The Factors Effecting Lymphovascular Invasion in Adenocarcinoma of the Colon and Rectum

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Abstract Adenocarcinomas of the colon and rectum are the most common gastrointestinal malignancy, and lymph node metastases are established as a prognostic factor. Lymphovascular invasion has been recognized as an indication of lymph node metastases. This prompted us to investigate the features of primary tumor that may serve as a risk factor for lymphovascular invasion in colorectal carcinoma. Clinical and pathologic tissue data of colorectal carcinoma treated in our hospital were retrieved from the computer files at Haydarpaşa Numune Education and Research Hospital, from June 1998 to December 2010, retrospectively. We excluded all patients who have two-thirds distal rectal carcinoma to rule out neoadjuvant treatment bias. Tissues from the specimens were stained with standard hematoxylin and eosin. Clinical data including age and sex of patient, location and diameter of tumor, perineural invasion, peritumoral lymphocytic infiltration, tumor grade, lymphovascular invasion, Pathologic T level (pT), and lymph node metastasis were recorded. Lymphovascular invasion was present only in 43 patients out of 108. Only pT and lymph node metastases were found to be statistically significant related to lymphovascular invasion ($p=0.04$ and $p<0.001$). Perineural invasion, pT, and peritumoral lymphocytic infiltration are the factors with $p<0.2$ in the univariate analysis that were investigated with multivariate analysis, but no factor was found as an independent prognostic factor for lymphovascular invasion.

Lymphovascular invasion is significantly related to lymph node metastases. Only pT is found as a factor that increases the lymphovascular invasion.

Keywords Colorectal adenocarcinoma · Lymph node metastasis · Lymphovascular invasion

Introduction

Adenocarcinomas of the colon and rectum are the most common gastrointestinal malignancy and afflict more than one million individuals worldwide [1]. Lymph node metastases (LNM) of adenocarcinoma of the colon and rectum have been established as an independent prognostic factor [2]. Therefore, the radical resection of the local lymph-draining tissue led to a significant improvement of oncological results by lowering the local recurrence rate [3, 4]. In addition, the histological identification of lymphovascular invasion (LVI) has long been recognized as a potential indication of LNM and prognostic indicator and predictor of patient outcome. Many studies have investigated the presence of LVI in colorectal cancer (CRC) and have determined it to be a strong stage-independent prognostic marker [5–7]. Patients with LVI usually have a higher chance of disease progression and poorer prognosis [6–8].

Because of advances in noninvasive surgical techniques by endoscopy, it has recently become possible to perform resection of many early colon and rectal lesions, particularly those with peduncles, and also colonoscopic polypectomy, transanal endoscopic microsurgery, or per anum excision for low-lying lesions. Knowledge of LVI will be helpful in differentiating the management modality after localized, nonmetastatic disease. There are several studies that report a high incidence of recurrence and LNM after a full-thickness local excision [9, 10]. This prompted us to study

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the features of the primary tumors that may serve as risk factors for LVI in CRC.

Patients and Methods

Clinical and pathologic tissue data from all consecutive patients with CRC treated in our hospital were retrieved from the computer files from June 2004 to December 2010, retrospectively. We excluded all patients who have two-thirds distal low rectal carcinoma to rule out neoadjuvant treatment bias. Therefore, no patient had received chemoradiation or radiation therapy before surgery. The study consisted of 108 patients with primary CRC who underwent surgical resection. All patients underwent a standard colectomy and regional lymphadenectomy according to tumor location. For proximal one-third rectal cancer, the mesorectum was divided at 5 cm distal to the tumor. Tissues from the specimens were fixed in 10 % buffered formalin, processed, and stained with standard hematoxylin and eosin without the use of any special stains. Hematoxylin and eosin-stained slides of all cases were reviewed to confirm the diagnosis and were evaluated for the presence of LVI. The presence of venous invasion was defined according to the following criteria: tumor cells lining the venous endothelial surface, tumor cell thrombi inside the lumen of the vein, or destruction of the vein wall by tumor cells. The presence of a smooth muscle wall and adjacent arterial structure confirmed the venous nature of the endothelium-lined channels. Lymphatic invasion was defined as the presence of tumor within an endothelium-lined space devoid of mural smooth muscle or elastic fibers, with the closest red blood cell located at least one high-power field from the border of the structure [11]. Clinical data including age and sex of patient, location and diameter of tumor, perineural invasion, peritumoral lymphocytic infiltration, tumor grade, LVI, pT, and LNM were recorded.

Statistical Analysis

Statistical analysis was performed using the chi-squared test and Mann–Whitney *U* test. Differences were considered to be statistically significant if $p < 0.05$. Logistic regression test was used for multivariate analysis and the factors with $p < 0.2$ in the univariate test that were included in the multivariate analysis. All analyses were performed using the SPSS (version 12.0).

Results

Only 108 patients who matched the inclusion criteria were enrolled in the analysis. The gender distribution, age, location and diameter of tumor, perineural invasion, peritumoral

lymphocytic infiltration, tumor grade, pT, and LNM were comparable in the two groups (Table 1). LVI presented only in 43 (39.81 %) out of 108 patients, and the remaining 65 (60.18 %) patients did not present LVI. This analysis included 56 (51.9 %) male and 52 (48.1 %) female patients with a median age of 66.4 years (range, 26–94 years) in the LVI group and 63.9 (range, 27–89 years) in the non-LVI group ($p = 0.323$). The median tumor diameter was 60.6 mm (range, 3–180 mm) in LVI group and 55.6 mm (range, 8–130 mm) in non-LVI group ($p = 0.698$). Of the 108 patients, 20 out of the 39 cases of right colon tumors, 1 out of the 5 cases of transverse colon tumors, 8 out of the 18 cases of left colon tumors, 9 out of the 26 cases of sigmoid colon tumors, and 5 out of 20 cases of rectosigmoid tumors had LVI ($p = 0.306$), while 2 out of 2 cases of pT1, 3 out of 16 cases of pT2, 31 out of 50 cases of pT3, and 7 out of 9 cases of pT4 expressed LVI ($p = 0.04$). Forty-two patients (44.4 %) that had evidence of LNM; 35 patients out of 42, with LNM; 8 patients out of 66, without LNM expressed LVI ($p < 0.001$). Fourteen of 28 patients with perineural invasion and 27 of 77 patients without perineural invasion expressed LVI ($p = 0.165$). Twenty-seven of 43 patients with peritumoral lymphocytic infiltration and 2 of 13 patients without peritumoral lymphocytic infiltration expressed LVI ($p = 0.107$). Five of 12 patients with histological grade I, 30 of 84 patients with histological grade II, and 8 of 12 patients with histological grade III expressed LVI ($p = 0.213$).

In a univariate analysis, factors associated with LVI were LNM and pT. Perineural invasion, pT, and peritumoral lymphocytic infiltration factors with $p < 0.2$ in the univariate analysis were investigated with multivariate analysis, but no factor was found as an independent prognostic factor for LVI (Table 2).

Discussion

LNM is the most important factor on prognosis and management of adenocarcinomas of the colon and rectum. The presence of LNM decreases survival and also increases distant metastasis. LNM is one of the factors that help to decide the need for neoadjuvant therapy. Prediction of LNM presence is not always possible before operation, and the factors that predict LNM before the management of CRC will be helpful. Colonoscopy widely provides diagnosis of CRC at an early stage. However, some tumors, although diagnosed in a small size, may already have LNM and even distant metastasis. The incidence of LNM in the submucosal invasive CRC has been reported to be approximately 7–15 %, and the treatment consists of endoscopic polypectomy, local excision for selected patients, or radical resection [12–14]. Many series in which long-term follow-up was performed have revealed that the local recurrence rate is high although submucosal invasive CRC can be expected

Table 1 The univariate analyses of demographic details of patients and tumor characteristics of the primary colorectal carcinoma on lymphovascular invasion

Demographics		Lymphovascular invasion		<i>p</i> value
		Present	Absent	
Age (mean, year)		66.4	63.9	0.323 ^a
Sex	Male	22 (51.2)	34 (52.3)	0.907 ^b
	Female	21 (48.8)	31 (47.7)	
Location of tumor	Right colon	20 (46.5)	19 (29.3)	0.306 ^b
	Transverse colon	1 (2.3)	4 (6.2)	
	Left colon	8 (18.6)	10 (15.4)	
	Sigmoid colon	9 (20.9)	17 (24.1)	
	Rectosigmoid colon	5 (11.6)	15 (2.1)	
Maximum diameter of tumor (mean, mm)		60.6	55.6	0.698 ^a
Histological grade	I	5 (12.5)	7 (11.1)	0.213 ^a
	II	30 (75.0)	54 (85.7)	
	III	5 (12.5)	2 (3.2)	
Perineural invasion	Present	14 (34.1)	14 (21.9)	0.165 ^b
	Absent	27 (65.9)	50 (78.1)	
Peritumoral lymphocytic infiltration	Present	27 (93.1)	43 (79.6)	0.107 ^b
	Absent	2 (6.9)	11 (20.4)	
pT	1	2 (4.7)	0 (0)	0.04 ^a
	2	3 (7.0)	13 (20.0)	
	3	31 (72.1)	50 (76.9)	
	4	7 (16.3)	2 (3.1)	
Lymph node metastasis	Present	35 (81.4)	7 (10.8)	<0.001 ^b
	Absent	8 (18.6)	58 (89.2)	

Only pathologic T and lymph node metastases are statistically related to lymphovascular invasion

^aMann–Whitney *U* test

^bChi-squared test

to be treated by local excision without adjuvant therapy [9, 10]. Therefore, some characters of primary tumor may determine the biological behavior of the tumor. Identification of the risk factors for LNM is crucial in selecting appropriate therapeutic modalities for submucosal invasive CRC. Various risk factors for LNM have been reported, such as the depth of submucosal invasion (sm3), peritumoral LVI, poorly differentiated cancer, and tumor cell dissociation [13–15]. Choi et al. demonstrated that LVI was a risk factor for LNM in the univariate analysis [16]. Lim et al. demonstrated that the presence of LVI was an independent unfavorable prognostic factor for the 5-year overall and 5-year disease-free survival of patients with sporadic

CRC. The presence of LVI status could be used to identify patients with sporadic primary CRC with aggressive tumors and as a factor that independently indicates an unfavorable prognosis [17]. In our study, it is recognized that 35 patients out of 42 with LNM and 8 patients out of 66 patients without LNM express LVI, and the strong relation between them is highlighted. In addition, vascular invasion, both venous and lymphatic, gives accessible information that may be used to enhance the power of existing staging systems to develop a more accurate prognostic profile. Vascular drainage increases the chance of visceral metastasis; circulating tumor cells have a greater possibility of distal implantation.

Table 2 Multivariate analysis of LVI predictors in patients with colorectal cancers

	B	S.E.	<i>p</i> value	Odds ratio	95.0 % CI for EXP(B)	
					Lower	Upper
Perineural invasion	0.136	0.533	0.798	1.146	0.403	3.255
pT	−0.535	0.496	0.281	1.707	0.646	4.510
Peritumoral lymphocytic infiltration	1.046	0.823	0.204	2.847	0.567	14.294

The perineural invasion, pT, and peritumoral lymphocytic infiltration were the factors with $p < 0.2$ in the univariate analysis on lymphovascular invasion that were analyzed in logistic regression multivariate analysis. No factors are statistically related to lymphovascular invasion; $p < 0.05$ was accepted significant

SE standard error

LVI could be recognized clearly on hematoxylin and eosin (H&E)-stained slides, despite the fact that some reports indicate that immunohistochemical stains with CD31 and D2-40 may improve the diagnosis [18, 19]. CD31 is a marker of endothelial cells and is also designated as “platelet endothelial cell adhesion molecule-1” (PECAM-1). D2-40 is a relatively new immunohistochemical stain that has been demonstrated to label lymphatic endothelium, but it is not reactive with vascular endothelium [18, 20]. Using D2-40 and CD31, microvessel counts in colorectal carcinoma correlate significantly with the presence of LVI and LNM [21]. On the other hand, usefulness of elastic staining is debated. Sternberg et al. believe that the use of elastic staining increases the probability of identifying vessel involvement by 38.5 % [22]. Another interesting point regarding the report on vascular involvement raised by Sternberg et al. is the inherent chance of missing venous invasion, estimated to be between 10 % and 29.6 %, because the destruction of some veins due to the disease is too advanced to enable proper identification. Finally, a potential source of nonuniformity in reporting vascular invasion is the differentiation by some authors between intra- or extramural spread. Immunohistochemical and elastic staining may affect the LVI, but we used only H&E dye stain.

Despite the fact that preoperative radiation may lead to some histological changes, such as tumor regression or even a complete response, Du et al. found that preoperative radiotherapy alone did not significantly reduce the occurrence of LVI: at the same pathologic stage, the positive rates of LVI in the two groups were not significantly different [23, 24]. Even for patients with tumor downstage, who could be considered sensitive to radiation, the LVI-positive rate was not significantly reduced, which implied that the killing effect of X-rays to cancer cells involved in the vessels may be limited [25]. The effect of neoadjuvant therapy on LVI invasion is out of the concept of this study, and to have a uniform patient group, we excluded the patients who had undergone neoadjuvant therapy. Okabe et al. demonstrated that LNM was significantly more common in the rectum than in the left and right colon and that T1 CRCs had a progressively higher risk of LNM as their location became more distal [26], including the distal tumors whose neoadjuvant therapy might show the real relationship.

Although we did not have any relation between gender, the site of tumor and differentiation, and LVI, Okabe and Son demonstrated early CRCs with the male gender, location in the left colon, macroscopically depressed lesion, moderate or poor differentiation, depth in sm2 or sm3, and the presence of lymphatic tumor emboli having a higher risk of LNM [26, 27]. In fact, in our study, LVI is only related to the pT of tumor. The high level of the pT increases the presence of LVI. In addition, Nascimbeni et al. also demonstrated that T1 CRC with LVI, sm3 depth of invasion, and

location in the lower third of the rectum have a high risk of LNM [13]. Although it was not shown in our study, the site of tumor may increase the LVI. For Huh et al., LVI presence and perineural invasion were independent predictors for LNM [28]. Huh et al. also demonstrated perineural invasion to be significantly associated with the depth of tumor invasion and positive LVI, but our study did not [29]. In our study, the factors present in literatures that increase the LNM were not related to LVI except for pT.

In conclusion, according to our study, LVI is significantly related to LNM as expected. Only pT is found as a factor that increases the LVI. It is crucial to have larger series and uniform patient groups to have more specific results. In addition, immunohistochemical staining and uniform reporting of LVI may show the real relation of the factors on LVI. With new evidences of the effects of neoadjuvant therapy on LVI leading to the inclusion of the distal rectum may increase the chance of showing real factors that affect the LVI.

References

1. Jemal A, Murray T, Samuels A et al (2003) Cancer statistics, 2003. *CA Cancer J Clin* 53(1):5–26
2. Wibe A, Syse A, Andersen E et al (2004) Oncological outcomes after total mesorectal excision for cure for cancer of the lower rectum: anterior vs. abdominoperineal resection. *Dis Colon Rectum* 47(1):48–58
3. Cecil TD, Sexton R, Moran BJ, Heald RJ (2004) Total mesorectal excision results in low local recurrence rates in lymph node-positive rectal cancer. *Dis Colon Rectum* 47(7):1145–1150
4. Heald RJ, Ryall RD (1986) Recurrence and survival after total mesorectal excision for rectal cancer. *Lancet* 28;1(8496):1479–1482
5. Brodsky JT, Richard GK, Cohen AM, Minsky BD (1992) Variables correlated with the risk of lymph node metastasis in early rectal cancer. *Cancer* 15;69(2):322–326
6. Compton CC, Fielding LP, Burgart LJ et al (2000) Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 124(7):979–994
7. Ross A, Rusnak C, Weinerman B et al (1999) Recurrence and survival after surgical management of rectal cancer. *Am J Surg* 177 (5):392–395
8. Koukourakis MI, Giatromanolaki A, Sivridis E, et al. (2005) Inclusion of vasculature-related variables in the Dukes staging system of colon cancer. *Clin Cancer Res* 15;11(24 Pt 1):8653–8660
9. Garcia-Aguilar J, Mellgren A, Sirivongs P et al (2000) Local excision of rectal cancer without adjuvant therapy: a word of caution. *Ann Surg* 231(3):345–351
10. Lamont JP, McCarty TM, Digan RD et al (2000) Should locally excised T1 rectal cancer receive adjuvant chemoradiation? *Am J Surg* 180(6):402–406
11. Meguerditchian AN, Bairati I, Lagacé R et al (2005) Prognostic significance of lymphovascular invasion in surgically cured rectal carcinoma. *Am J Surg* 189(6):707–713
12. Nivatvongs S (2000) Surgical management of early colorectal cancer. *World J Surg* 24(9):1052–1055

13. Nascimbeni R, Burgart LJ, Nivatvongs S, Larson DR (2002) Risk of lymph node metastasis in T1 carcinoma of the colon and rectum. *Dis Colon Rectum* 45(2):200–206
14. Wang HS, Liang WY, Lin TC et al (2005) Curative resection of T1 colorectal carcinoma: risk of lymph node metastasis and long-term prognosis. *Dis Colon Rectum* 48(6):1182–1192
15. Tominaga K, Nakanishi Y, Nimura S et al (2005) Predictive histopathologic factors for lymph node metastasis in patients with non-pedunculated submucosal invasive colorectal carcinoma. *Dis Colon Rectum* 48(1):92–100
16. Choi PW, Yu CS, Jang SJ et al (2008) Risk factors for lymph node metastasis in submucosal invasive colorectal cancer. *World J Surg* 32(9):2089–2094
17. Lim SB, Yu CS, Jang SJ et al (2010) Prognostic significance of lymphovascular invasion in sporadic colorectal cancer. *Dis Colon Rectum* 53(4):377–384
18. Walgenbach-Bruenagel G, Tolba RH, Varnai AD et al (2006) Detection of lymphatic invasion in early stage primary colorectal cancer with the monoclonal antibody D2-40. *Eur Surg Res* 38(5):438–444, Epub 2006 Aug 15
19. Kingston EF, Goulding H, Bateman AC (2007) Vascular invasion is underrecognized in colorectal cancer using conventional hematoxylin and eosin staining. *Dis Colon Rectum* 50(11):1867–1872
20. Fogt F, Zimmerman RL, Ross HM et al (2004) Identification of lymphatic vessels in malignant, adenomatous and normal colonic mucosa using the novel immunostain D2-40. *Oncol Rep* 11(1):47–50
21. Saad RS, Kordunsky L, Liu YL et al (2006) Lymphatic microvessel density as prognostic marker in colorectal cancer. *Mod Pathol* 19(10):1317–1323
22. Sternberg A, Amar M, Alfici R, Groisman G (2002) Conclusions from a study of venous invasion in stage IV colorectal adenocarcinoma. *J Clin Pathol* 55(1):17–21
23. Janjan NA, Khoo VS, Abbruzzese J et al (1999) Tumor downstaging and sphincter preservation with preoperative chemoradiation in locally advanced rectal cancer: the M. D. Anderson Cancer Center experience. *Int J Radiat Oncol Biol Phys* 15:44(5):1027–1038
24. Du CZ, Xue WC, Cai Y et al (2009) Lymphovascular invasion in rectal cancer following neoadjuvant radiotherapy: a retrospective cohort study. *World J Gastroenterol* 14;15(30):3793–3798
25. Cui Y, Yang H, Wu S et al (2002) Molecular mechanism of damage and repair of mouse thymus lymphocytes induced by radiation. *Chin Med J (Engl)* 115(7):1070–1073
26. Okabe S, Shia J, Nash G et al (2004) Lymph node metastasis in T1 adenocarcinoma of the colon and rectum. *J Gastrointest Surg* 8(8):1032–1040
27. Son HJ, Song SY, Lee WY et al (2008) Characteristics of early colorectal carcinoma with lymph node metastatic disease. *Hepatogastroenterology* 55(85):1293–1297
28. Huh JW, Kim HR, Kim YJ (2010) Lymphovascular or perineural invasion may predict lymph node metastasis in patients with T1 and T2 colorectal cancer. *J Gastrointest Surg* 14(7):1074–1080
29. Huh JW, Kim HR, Kim YJ (2010) Prognostic value of perineural invasion in patients with stage II colorectal cancer. *Ann Surg Oncol* 17(8):2066–2072