

## Vascular Invasion, but not Lymphatic Invasion, of the Primary Tumor Is a Strong Prognostic Factor in Patients with Colorectal Cancer

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**Abstract.** *Background:* We previously showed that the presence of vascular invasion, but not lymphatic invasion, was a strong prognostic factor for breast cancer. Lymphatic invasion may represent mainly the selective affinity of cancer cells for lymph nodes. The present study was undertaken to evaluate the presence of vascular invasion that may reflect systemic disease as a predictor of disease recurrence in colorectal cancer, separate from lymphatic invasion of the primary tumor. *Patients and Methods:* We retrospectively evaluated the cases of 177 consecutive patients with primary colorectal cancer who underwent colorectal resection. We examined the relationship between recurrence and the prognostic significance of clinicopathological factors, particularly lymphatic and vascular invasion. *Results:* The presence of vascular invasion (v) was significant, while that of lymphatic invasion (ly) was not significant in univariate analysis. The presence of vascular invasion was an independent prognostic factor in multivariate analysis. Among the 60 patients in the ly-/v- group, one (1.7%) had disease recurrence, and among the 33 patients in the ly+/v- group, one (3.0%) had disease recurrence. On the other hand, among the 71 patients in the ly+/v+ group, 16 patients (22.5%) suffered recurrence, and among the 13 patients in the ly-/v+ group, four (30.8%) suffered recurrence. It is interesting to note that despite the presence of lymphatic invasion, the group without vascular invasion (ly+/v-) had a few patients with distant metastases, a result which is

similar to that of the ly-/v- group. *Conclusion:* The presence of vascular invasion, but not lymphatic invasion, could be an indicator of high biological aggressiveness and may be a strong prognostic factor for colorectal cancer.

The correct definition of poor prognostic factors for colorectal cancer may help guide more aggressive adjuvant treatment protocols. Pathological staging is currently the most accurate predictor of prognosis in colorectal cancer. The commonly used staging systems for colorectal cancer, including Dukes and TNM (tumors/ nodes/metastases), depend on the degree of depth of tumor invasion and the number of lymph nodes involved in metastasis, and serve as a benchmark for predicting the prognosis (1, 2). Tumor cells invade blood vessels and lymphatic vessels; lymphovascular invasion (LVI) is a critical step in tumor cell dissemination and metastasis in various types of cancers (2-6). The prognostic significance of LVI in colorectal cancer has been investigated (2, 7-10), however, LVI is not incorporated into most of the internationally-recognized staging systems. The prognostic significance of LVI, including vascular invasion and lymphatic invasion, remains unclear.

We previously reported that the presence of vascular invasion, but not lymphatic invasion, was an indicator of high tumor biological aggressiveness and may be a strong prognostic factor for breast cancer (3). Tumor cells invade the lymphatic vessels, and this invasion allows the cells to then penetrate the lymphatic system. Both experimental tumor models and human clinicopathological data indicate that the growth of lymphatic vessels near solid tumors is often associated with lymph node metastasis (11-13). The presence of lymphatic invasion in colorectal cancer could be a potential indicator of the ability of cancer cells to metastasize to lymph nodes. Lymphatic invasion may represent mainly the selective affinity of cancer cells for lymph nodes. On the other hand, the phenomenon of tumor cells invading blood vessels, not lymphatic vessels, is a

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critical step in tumor cell dissemination and metastasis for predicting disease recurrence or prognosis. The vascular invasion of the primary tumor (denoted herein as “v”) may thus reflect systemic disease. We, therefore, hypothesized that vascular invasion of the primary tumor would reflect the risk of recurrent disease and the prognosis more accurately than lymphatic invasion (“ly”) in patients with colorectal cancer. To contribute to staging information, vascular and lymphatic invasion were evaluated as potential prognostic factors. In the present study, we retrospectively investigated the relationship between vascular invasion with or without lymphatic invasion and recurrence in patients with operable colorectal cancer.

**Patients and Methods**

We retrospectively investigated the cases of 177 consecutive patients with primary colorectal cancer who underwent surgery at the Department of General Surgical Science, Gunma University Hospital, between January 2007 and December 2009. Patients with previously diagnosed colorectal cancer or incomplete clinical information were excluded. None of the patients had received preoperative chemotherapy or radiation. All patients were diagnosed with colon or rectal cancer and underwent surgical resection of the primary tumor. Informed consent was obtained from all patients.

The details extracted from the database were age, sex, primary tumor location, depth of tumor invasion, lymph node metastasis, lymphatic or vascular invasion of the primary tumor, administration of adjuvant therapy, and a serum tumor marker (carcinoembryonic antigen; CEA). The overall median follow-up period was 3.45 years, and none of the patients died of surgical complications.

*Statistical analysis.* The colorectal cancer cases were divided into two groups on the basis of the presence or absence of recurrence. We conducted univariate statistical analysis using Fisher’s exact test or the  $\chi^2$  test with or without Yates’ correction. To compare the two groups, we used Student’s *t*-test. To test the independence of the risk factors, we entered the variables into a multivariate logistic regression model with a likelihood of  $p < 0.05$ . Relapse-free (RFS) and overall (OS) survival were calculated using the Kaplan–Meier method. The log-rank test was used to evaluate differences between OS and the recurrence-free interval. Differences were considered significant at  $p < 0.05$ .

**Results**

We divided the cases of patients with colorectal cancer into two groups based on the presence of recurrence. Among the 177 patients, 22 (12.4%) had recurrent disease. Table I summarizes not only patients’ characteristics but also the results of the univariate analysis conducted to determine the relationship between the examined clinicopathological variables and recurrent disease. The univariate analysis revealed that lymph node metastasis, depth of tumor invasion, and vascular invasion were statistically significantly different between the two groups. Lymphatic invasion was frequently seen in patients with recurrent disease, but was

Table I. Patients’ characteristics and clinicopathological features associated with recurrent disease. Values are expressed as mean±SD.

	Recurrence		p-Value
	Negative 155	Positive 22	
Age (years)	66.0±12.1	67.1±15.3	0.404
Gender (Male/Female, n)	99/56	12/10	0.397
Location (colon/rectum, n)	94/61	16/6	0.391
Depth of invasion			<0.001
T1/Tis	47	0	
T2	36	2	
T3	59	12	
T4	13	8	
Lymph node metastasis (n)	38	14	<0.001
Lymphatic invasion (positive, n)	87	17	0.059
Vascular invasion (positive, n)	64	20	<0.001
CEA (≥3 ng/ml, n)	38	9	0.170
Adjuvant therapy (n)	37	9	0.020

CEA: Carcinoembryonic antigen.

not significant. The multivariate analyses revealed that vascular invasion ( $p=0.034$ ) and depth of tumor invasion ( $p=0.006$ ) were independent negative prognostic factors. Lymph node metastasis ( $p=0.052$ ) lost its significance in the multivariate analysis.

As shown by the Kaplan–Meier curves, the RFS was significantly shorter for patients with vascular invasion ( $p < 0.009$ ; Figure 1a), as was the OS ( $p < 0.039$ ; Figure 1b). The two types of survival curves indicate a significantly lower rate of survival among patients with vascular invasion. On the other hand, the OS shown by the Kaplan–Meier curves was shorter for patients with lymphatic invasion ( $p=0.034$ ), while the RFS did not differ among patients with lymphatic invasion ( $p=0.079$ ) (Figure 2).

Lymphatic invasion without vascular invasion does not affect the risk of recurrent disease or the prognosis. We found that the presence of vascular invasion, which may reflect systemic disease, was an independent risk factor of recurrent disease. Conversely, our results did not show that lymphatic invasion was of independent prognostic value. Among the 60 patients in the ly–/v– group, only one (1.7%) suffered disease recurrence, and among the 33 patients in the ly+/v– group, only one (3.0%) suffered disease recurrence. On the other hand, among the 71 patients in the ly+/v+ group, 16 (22.5%) experienced recurrence, and among the 13 patients in the ly–/v+ group, four (30.8%) experienced recurrence. The RFS curves for the various groups based on lymphatic invasion and vascular invasion of the primary tumor are shown in Figure 3. The ly+/v– group exhibited almost the same RFS curve as the ly–/v– group.

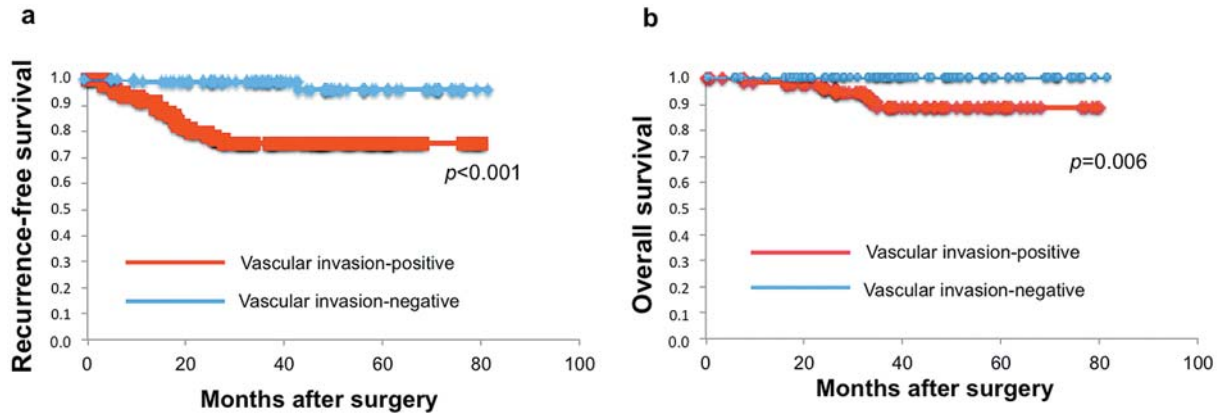


Figure 1. The Relapse-free survival and overall survival revealed by Kaplan–Meier curves were significantly shorter among patients with colorectal cancer with vascular invasion in the primary tumor. With a median follow-up duration of 39.7 months for RFS and 41.4 months for OS, both survival curves suggest a significantly lower rate of survival among patients with vascular invasion.

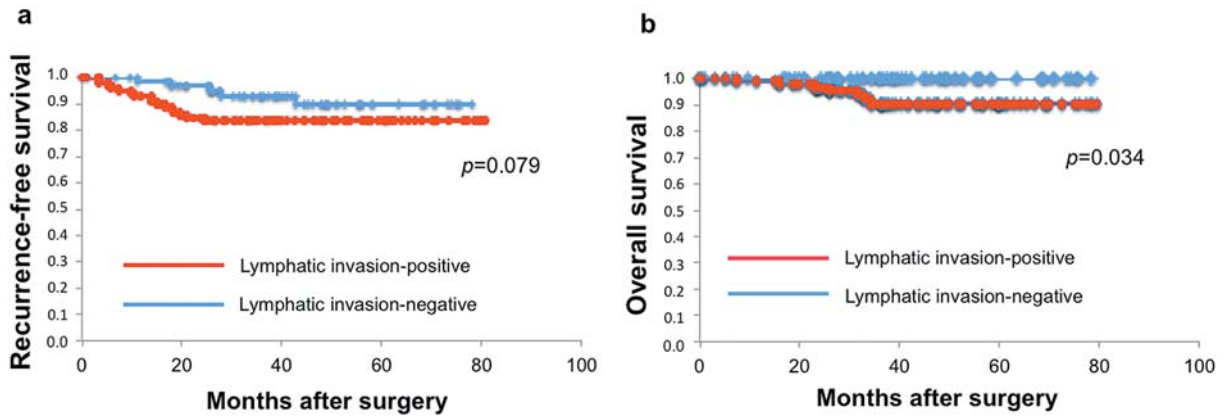


Figure 2. The overall survival revealed by the Kaplan-Meier curves was significantly shorter among colorectal cancer patients with lymphatic invasion in the primary tumor, while RFS curves did not differ among patients with lymphatic invasion.

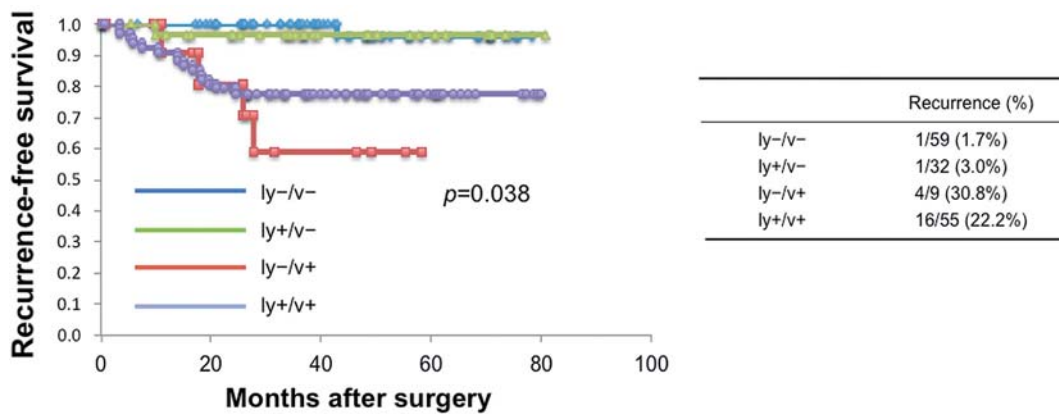


Figure 3. Relapse-free survival (RFS) curves by vascular invasion (v) and lymphatic invasion (ly) of the primary tumor. The RFS curve for the ly+/v- group was almost the same as that for the ly-/v- group.

## Discussion

The routine assessment of LVI is now part of the minimum dataset for colorectal cancer pathology reporting. The prognostic significance of LVI in colorectal cancer has been investigated (2, 7-10), but the use of LVI in clinical management decisions remains a matter of debate. The invasion of blood vessels or lymphatic vessels by tumor cells is a critical step in tumor cell dissemination and metastasis for predicting disease recurrence or prognosis (2-6). As described above, tumor cells invade the lymphatic vessels, and this allows cells to penetrate into the lymphatic system. Lymphatic invasion may reflect the selective affinity of colorectal cancer cells for lymph nodes. Recently, there has been increasing interest in defining lymphatic and vascular invasion, separately (7, 14, 15). We conducted the present study to investigate whether the presence of vascular invasion, which reflects systemic disease, is a predictor of disease recurrence in colorectal cancer, separate from lymphatic invasion of the primary tumor.

The key observations made in this study can be summarized as follows: (i) the presence of vascular invasion was significant, but lymphatic invasion was not significant in the univariate analysis; (ii) the presence of vascular invasion was an independent prognostic factor in the multivariate analysis; and (iii) the presence of the lymphatic invasion without vascular invasion of the primary tumor was not associated with the risk of recurrent disease. These results suggest that the presence of vascular invasion, but not lymphatic invasion, could be considered an indicator of high biological aggressiveness and may be a strong prognostic factor in colorectal cancer.

LVI has been reported to be a prognostic factor in patients with colorectal cancer (2, 7-10). In our study, a univariate analysis of the risk of recurrent disease using clinicopathological variables revealed that correlation with vascular invasion, but not lymphatic invasion, was statistically significant. Lymphatic invasion was related to lymph node metastasis. Many previous studies have demonstrated the relationship between lymphatic invasion and lymph node metastasis (16-18), and the present results are consistent with those studies. It is interesting to note that despite the presence of lymphatic invasion, the present patient group without vascular invasion (ly+/v-) had a few patients with distant metastases, similar to the ly-/v- group. Vascular invasion may represent systemic disease better than lymphatic invasion. In fact, we previously reported that the presence of vascular invasion, but not lymphatic invasion, was an indicator of high biological aggressiveness and may be a strong prognostic factor for breast cancer (3). We therefore investigated the utility of vascular invasion as an additional useful prognostic indicator. We found that in order to predict systemic disease, it is useful to identify the subset of patients with vascular invasion among patients colorectal cancer with or without lymphatic invasion.

This study has several potential limitations. The primary limitation is our use of retrospective methods of data collection. In addition, the number of cases was relatively small. However, the clinical implications of the data we obtained are extremely important. Additional research is needed to explore the significance of vascular invasion in prognosis and in metastatic disease.

In conclusion, the present findings suggest that the presence of vascular invasion, but not that of lymphatic invasion, could be considered an indicator of high biological aggressiveness. Patients with vascular invasion may require stronger adjuvant therapies because of the high risk of distant recurrences. Analyses from large randomized trials are warranted to evaluate the usefulness of vascular invasion as a prognostic factor.

## Competing Interest Statement

The Authors declare that they have no competing financial interests.

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