Objective Criteria for Crohn-like Lymphoid Reaction in Colorectal Cancer

Hideki Ueno, MD, PhD[1], Yojiro Hashiguchi, MD, PhD,[1] Hideyuki Shimazaki, MD, PhD,[2] Eiji Shinto, MD, PhD,[1] Yoshiki Kajiwara, MD, PhD,[1] Kuniaki Nakanishi, MD, PhD,[2] Kei Kato, MD, PhD,[2] Kazuya Maekawa, MD,[2] Kosuke Miyai, MD,[3] Takahiro Nakamura, PhD,[4,5] Junji Yamamoto, MD, PhD,[1] and Kazuo Hase, MD, PhD[1]

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Abstract

*We aimed to determine semiquantitative evaluation criteria for Crohn-like lymphoid reaction (CLR). We reviewed 1,032 patients with colorectal cancer and evaluated CLR by counting all peritumoral lymphoid aggregates (LAs) and by measuring the maximum diameter of the largest LA. The maximum diameter of the largest LA, rather than the number, had a significant impact on survival. Active CLR determined by the 1-mm rule was significantly associated with MLH1/MSH2 immunohistochemical staining deficiency. The group with LAs 1 mm or larger had lower recurrence (*P = .0008) and a higher survival rate (P < .0001) than that without LAs 1 mm or larger. These results were similarly observed in another cohort of 500 patients with colorectal cancer. The k values for CLR evaluation among 8 observers were 0.67 for the 1-mm rule and 0.50 for Graham's criteria. The size of the largest LA best reflects the specific characteristics of CLR, and the 1-mm rule is expected to improve assessment reproducibility.*

The lymphocytic reaction observed in resected colorectal cancer (CRC) specimens has long been recognized as an indicator of host immune responses to tumor cells.[1] A significant correlation between enhanced lymphocytic reaction and a favorable prognostic outcome had been observed in various activities involving lymphocytic systems, including peritumoral lymphocytic infiltration,[2-5] tumor lymphocyte infiltration of cancer nests,[3,6,7] and lymphoid hyperplasia in regional lymph nodes.[5,8,9]

The histologic feature of nodular lymphoid aggregates (LAs) at the periphery of the carcinoma also reflects the activation of the lymphatic system against tumors. Pihl et al[9] first paid attention to this characteristic and coined the term *perivascular lymphocyte cuffs*, defined as the areas occupied by sheaths of small, dark lymphocytes in the muscle and pericolic/rectal fat at the tumor periphery. They demonstrated that this phenomenon was a putative manifestation of host immunological response to invasive carcinoma.[9,10] Subsequently, Graham and Appelman[11] referred to peritumoral LAs as a Crohn-like reaction (CLR) because of their resemblance to part of the inflammatory component of Crohn disease. We now use the term *CLR* for all nodular LAs, irrespective of whether they are located adjacent to the venules,[11] and currently the term *CLR* appears to be more familiar to pathologists than *perivascular lymphocyte cuffs*. Many studies have shown that CLR is a feature associated with favorable outcomes,[3,5,11-14] and it has recently been demonstrated to be associated with mismatch repair (MMR) status and to be one of the histologic markers of microsatellite instability (MSI).[15-20]

For assessing CLR, the method proposed by Graham and Appelman (Graham's method)[11] is widely used.[12,13,15,20] According to this method, CLR is classified into 3 grades

based on the number and size of LAs and number of germinal centers. More specifically, grade 0 is defined as no LA or, at most, 1 single small LA in all tumor sections; grade 1, occasional, small LAs with rare or absent germinal centers; and grade 2, numerous large LAs with frequent germinal centers. More recently, Harrison et al[21] combined grades 0 and 1 into what they termed as the inconspicuous group and classified grade 2 as the conspicuous group.

As is clear from the definition, according to Graham's method,[11] CLR grading is determined in a subjective manner and each category is multifactorial; however, few studies have been conducted to assess the reproducibility of such evaluations.[12] There have also been few attempts to determine the optimal criteria for assessing CLR status. In the present study, we examined the impact of histologic characteristics associated with CLR (ie, the number and size of LAs) on postoperative survival outcome. We then attempted to identify semiquantitative CLR criteria favoring objective evaluation in routine practice.

Materials and Methods

Patients

A total of 1,032 patients with CRC who had undergone potentially curative surgery at the Department of Surgery, National Defense Medical College, Tokorozawa, Japan, between 1980 and 1999 and who had no distant metastasis at the time of surgery were enrolled. All CRCs were pathologically confirmed as having penetrated into or through the muscularis propria layer. This data set (first cohort) included 601 men and 431 women (average age, 61.8 years; range, 21-91 years). The follow-up period was a minimum of 5 years or until death. No patient received preoperative adjuvant therapy or postoperative therapy, such as radiation therapy or intensive chemotherapy with intravenous administration of chemotherapeutic agents.

To validate the results, we enrolled 500 consecutive patients with CRC who had undergone curatively intended surgery as the second cohort. T1 CRC patients were excluded. All patients underwent surgery at the above-mentioned institute between 2000 and 2005. The median age of these patients was 66 years (range, 18-96 years); 280 and 220 tumors were resected from male and female patients, respectively. No patient received preoperative adjuvant therapy. Regarding postoperative adjuvant chemotherapy, 245 patients received chemotherapy, 96 received 5-fluorouracil/leucovorin (FU/LV), 56 received uracil/tegafur (UFT)/LV, and 93 received oral anticancer drugs such as UFT, 5'-deoxy-5-fluorouridine, carmofur, or 5-FU. No adjuvant therapy was administered to 247 patients, and no detailed information on postoperative adjuvant therapy could be obtained for 8 patients. Detailed prognostic information, including the recurrence pattern at 5 years after surgery, was obtained for 483 patients (96.6%); the median follow-up period for the 378 survivors was 68 months.

Histologic Evaluation

Surgically resected specimens were routinely processed according to the guidelines of the Japanese General Rules for Clinical and Pathologic Studies on Cancer of the Colon, Rectum, and Anus.[22] After fixation in formalin solution, the tumors were sequentially sectioned along the long axis of the intestine to include the tumor center. One of the authors (H.U.) performed histopathologic evaluation of CLR and tumor budding using H&E-stained glass slides prepared from a single longitudinal section of the whole tumor, including its deepest part. The median number of slides examined per patient was 2 (range, 1-9) for the first cohort and 2 (range, 1-10) for the second cohort.

Crohn-like Lymphoid Reaction

Nodular LAs lining the tumor periphery were evaluated with regard to the CLR status (**Image 1**). The following were not evaluated as LAs associated with CLR: (1) LAs from mucosa-associated lymphoid tissue (ie, located within the mucosal layer or just below the muscularis mucosae), (2) LAs judged to be part of small lymph nodes rather than LAs associated with CLR on the basis of the existence of circumferential connective tissue around LAs, and (3) nonnodular LAs, including irregularly shaped as well as long and narrow ones. We regarded small LAs as those in which the number of lymphocytes appeared to increase in comparison to those in the background but which had no definite "aggregation," so we created a yardstick of 300 mm or larger in size for positive determination of LAs.

To examine the prognostic impact of the number of LAs, we evaluated the following parameters: (1) the total number of LAs observed on all slides covering the entire tumor, (2) the average number of LAs observed per slide, and (3) the number of LAs in a ×2 microscopic objective lens field where CLR was most intense. In addition, the largest LA in each patient was identified and its maximum diameter was determined with a calibrated ocular reticule (Image 1). The outline of LAs for measurement was determined under low-power magnification.

Tumor Budding

Tumor budding was determined according to a previously described method.[23] It was defined as either a single cancer cell or clusters of fewer than 5 cancer cells observed in the invasive frontal region. To determine the degree of tumor budding, the clusters were counted under a magnification of ×200 in the field where budding was most intensive. Tumors

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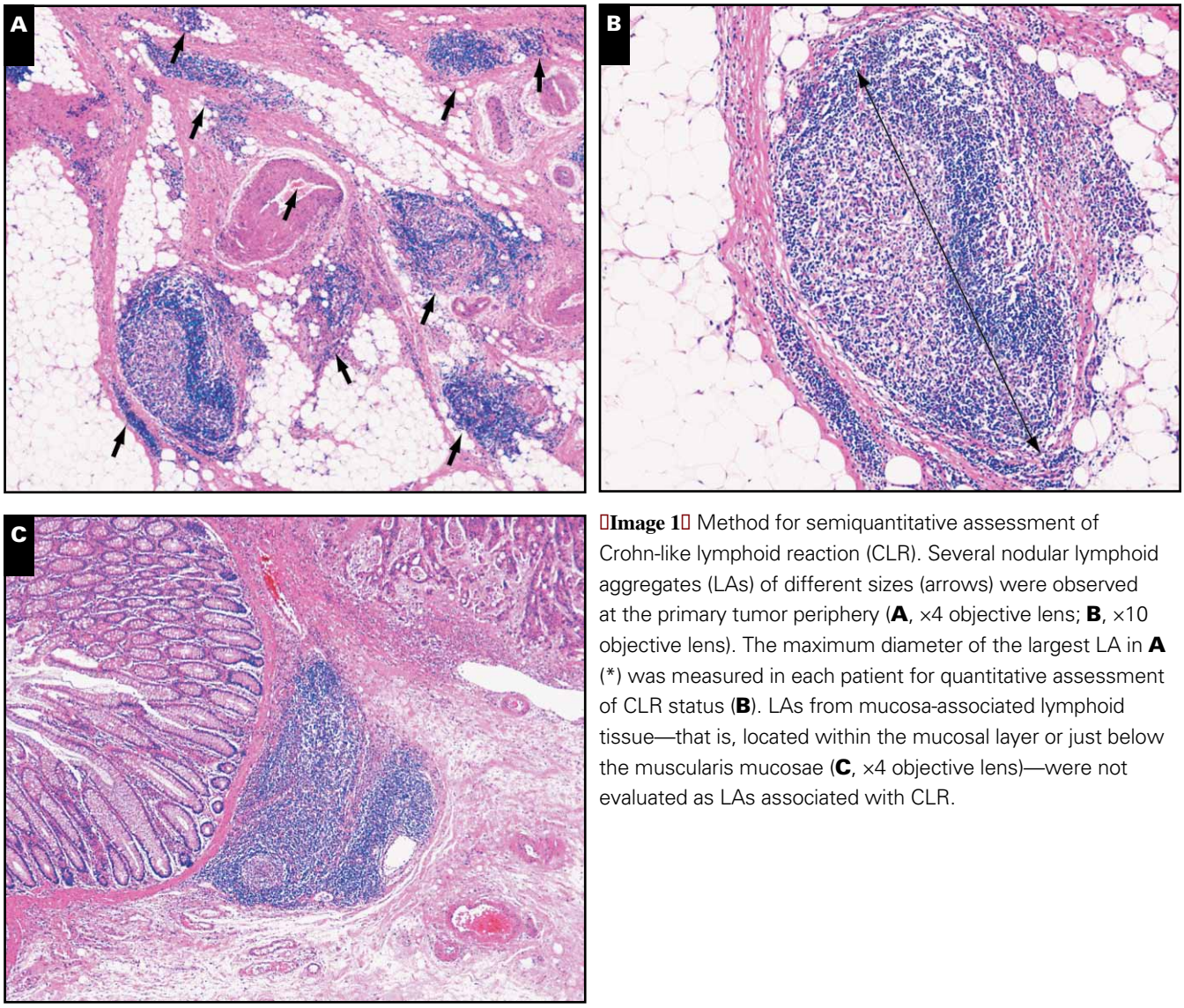

Image 1 Method for semiquantitative assessment of Crohn-like lymphoid reaction (CLR). Several nodular lymphoid aggregates (LAs) of different sizes (arrows) were observed at the primary tumor periphery (**A**, ×4 objective lens; **B**, ×10 objective lens). The maximum diameter of the largest LA in **A** (*) was measured in each patient for quantitative assessment of CLR status (**B**). LAs from mucosa-associated lymphoid tissue—that is, located within the mucosal layer or just below the muscularis mucosae (**C**, ×4 objective lens)—were not evaluated as LAs associated with CLR.

with less than 5, 5 to 9, and 10 or more budding foci were classified as G1, G2, and G3, respectively.

MLH1 and MSH2 Protein Immunohistochemistry

A total of 225 T3 or T4 CRC patients who underwent curative surgery between 1995 and 1999 were evaluated. MLH1 and MSH2 protein expression was determined by immunohistochemistry of formalin-fixed, paraffin-embedded tumor tissue sections using mouse monoclonal antibodies G168-728 (1:100; Pharmingen, San Diego, CA) and G219-1129 (1:500; Pharmingen). Tumors showing complete loss of nuclear MLH1 or MSH2 expression were classified as MLH1 or MSH2 negative. Nuclear immunostaining of normal epithelial cells, lymphocytes, and stromal cells served as internal positive controls in each case. Tumors with normal nuclear expression of both proteins were considered to exhibit intact MMR protein expression, whereas those classified as MLH1 negative or MSH2 negative were considered to show defective expression.

Interobserver Studies

An interobserver study was conducted by circulating glass slides from 100 patients serially treated at the National Defense Medical College Hospital between 1996 and 1998 to examine interobserver reproducibility in evaluating the histologic lymphoid reaction (ie, CLR by Graham's criteria,[11] CLR by the semiquantitative method, and lymphoid infiltration by the criteria of Jass et al[24]). Glass slides prepared from a single longitudinal section of the whole tumor, including the deepest part of the tumor, were used for this study; the median number of slides evaluated per patient was 2 (range, 1-5). This study involved H.U. and an additional 7 observers (H.S., E.S., Y.K.,

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K.N., K.K., K. Maekawa, and K. Miyai), who evaluated CLR using the semiquantitative method for the first time; prior to examination, they understood the concept only on the basis of the scheme shown in Image 1. No direct hands-on instruction was provided to them regarding microscopic evaluation of CLR.

Statistical Analyses

Disease-specific and overall survival rates were calculated using the Kaplan-Meier method, and comparisons were made using the log-rank test. The association between parameters linked with CLR and other clinicopathologic characteristics of known prognostic significance (tumor differentiation pattern, depth of tumor penetration, nodal involvement, and lymphatic and venous invasion, which previously had been recorded in pathological reports) as well as recurrence patterns were analyzed by the c^2 test or the Mann-Whitney *U* test. Cox proportional hazards regression analysis was used to determine the impact of conventional and novel histologic parameters on disease-specific survival. For interobserver assessments of histologic lymphoid reaction, the Fleiss k value among the 8 observers was calculated to evaluate variability. Statistical calculations were performed using StatView version 5.0 software (SAS Institute, Cary, NC) and R software (version 2.8.1).

Results

Survival Implication of the Number and Size of LAs

Survival implications of LAs in terms of their number and size are shown in Table 1. Although we found that the total number of LAs varied widely, there was no significant correlation between the number and recurrence rate or 5-year disease-specific survival rate. The results were similar when we analyzed the prognostic impact of the number of LAs in a ×2 microscopic objective lens field where CLR was most intense.

In contrast, we found that a value of 1 mm for the maximum LA diameter was the cutoff point that distinguished the recurrence rate as well as the 5-year disease-specific survival rate (Table 1). The recurrence rate was significantly lower in the group with LAs 1 mm or larger (16.2%) than in the group without LAs 1 mm or larger (27.5%; $P = .0008$).

In addition, more LAs were observed in patients with LAs 1 mm or larger (average, 36.3; 95% confidence interval (CI), 30.8-42.0) than in those without LAs 1 mm or larger (average, 10.5; 95% CI, 9.4-11.5; $P < .0001$). However, there was no significant difference in the total number of LAs between the groups with and without recurrence when patients with LAs 1 mm or larger (34.4 [n = 34] and 36.7 [n = 176], respectively) and those without LAs 1 mm or larger (11.6 [n = 226] and 10.0 [n = 596], respectively) were examined separately.

Table 1
Survival Outcome According to the Number and Size of Peritumoral Lymphoid Aggregates (LAs), First Cohort (n = 1,032)

	Recurrence Rate		DSS Rate at 5 y After Surgery	
Characteristics of LAs (No. of Cases)	**No. (%) of Cases**	***P* Value[a]**	**%**	***P* Value[a]**
Total No. of LAs				
0-4 (325)	78 (24.0)	NS	81.6	NS
5-9 (226)	57 (25.2)		82.0	
10-19 (249)	66 (26.5)		82.3	
20-49 (168)	46 (27.4)		80.9	
≥50 (64)	13 (20.3)		85.8	
Average No. of LAs per slide				
0-4 (519)	132 (25.4)	NS	80.1	NS
5-9 (280)	80 (28.6)		81.6	
10-19 (168)	37 (22.0)		85.2	
20-49 (53)	8 (15.1)		88.6	
≥50 (12)	3 (25.0)		91.7	
No. of LAs in the microscopic field of ×2 objective lens				
0-4 (478)	120 (25.1)	NS	80.7	NS
5-9 (346)	93 (26.9)		81.8	
10-14 (119)	29 (24.4)		86.1	
15-19 (45)	8 (17.8)		84.0	
≥20 (44)	10 (22.7)		84.1	
Maximum diameter (D) of the largest LA, mm				
<0.5 (269)	72 (26.8)	0.0008[b]	78.9	<.0001[b]
0.5-0.9 (553)	154 (27.8)		79.7	
1.0-1.4 (153)	25 (16.3)		91.3	
1.5-1.9 (42)	7 (16.7)		92.6	
≥2.0 (15)	2 (13.3)		92.9	

DSS, disease-specific survival; NS, not significant.
[a] Log-rank test.
[b] D <1.0-mm group (n = 825) vs D ≥1.0-mm group (n = 207).

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Figure 1 demonstrates the favorable impact that LAs 1 mm or larger have on disease-specific and overall survival. Disease-specific survival at 5 years after surgery according to the presence or absence of LAs 1 mm or larger was 100% (n = 21) and 94.7% (n = 102), respectively, in stage I; 96.1% (n = 107) and 89.8% (n = 328), respectively, in stage II; and 85.8% (n = 78) and 66.4% (n = 396), respectively, in stage III **Figure 2**. The superior survival outcome associated with LAs 1 mm or larger was statistically significant in stages II (P = .0271) and III (P = .0015). There was a marginal difference in the recurrence rate between patients with 1 LA 1 mm or larger and those with 2 or more LAs 1 mm or larger (20.4% and 11.3%, respectively; P = .0771); however, no statistical difference was observed in the disease-specific and overall survival rates between the 2 groups.

Clinicopathologic Features of CLR According to the 1-mm Rule

On the basis of the above results, we evaluated CLR status as being active for tumors with 1 or more LAs 1 mm or larger and as inactive for tumors with no LA 1 mm or larger. Active CLR was significantly associated with younger patients, right-sided colonic cancer, poorly differentiated tumors, greater number of lymph nodes retrieved, and involvement of fewer lymph nodes **Table 2**.

Regarding MMR protein expression, 13 (5.8%) and 4 (1.8%) tumors showed loss of staining for MLH1 and MSH2, respectively. The incidence of defective expression of these MMR proteins was significantly higher in tumors

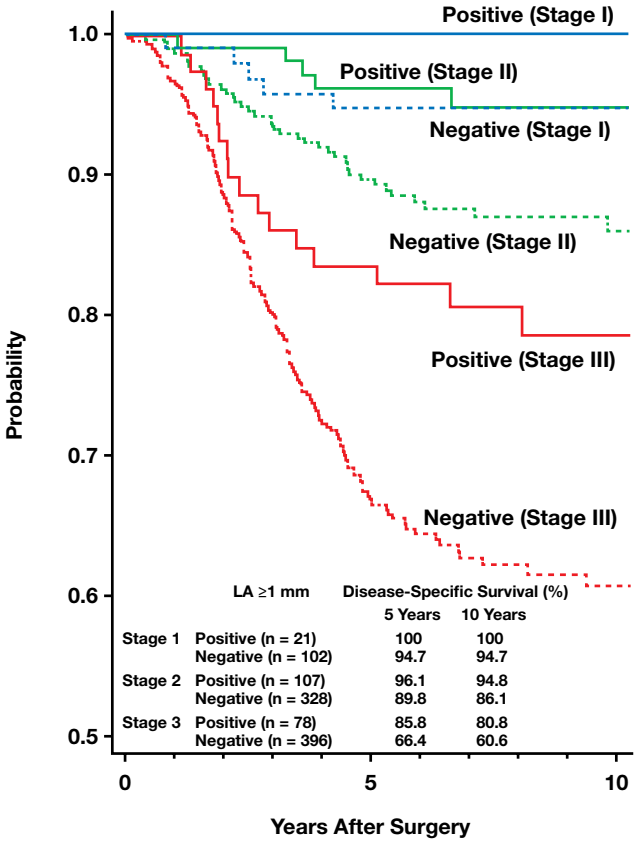

Figure 2 Disease-specific survival curves with or without lymphoid aggregates (LAs) ≥1 mm according to the tumor stage (first cohort).

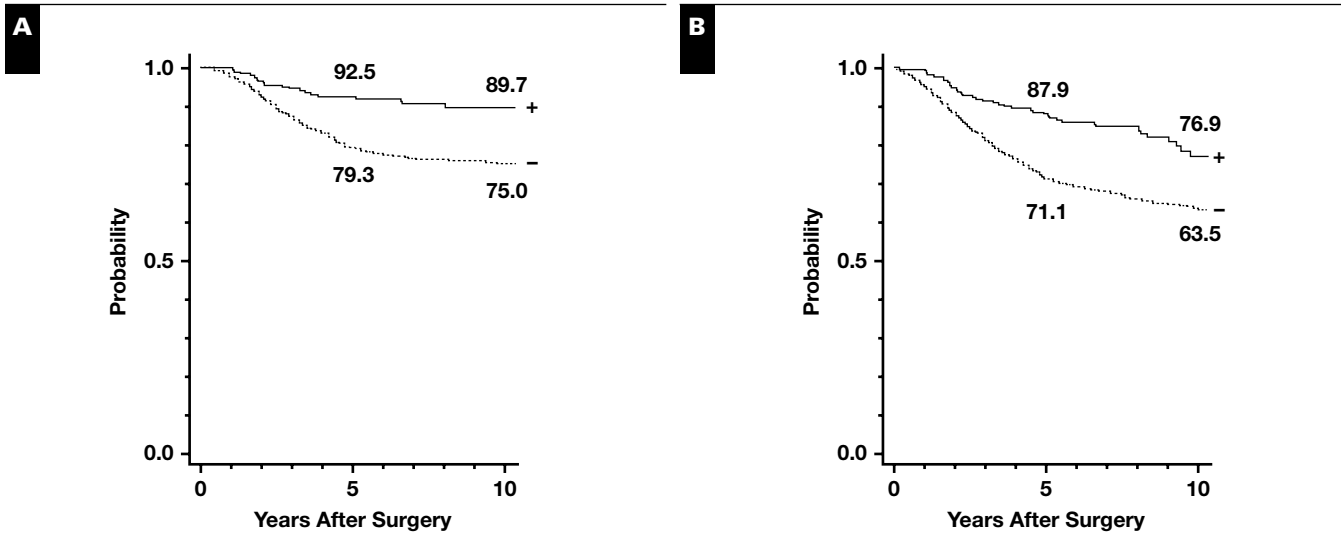

Figure 1 Disease-specific (**A**) and overall (**B**) survival curves with (+) (n = 206) or without (–) (n = 826) lymphoid aggregates ≥1 mm (first cohort). P < .0001.

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with active CLR (18.2%) than in those with inactive CLR (5.7%) (P = .0236).

Recurrence Mode and Survival Impact of CLR by Multivariate Analysis

Active or inactive CLR status as defined by the 1-mm rule was relevant to recurrence in the lung (5.2% and 13.1%, respectively; P = .0014), liver (6.2% and 12.9%, respectively; P = .0066), and lymph node plus the local site (7.6% and 13.0%, respectively; P = .0312).

The results of multivariate analysis using the Cox proportional hazards regression model are shown in Table 3. Active CLR status was found to have an independent survival impact on disease-specific survival together with other histologic parameters such as T stage, N stage, and tumor budding grade.

Survival Impact of CLR in the Second Cohort

The total number of patients who had a tumor with 1 or more LAs 1 mm or larger was 186 (37.2%). Active CLR according to the 1-mm rule also had a favorable impact on disease-specific survival in the second cohort (Table 3). Similar to the results obtained in the first cohort, CLR status as evaluated by the 1-mm rule was selected as an independent prognostic parameter, along with T stage, N stage, venous invasion, and tumor budding.

Interobserver Study

The Fleiss k value for CLR according to the 1-mm rule (active/inactive) was 0.67, whereas it was 0.50 for CLR assessed using Graham's criteria,[11] 0.56 for CLR assessed using criteria from Harrison et al,[21] and 0.15 for lymphocytic infiltration assessed using the criteria from Jass et al.[24]

Table 2
Status of Crohn-like Lymphoid Reaction (CLR) by the 1-mm Rule and Other Clinicopathologic Characteristics, First Cohort (n = 1,032)[a]

Variables (No. of Cases)	Inactive CLR (n = 822)	Active CLR (n = 210)	*P* Value
Sex			.5440
Male (601)	474 (78.9)	127 (21.1)	
Female (431)	348 (80.7)	83 (19.3)	
Age, y	62.4	59.5	.0009
Location			.0768[b]
Right-sided colon (217)	166 (76.5)	51 (23.5)	
Left-sided colon (462)	365 (79.0)	97 (21.0)	
Rectum (353)	291 (82.4)	62 (17.6)	
Histologic type			.0115[c]
Well (322)	261 (81.1)	61 (18.9)	
Moderately (658)	520 (79.0)	138 (21.0)	
Poorly (17)	9 (52.9)	8 (47.1)	
Mucinous (33)	30 (90.9)	3 (9.1)	
Signet-ring cell (2)	2 (100.0)	0	
Depth of penetration			.0622[d]
T2 (152)	127 (83.6)	25 (16.4)	
T3 (812)	646 (79.6)	166 (20.4)	
T4 (68)	49 (72.1)	19 (27.9)	
No. of LNs retrieved	25.1	28.5	.0110
No. of LNs involved			.0044[d]
0 (558)	428 (76.7)	130 (23.3)	
1-3 (321)	261 (81.3)	60 (18.7)	
4 (153)	133 (86.9)	20 (13.1)	
Venous invasion			.0253
Negative (267)	200 (74.9)	67 (25.1)	
Positive (765)	622 (81.3)	143 (18.7)	
Tumor budding			.1587[d]
Grade 1	382 (78.6)	104 (21.4)	
Grade 2	179 (76.8)	54 (23.2)	
Grade 3	261 (83.4)	52 (16.6)	
MLH1/MSH2 expression			.0236
Intact (208)	181 (87.0)	27 (13.0)	
Defective (17)	11 (64.7)	6 (35.3)	

LN, lymph node.
[a] Values are presented as No. (%) of cases unless otherwise indicated.
[b] Right-sided colon vs rectum (c^2 test).
[c] Poorly vs others (Fisher exact test).
[d] Mann-Whitney U test.

Discussion

A purely subjective method of assessing CLR had been employed in some studies whereby CLR status has been classified as either negative or positive without any criteria being specifically defined.[5,10,19] Graham's method,[11] which has been used most frequently in reported studies,[12,13,15,20] includes multiple factors as criteria for each category; however, these are also determined in a subjective manner. For example, Graham's grade 2, which is equivalent to the "conspicuous" grade according to the 2-tiered grading system by Harrison et al,[21] is defined as "numerous large LAs with frequent germinal centers." Although few studies have evaluated the degree of interobserver evaluation agreement,[21] we have some concerns about a lack of reproducibility in these approaches, which provide no quantitative criteria for determining the degree of CLR.

Over the previous decade, the number of LAs has often been used as the standard LA criterion. For example, CLR was scored as present in some studies when at least 3 nodular LAs[25] or 4 nodular LAs[17,18] were counted under a low-power microscopic field. In other studies, 2 or more large LAs in a section[26] or a minimum of 3 LAs per section[16] were regarded as the benchmark for the presence of CLR. However, the actual size of the LAs evaluated was not clarified in these studies. In the present study, if all LAs were counted irrespective of size, no positive correlation would have been observed between the number of LAs and postoperative prognostic outcomes, such as recurrence and disease-specific survival. Our results indicate that the activation of CLR as an antitumor immune response was most markedly characterized by the size of the largest LA. Interestingly, there was no linear correlation between the maximum diameter of the largest LA and prognostic outcome, and we observed a clear cutoff value of 1

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Table 3
Univariate and Multivariate Analyses for Disease-Specific Survival Using the Cox Proportional Hazards Regression Model

	First Cohort (n = 1,032)					Second Cohort (n = 500)				
		Univariate		Multivariate			Univariate		Multivariate	
Variables	No.	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	No.	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Tumor differentiation										
Well	322	1				210	1			
Moderate	658	1.4 (1.0-2.0)	.0336			247	1.6 (1.0-2.7)	.0445		
Others	52	2.2 (1.2-3.9)	.0067			43	1.7 (0.7-3.7)	.2155		
T stage										
T2	152	1				26	1		1	
T3	812	3.7 (1.9-7.3)	.0001	2.0 (1.0-4.0)	.0441	369	3.5 (0.5-25.5)	.2129	5.5 (0.7-39.8)	.0947
T4	68	6.9 (3.2-14.8)	<.0001	3.9 (1.8-8.6)	.0006	105	8.0 (1.1-59.1)	.0405	8.1 (1.1-60.7)	.0407
N stage										
N0	558	1				248	1		1	
N1	321	3.3 (2.3-4.7)	<.0001	2.2 (1.6-3.2)	<.0001	194	3.7 (2.1-6.5)	<.0001	3.2 (1.8-5.7)	<.0001
N2	153	6.8 (4.7-9.8)	<.0001	4.1 (2.8-6.0)	<.0001	58	5.4 (2.8-10.4)	<.0001	4.0 (2.0-8.0)	<.0001
Venous invasion										
Negative	267	1				76	1		1	
Positive	765	1.7 (1.2-2.4)	.0041			424	5.0 (1.6-15.8)	.0065	3.8 (1.2-12.1)	.0244
Tumor budding										
Grade 1	486	1				149	1		1	
Grade 2	233	3.0 (2.0-4.6)	<.0001	2.1 (1.4-3.2)	.0005	156	1.6 (0.8-3.6)	.2140	1.3 (0.6-2.9)	.4619
Grade 3	313	5.3 (3.7-7.7)	<.0001	3.2 (2.2-4.7)	<.0001	195	4.6 (2.3-9.0)	<.0001	2.3 (1.2-4.7)	.0186
Status of CLR by the 1-mm rule										
Inactive	822	1				314	1		1	
Active	210	0.4 (0.3-0.6)	<.0001	0.4 (0.3-0.7)	.0004	186	0.3 (0.2-0.6)	.0002	0.4 (0.2-0.8)	.0041

CI, confidence interval; CLR, Crohn-like lymphoid reaction; HR, hazard ratio; N, lymph node; T, tumor.

mm, which made a large difference to the recurrence rates in the 2 cohorts analyzed in this study.

Active CLR as defined by the 1-mm rule was also related to younger patient age, poor tumor differentiation, the number of lymph nodes retrieved, and absence of lymph node involvement. Younger patient age[16-18] and poor tumor differentiation[15,18,26] are generally recognized as clinicopathologic characteristics of MSI-associated tumors. As expected, we found a significant correlation between active CLR status as defined by the 1-mm rule and MMR protein status. Only MLH1 and MSH2 were used for MMR in the present study. Thus, a small number of cases with PMS2- or MSH6-only loss may have been missed. We can state that the 1-mm rule is a semiquantitative benchmark for CLR that can also be used as a predictive marker for MSI status.

We are not aware of any publication dealing with CLR status and the number of lymph nodes retrieved. Pihl et al[10] demonstrated a clear correlation between paracortical hyperplasia in regional lymph nodes and perivascular lymphocyte cuffing in the primary tumor. We also found that CLR was associated with the status of histologic features in regional lymph nodes, such as paracortical hyperplasia and germinal center hyperplasia in rectal cancer.[5] The correlation between active CLR and an increased number of retrieved lymph nodes can be explained by the hypothesis that the host immune response to cancer is systematically expressed in both primary tumor and regional lymph nodes.

Based on our results, active CLR was related not only to a low incidence of locoregional recurrence but also to a low rate of recurrence in distant organs such as the lung and liver. In both cohorts, CLR status as defined by the 1-mm rule had an independent impact on disease-specific survival together with T stage, N stage, and tumor budding. Of note, CLR had a greater impact on survival outcome than conventional histologic parameters such as tumor differentiation and venous invasion, which are presently regarded as important prognostic indicators.[27]

It is well known that lymphocyte infiltration into cancer nests or stroma is a morphological expression of the host antitumor response,[2-7] and this has been demonstrated to have an interactive effect with CLR that confers a survival advantage.[5,14] However, few reports have demonstrated a significant correlation between lymphocytic infiltration quantitatively assessed on H&E slides and survival outcome; some argued that peritumoral lymphatic infiltration was potentially difficult to evaluate objectively.[2,28] In contrast, CLR could be more easily defined and expressed in quantitative terms[10] and a more suitable histopathologic parameter for use in routine practice as an index of the host immune response to cancer. We believe that the 1-mm rule can enhance this attribute of

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CLR. We cannot determine the actual reason for interobserver variability in the identification of CLR based on the 1-mm rule, and we believe that future study is required to minimize this problem. The appearance of a germinal center is one of the criteria of Graham's method.[11] However, the standard for LA assessment becomes multifactorial when this feature is added to the criteria. This may be the reason why the presence or absence of a germinal center has not been included in the methods adopted in recent studies.[17,18]

In conclusion, CLR may be evaluated semiquantitatively by assessing the maximum diameter of the largest LA within a tumor. The 1-mm rule can offer simplicity and improved reproducibility in evaluating CLR, which can contribute to effective selection of CRC patients with a favorable survival outcome.

From the [1]Department of Surgery, [2]Department of Laboratory Medicine, [3]Department of Basic Pathology, and [4]Laboratory for Mathematics, National Defense Medical College, Tokorozawa, Japan, and the [5]Laboratory for Statistical Analysis, Center for Genomic Medicine, RIKEN, Wako, Japan.

Address reprint requests to Dr Ueno: Dept. of Surgery, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan; e-mail: ueno@ndmc.ac.jp.

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