these factors indicated that Lk was independently associated with perioperative blood transfusion ($P < 0.001$).

Kaplan–Meier Estimate of 5-Year DFS

All the patients were included in the survival analysis. The overall follow-up period ranged from 2 to 207 months (median, 116 months), and the mean DFS was 55.4 months corresponding to a 5-year follow-up. Because a cumulative DFS probability of 50% was not yet reached by the end of 5-year follow-up, the overall median DFS time was not determined. The overall DFS rate was 87.0% (27 cases with recurrence and 180 cases without recurrence). Five-year cumulative DFS of patients with Lk was remarkably worse (58.3%), which corresponded to stage III CRC (63.2%), compared with those without Lk (88.7%; $P < 0.001$, Fig. 1a). Lymphatic involvement (ly; $P = 0.119$) and vascular involvement (v; $P = 0.086$) tended to indicate poor prognosis (Supplemental Fig. 1a, b), and patients with both ly and v involvement ($n = 28$) showed significantly poor prognosis (DFS, 84.9%) compared with the counterpart ($n = 179$; 100.0%; $P = 0.033$; Supplemental Fig. 1c).

When separately analyzed on tumor position, Lk still significantly affected adversely on long-term prognosis in both colon and rectum (Fig. 1b, c), and there was no significant difference between DFS of patients with Lk in colon cancer (60.0%) and that in rectal cancer (57.1%). In addition, Lk was the only significant prognostic factor, and there was no factor which had prognostic potential ($P < 0.1$) both in colon and rectum when separately analyzed (data not shown).

Contribution of Lk to the Risk of Recurrence with Multivariable Analysis

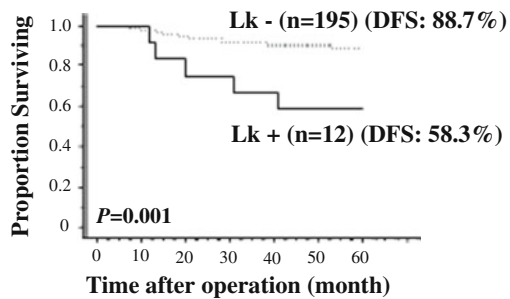
Cox proportional hazards model was applied to estimate the effect of Lk on DFS. Lk was the only significant prognostic

Table 1 Characteristics and those in correlation with anastomotic leakage (Lk)

Variables	No. of patients	Percentage	Lk			<i>P</i> ^a values
			Present	Absent	Present rate (%)	
Gender						
Male	127	61	10	117	7.9	0.13
Female	80	39	2	78	2.5	
Age (years)						
<60	94	45	4	90	4.3	0.55
>60	113	55	8	105	7.1	
Tumor position						
Ccolon	131	63	5	126	3.8	0.13
Rectum	76	37	7	69	9.2	
Differentiation						
Non-poor	194	94	12	182	6.2	0.36
Poor ^b	13	6	0	13	0.0	
T factor						
T3	199	96	10	189	5.0	0.07
T4	8	4	2	6	25.0	
Lymphatic involvement (ly)						
Negative	16	8	0	16	0.0	0.61
Positive	191	92	12	179	6.3	
Vascular involvement (v)						
Negative	19	9	1	18	5.3	0.92
Positive	188	91	11	177	5.9	
Preoperative CEA						
Normal (<2.5 ng/ml)	138	67	5	133	3.6	0.110
Elevated (>2.5 ng/ml)	69	33	7	62	10.1	
Preoperative CA19-9						
Normal (<37 ng/ml)	183	88	10	173	5.5	0.64
Elevated (>37 ng/ml)	24	12	2	22	8.3	
Obstruction						
Yes	16	8	1	15	6.3	0.94
No	191	92	11	180	5.8	
Lk						
Yes	12	6	n/a	n/a	n/a	n/a
No	195	94	n/a	n/a	n/a	
Number of total dissected lymph node						
<6	5	2	0	5	0.0	0.78
6–10	27	13	1	26	3.7	
11–15	34	17	3	31	8.8	
>15	141	68	8	133	5.7	
Laparoscopy-assisted operation						
Yes	8	4	0	8	0.0	0.47
No	199	96	12	187	6.0	
Adjuvant chemotherapy						
Yes	180	87	9	171	5.0	0.2
No	27	13	3	24	11.1	
Perioperative transfusion						
Yes	45	22	10	35	22.2	<0.001
No	162	78	2	160	1.2	

OR odds ratio, LNDE lymph node dissection extent, n/a not applicable
^a Compared by Fisher’s exact test or chi-square test
^b Poor consists of poorly differentiated, mucinous, and undifferentiated types

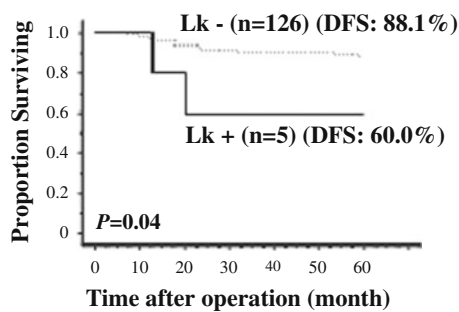
A. total stage II CRC (n=207)



No. at risk

Lk +	12	12	10	9	8	7	7
Lk -	195	194	185	179	177	177	173

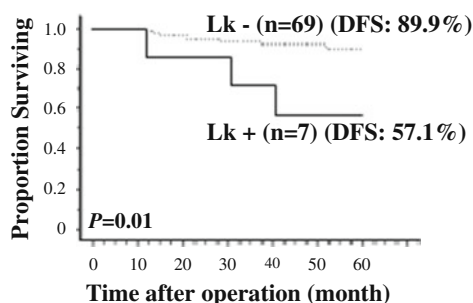
B. colon cancer (n=131)



No. at risk

Lk +	5	5	4	3	3	3	3
Lk -	126	125	119	115	114	114	111

C. rectal cancer (n=76)



No. at risk

Lk +	7	7	6	6	5	4	4
Lk -	69	69	66	64	63	63	62

Fig. 1 Kaplan–Meier curve of 5-year DFS according to anastomotic leakage (Lk): **a** Total stage II CRC ($n=207$). **b** Colon cancer ($n=131$). **c** Rectal cancer ($n=76$)

factor, and there was no other factor which had prognostic potential ($P<0.1$). The crude hazard ratio (HR) of Lk-positive compared to Lk-negative was 4.38 (95% confidence interval (CI), 1.66–11.58; $P=0.003$), which indicated Lk increased the risk of recurrence of CRC and cancer-related death by more than four times that of without Lk. The effect of Lk on recurrence in colon and rectal cancer

group gave similar results: crude HR (95%CI) was 4.1 (0.9–17.9) for the colon group and 4.9 (1.3–19.0) for the rectal group.

Before multivariable analyses were adopted to estimate adjusted effect of Lk on DFS, we further confirmed that there was no interaction effect between cancer position (colon or rectum) and Lk ($P=0.874$); taking into account that evaluation in each group would result in a small sample size and thus decrease the power of the study, we finally combined them together. Potential confounders of variables were included in the multivariable analysis (Table 2). The adjusted HR of Lk became 5.27 (95%CI, 1.54–18.10; $P=0.008$) in comparison to Lk-negative. We also performed an analysis by using propensity score to adjust the effect of Lk by transforming all other confounding variables into a single estimator and revealed that, after the adjustment, the HR of Lk became 4.21 (95%CI, 1.24–14.33; $P=0.021$). These findings suggested that Lk seems to be an independent and significant risk factor of poorer DFS (Table 2).

Lk was Associated with Hematogenic Recurrence Rather than Local or Peritoneal Recurrence in Stage II CRC

Next, first recurrence site in patients with stage II CRC was analyzed according to Lk. Interestingly, Lk was correlated with hematogenic recurrence ($P=0.003$ by Fisher's exact test) rather than local recurrence or peritoneal dissemination ($P=0.605$; Table 3). Therefore, Lk may cause systemic micrometastasis, leading to systemic recurrence.

Effect of Lk on DFS When Taking Systemic Inflammatory Response into Account

Recently, a systemic inflammatory response, as evidenced by raised circulating levels of C-reactive protein (CRP), has been reported to be associated with poor survival in patients who underwent potentially curative resection for CRC.¹⁸ These reports may explain the above implication of Lk in systemic recurrences, hence circulating level of CRP was analyzed, which was measured as a part of routine blood examination either before or after potentially curative resection for stage II CRC. CRP level was classified as raised (≥ 1.0 mg/dl) or normal (<1.0 mg/dl) from a clinical practice view. Lk was significantly correlated with CRP level at 1 or 2 weeks after curative operation ($P=0.018$, 0.003, respectively, by Fisher's exact test; Supplemental Table 2). Moreover, the sustained elevation of CRP level at 2 weeks after operation predicted significantly worse prognosis (DFS, 75.0%) than its counterpart (89.3%; $P=0.022$, compared by log-rank test, Supplemental Fig. 2), while preoperative CRP and CRP at 1 week after operation did not show prognostic significance (data not shown). The multivariable prognostic analysis including CRP at 2 weeks

Table 2 Prognostic analysis of stage II patients according to 5-year DFS (*n*=207)

Variables	Univariable analysis		Multivariable analysis			
			Model 1		Model 2	
	HR (95%CI)	<i>P</i> ^b values	HR (95%CI)	<i>P</i> ^b values	HR (95%CI)	<i>P</i> ^b values
Lk	4.38 (1.66–11.58)	0.003	5.27 (1.54–18.10)	0.008	4.21 (1.24–14.33)	0.021
Gender (male)	1.87 (0.79–4.43)	0.154	1.76 (0.71–4.34)	0.221	n/d	n/d
Age >60	1.26 (0.58–2.71)	0.559	1.24 (0.56–2.73)	0.603	n/d	n/d
Tumor position (colon)	0.99 (0.46–2.17)	0.988	1.12 (0.47–2.69)	0.797	n/d	n/d
Poor differentiation ^c	0.56 (0.08–4.14)	0.572	0.59 (0.07–5.29)	0.637	n/d	n/d
T factor (T4)	1.02 (0.14–7.51)	0.985	0.65 (0.07–5.66)	0.693	n/d	n/d
Lymphatic involvement (ly)	22.90 (0.05–9651.67)	0.310	n/d	n/d	n/d	n/d
Vascular involvement (v)	23.51 (0.09–6204.78)	0.267	n/d	n/d	n/d	n/d
Preoperative CEA elevation	1.21 (0.55–2.64)	0.636	1.13 (0.48–2.68)	0.783	n/d	n/d
Preoperative CA19-9 elevation	0.59 (0.14–2.48)	0.470	0.57 (0.13–2.55)	0.458	n/d	n/d
Obstruction	1.54 (0.46–5.11)	0.482	1.89 (0.47–7.56)	0.368	n/d	n/d
Number of total dissected lymph node					n/d	n/d
<6	reference		reference		n/d	n/d
6–10	1.60 (0.21–12.01)	0.649	0.50 (0.05–5.53)	0.570	n/d	n/d
11–15	1.26 (0.43–3.75)	0.674	0.48 (0.05–5.05)	0.542	n/d	n/d
>15	1.29 (0.48–3.50)	0.615	0.40 (0.04–3.68)	0.416	n/d	n/d
Laparoscopy-assisted operation	0.96 (0.13–7.05)	0.956	1.15 (0.15–8.79)	0.895	n/d	n/d
Adjuvant chemotherapy	0.90 (0.31–2.59)	0.838	0.95 (0.29–3.08)	0.928	n/d	n/d
Perioperative transfusion	1.28 (0.54–3.03)	0.575	0.70 (0.22–2.24)	0.547	n/d	n/d
Propensity score	n/d	n/d	n/d	n/d	1.16 (0.07–18.50)	0.918

DFS disease-free survival, HR hazard ratio, CI confidence interval, n/d not determined

^a End-point: date of death or April 30, 2007, no patient was lost to follow-up

^b Significance based on Cox’s proportional hazard model

^c Poor consists of poorly differentiated, mucinous, and undifferentiated types

There was no event in ly or v negative cases, so that these variables were excluded from multivariable analysis

Multivariable model 2 indicates the adjusted effect of Lk by applying propensity score which is a conditional probability of presenting Lk given by other clinicopathological factors including gender, age, tumor position, differentiation, vascular involvement, preoperative CEA elevation, and perioperative transfusion

after operation (*n*=175) showed that Lk still indicated prognostic potential (HR, 3.70, *P*=0.075; Table 4). This result suggests that Lk is more strongly associated with recurrence independent of sustained systemic inflammation.

Discussion

The present study showed that an anastomotic leakage (Lk) was closely associated with an adverse impact on long-term

DFS (5-year DFS, 58.3%) in patients who underwent potentially curative resection for stage II CRC, and it was the most robust independent prognostic factor. This DFS was comparable to that of patients with stage III CRC. Although intramural vessel involvement may be available for the selection of low-risk patients (DFS, 100.0%), it was insufficient for the patient selection who have high risk of recurrence and would be rather low-risk selection (Supplemental Fig. 1). Therefore, with regard to patient selection, Lk alone may be potential classifier of stage II CRC. Lk has

Table 3 Association of Lk with first recurrence site in stage II patients

Lk	Local or peritoneal recurrence		<i>P</i> ^a values	Hematogenic recurrence		<i>P</i> ^a values
	Present	Absent		Present	Absent	
Yes	1	11	0.605	4	8	0.003
No	14	181		8	187	

^a Significance based on Fisher’s exact test

Table 4 Multivariate analysis of Lk effect on 5-year DFS in stage II CRC patients taken CRP into account ($n=175$)

Variables	Model 1		Model 2	
	HR (95%CI)	P^b values	HR (95%CI)	P^b values
Lk	3.05 (0.79–11.83)	0.106	3.70 (0.88–15.62)	0.075
Post-CRP (2w)	0.53 (0.21–1.35)	0.182	n/d	n/d
Gender (male)	1.97 (0.73–5.30)	0.178	n/d	n/d
Age>60	1.34 (0.59–3.14)	0.464	n/d	n/d
Tumor position (colon)	1.12 (0.43–2.91)	0.823	n/d	n/d
Poor differentiation ^c	1.02 (0.12–8.45)	0.986	n/d	n/d
T factor (T4)	0.53 (0.05–5.14)	0.583	n/d	n/d
Preoperative CEA elevation	1.30 (0.52–3.22)	0.572	n/d	n/d
Preoperative CA19-9 elevation	0.21 (0.03–1.66)	0.139	n/d	n/d
Obstruction	1.50 (0.33–6.90)	0.602	n/d	n/d
Number of total dissected lymph node			n/d	n/d
<6	Reference		n/d	n/d
6–10	6863.02	0.938	n/d	n/d
11–15	10138.02	0.935	n/d	n/d
>15	7343.4	0.937	n/d	n/d
Laparoscopy-assisted operation	1.17 (0.15–9.12)	0.884	n/d	n/d
Adjuvant chemotherapy	0.79 (0.23–2.75)	0.710	n/d	n/d
Perioperative transfusion	0.86 (0.26–2.84)	0.803	n/d	n/d
Propensity score	n/d	n/d	1.50 (0.16–13.88)	0.724

DFS disease-free survival, HR hazard ratio, CI confidence interval, n/d not determined, post-CRP (2w), CRP level at 2 week after operation

^a End-point: date of death or April 30, 2007, no patient was lost to follow-up

^b Significance based on Cox's proportional hazard model

^c Poor consists of poorly differentiated, mucinous, and undifferentiated types

Variables with no event were excluded from multivariate analysis

Multivariable model 2 indicates the adjusted effect of Lk by applying propensity score which is a conditional probability of presenting Lk given by other clinicopathological factors and CRP level

been reported to be a risk factor of local recurrences in curatively operated CRC patients^{10–12,19} which included several stage CRCs. However, to our knowledge, our study is the first report concerning Lk with high risk of recurrence limited in stage II disease. Interestingly, in our study, Lk was significantly implicated in systemic recurrence ($P=0.003$) rather than local recurrence in stage II.

In our study, there was no prognostic difference between colon cancer and rectal cancer. Although tumor position did not affect Lk and long-term prognosis in this study, anastomosis and prognosis in rectal cancer is thought to be affected by various factors compared with that in colon cancer.^{10,20–23} However, even when separately analyzed on tumor positions, Lk was still significant prognostic factor (Fig. 1b, c).

Adjuvant chemotherapy for stage II CRC has been controversial at present because stage II patients show good prognosis and only a part of high-risk stage II patients may benefit in prognosis from previous studies.^{6,24,25} Neverthe-

less, at present, standard chemotherapy is not recommended for stage II CRC patients because of excellent prognosis. Our current study included many such patients even with Lk who actually underwent adjuvant chemotherapy, but which did not include the most active agents such as oxaliplatin, CPT-11, bevacizumab, or cetuximab, suggesting that Lk anyway showed high risk for stage II CRC irrespective of adjuvant therapy. Therefore, our current result is worthy of further study on high-risk patient selection in stage II CRC and also on more powerful adjuvant chemotherapy such as FOLFOX in stage II patients with Lk in order to elucidate the benefit of adjuvant chemotherapy for these patients. In addition, neoadjuvant chemo-radiotherapy for locally advanced rectal cancer is now becoming standard. However, during the terms of this current study, we did not think that neoadjuvant treatment is really effective for such patients from a prognostic point of view. Thus, Lk in patients with neoadjuvant treatment should be also studied in the future.

Several parameters have been reported as independent prognostic factor or chemosensitive marker for patient selection allowing for the application of adjuvant chemotherapy in stage II CRC.^{6,24,26} The number of evaluated lymph nodes,²⁷ T4 factor (direct invasion into adjacent structure),^{16,28} tumor budding/infiltrating,²⁹ vascular involvement,^{16,28} or perforation through the tumor²⁸ were such high-risk potential markers. In the present study, vascular involvement tended to be a prognostic factor, however, it was not insufficient to select high-risk patients. On the other hand, the number of evaluated lymph nodes and T4 factor did not indicate any prognostic significance in our current cohort of stage II CRC. Several molecular and genetic markers have also been reported to indicate poor prognosis of stage II CRC such as the DNA aneuploid,³⁰ 17p or 18q allelic imbalance,⁷ gene expression profiling by cDNA microarray,⁸ and micrometastasis detected by reverse transcriptase-polymerase chain reaction of CEA³¹ or CK20.³² In addition, microsatellite instability (MSI) has been reported as chemoresistant marker.⁹ Actually, the largest stage II colon cancer trial (ECOG 5202, the US Gastrointestinal Intergroup including the National Cancer Institute of Canada) is ongoing, in which patients are now selected prospectively for adjuvant chemotherapy based on 18q loss of heterozygosity and MSI status.³³ Nevertheless, all such genetic and molecular tools are unsuitable for routine application at present because they are costly and time-consuming methods and have not been validated yet. In this meaning, Lk is easily available for patient selection at any minute.

Viable cancer cells in the lumen may be present at the site of the anastomosis at the time of surgery, which can be detected on suture or staple lines of anastomosis,³⁴ and on the occasion of Lk, those may be capable of implantation and subsequent local recurrence.³⁵ However, this theory alone did not explain the association of Lk with systemic recurrence in the present study. Systemic inflammatory response, as evidenced by raised circulating concentrations of CRP, has been reported to predict recurrence and disease-specific survival in curatively operated CRC patients.¹⁸ Consistently, the sustained CRP elevation at either 1 or 2 weeks after operation was significantly associated with Lk, and especially, CRP at 2 weeks after operation per se predicted poor prognosis ($P=0.022$) in the present study. CRP may reflect the inflammatory response promoted by various cytokines which are presumably released from leukocytes in the malignant process.³⁶ On the other hand, a raised CRP level was thought to be related to the reduction of circulating lymphocytes.³⁷ In addition, the reduction of lymphocytes in the peripheral blood was shown to reflect the immune suppression in patients with malignant tumor,³⁸ and tumor-induced immune suppression adversely affects their prognosis.³⁹

Perioperative allogeneic blood transfusion was reported to be an independent risk factor for Lk in a dose-dependent manner.²³ Also in the present study, perioperative blood transfusion affected Lk most robustly even when CRP was included in the multivariable logistic analysis (data not shown). Allogeneic blood transfusion impairs the cell-mediated immune response⁴⁰ and predisposes to postoperative infectious complication,⁴¹ and cell-mediated immune responses, which include mainly macrophage and T-lymphocyte, has been thought to affect the healing process.⁴² Tadros T. et al. reported that perioperative blood transfusion impaired the healing of experimental intestinal anastomosis in an animal model using bursting pressure of anastomosis, in addition, cell-mediated immune response, as evidenced by exogenous IL-2, reversed the negative effects of blood transfusion on anastomotic repair.⁴³ Taken together, Lk may lead to systemic recurrences partly through cancer immune suppression together with sustained CRP elevation and perioperative blood transfusion. Conversely, we could also say that Lk is favored by a local depression of the immune system for the presence of undetected micrometastasis.

Recently, it has been suggested that tumor progression such as invasion and metastasis is coordinated by both cancer cells and host stromal cells, which consist tumor microenvironment.^{44–46} A variety of host bone marrow-derived cells, which include inflammatory cells, cancer-associated fibroblasts, and endothelial progenitor cells compose of a tumor microenvironment.^{47–49} Host inflammatory cells produce much more TGF- β than tumor cells, leading to inhibition of host tumor immune surveillance,^{50,51} which may lead to cancer cell escape and intravasate into circulation. Local inflammation caused by Lk may additionally affect the above mechanism and may result in metastasis-prone phenotype. However, in order to answer the reason why Lk was associated with systemic recurrence, further experimental studies, such as comparison of circulating cancer cells or cytokines in both patients and experimental model, may be needed.

In conclusion, we showed that Lk was the most robust independent prognostic factor among the clinicopathological factors in stage II CRC. These results suggest that Lk may be appropriate for the selection of high-risk patients. And, Lk was associated with systemic recurrence in both colon and rectal cancer. Because Lk necessarily occurs at a given rate in spite of perioperative treatment with maximal attention and it is immediately available for clinical use from cost and technical point of view, Lk could be a factor for selecting high-risk patients. As only 12 patients (out of 207) had an Lk in this study, the prognostic impact of Lk should be validated in a larger study. On the other hand, because the DFS of patients without Lk was still 88.7%, further molecular tools would be necessary.

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