Cancer Res Treat. 2008;40(2):75-80

Clinical Significance of Lymph Node Micrometastasis in Stage I and II Colon Cancer

Sun Jin Park, M.D.[1], **Kil Yeon Lee,** M.D.[1], PhD. and **Si Young Kim,** M.D., Ph.D.[2]

Departments of [1]Surgery and [2]Medical Oncology/Hematology, Kyung Hee University College of Medicine, Seoul, Korea

Purpose: **A 25% rate of recurrence after performing complete resection in node-negative colon cancer patients suggests that their nodal staging is frequently suboptimal. Moreover, the value of occult cancer cells in tumor-free lymph nodes still remains uncertain. The authors evaluated the prognostic significance of the pathologic parameters, including the lymph node occult disease (micrometastases) detected by immunohistochemistry, in patients with node-negative colon cancer.**

Materials and Methods: **The study included 160 patients with curatively resected stage I or II colon cancer and they were without rectal cancer. 2852 lymph nodes were re-examined by re-do hematoxylin and eosin (H-E) staining and immunohistochemical staining. The detection rates were compared with the clinicopathologic characteristics and with the cancer-specific survival.**

Results: **Occult metastases were detected in 8 patients (5%). However, no clinicopathologic parameter was found to be correlated with the presence of micrometastasis. Twenty patients developed recurrence at a median follow-up of 45.7 months: 14 died of colon cancer and 9 died from noncancer-related causes. Univariate analysis showed that lymphatic invasion and the number of retrieved lymph nodes significantly influenced survival, and multivariate analysis revealed that the stage, the number of retrieved lymph nodes and lymphatic invasion were independently related to the prognosis.**

Conclusions: **Inadequate lymph node retrieval and lymphatic invasion were found to be associated with a poorer outcome for node-negative colon cancer patients. The presence of immunostained tumors cells in pN0 lymph nodes was found to have no significant effect on survival, but these tumor were identified by re-do H-E staining. Maximal attention should be paid to the total number of lymph nodes that are retrieved during surgery for colon cancer patients.** ***(Cancer Res Treat. 2008;40:75-80)***

Key Words: **Colonic neoplasms, Lymphatic metastasis, Immunohistochemistry, Survival**

INTRODUCTION

Colorectal carcinoma (CRC) is the second most common gastrointestinal malignancy in Korea, and the incidence of CRC continues to steadily increase (1). The prognosis for patients with colorectal cancer is mainly predicted with using the clinicopathologic stage (TNM classification), but one of the main prognostic parameters is represented by lymph node (LN) metastases. Approximately 39% of all colorectal cancer patients have histologically node-negative (N0) localized disease (2). Although these patients with localized colon cancer can potentially be cured by surgical resection alone, a 25% rate of recurrence suggests that their nodal staging is frequently suboptimal (3). The presence of micrometastases (MCMs) within LNs that are not detected by conventional hematoxylin and eosin (H-E) staining provides a possible explanation for this discrepancy. However, although the presence of LN MCMs in CRC patients has been investigated, no consensus has been reached regarding the prognostic significance of this finding (4-13).

The aim of this study was to examine a wide range of clinicopathologic variables in a retrospective series of patients with curatively resected stage I or II colon cancer, and we also wanted to determine whether these findings were associated with tumor recurrence.

Correspondence: Si Young Kim, Department of Medical Oncology/Hematology, Kyung Hee University Medical Center, 1, Hoegi-dong, Dongdaemun-gu, Seoul 130-702, Korea. (Tel) 82-2-958-8721, (Fax) 82-2-959-7192, (E-mail) sykim@khu.ac.kr.

Received March 19, 2008, Accepted June 14, 2008

This work was supported by the second phase of the Brain Korea 21 Project in 2007.

MATERIALS AND METHODS

1) Patients

Between March 1996 and June 2005, 160 patients who met the following inclusion criteria underwent resection for their colon cancer: (1) colon carcinoma was proven by biopsy (rectal cancer and rectosigmoid colon cancer were excluded) and the treatment was curative colon resection, (2) R0 resection was done (on the pathologic examinations), (3) stage I or II disease (any T stage with no lymph node or distant metastasis); (4) no other synchronous or metachronous malignant tumor, (5) the carcinoma did not arise from familial adenomatous polyposis,

hereditary nonpolyposis colorectal cancer, ulcerative colitis or Crohn's disease. One hundred three (64.4%) men and 57 (35.6%) women were included in this study. The median age of the patients was 62 years (range: 24～86 years)(Table 1).

2) Follow-Up

The mean follow-up period was 45.7 months (range: 1～137 months). All the patients were assessed for local control and distant metastases by performing clinical examination, chest radiography, abdominal ultrasonography and abdominal computed tomography scanning and measuring the carcinoembryonic antigen (CEA) serum level. Follow-up examinations were performed every 3 months during the first two years, biannually for the next three years and then annually. The survival times were calculated from the date of surgery to the date of death or the last follow-up, and these dates were confirmed using the data registered at the National Cancer Center.

Table 1. Clinicopathologic characteristics of the patients with stage I and II colon cancer

	No. of cases (%)
Gender	
Male	103 (64.4)
Female	57 (35.6)
Median age (range)	62 (24～86)
pT stage	
T1～2	34 (21.3)
T3	123 (76.9)
T4	3 (1.9)
Tumor location	
Ascending colon and hepatic flexure	57 (35.6)
Transverse colon	18 (11.3)
Splenic flexure and descending colon	11 (6.9)
Sigmoid colon	74 (46.3)
Tumor differentiation	
Well	56 (35.0)
Moderate	97 (60.6)
Poor	7 (4.4)

3) Methods

(1) Pathologic variables: With regard to the cancer's location, two anatomic sites were considered, i.e., the proximal colon (from the cecum to the transverse colon), and the distal colon (from the splenic flexure to the sigmoid colon). The tumor's gross shape was classified as exophytic or nonexophytic. The tumor's maximal diameter was categorized as ≤ or >5 cm. The tumors were classified as mucinous or nonmucinous, according to the amount of the mucinous component (≥ and <50% of the tumor volume, respectively). Well and moderately differentiated tumors were categorized as low-grade tumors, whereas poorly differentiated, undifferentiated and mucinous tumors were categorized as high-grade.

(2) Immunohistochemical evaluation: The original histologic slides of the tumor tissues were reviewed by an experienced pathologist. 2852 LNs from 160 patients were examined (mean number of LNs: 17.8 LNs/patient). For each LN, two new slices of 5 μm thickness were obtained from the original paraffin blocks. The sections were deparaffinized in xylene and then they were rehydrated. One section was stained with H-E and the other was subjected to immunohistochemistry (IHC). The IHC staining procedures were conducted using an automated immunostainer (Vision Biosystems, VIC, Australia). The sections were incubated at a dilution of 1 : 400 with a monoclonal antibody for anti-cytokeratin 20 (CK20; clone K 20.8, Dako, Carpentaria, CA). The immunostaining was developed using 3,3'-diaminobenzidine as a chromogen. Appropriate positive controls were added to each automated IHC run to confirm the sensitivity and specificity of the antibody (sections of the CK20-positive CRC tissue served as the positive controls). The immunostained slides were evaluated by a pathologist who had no knowledge of the pathologic data or the patients' outcomes.

(3) Statistical Analysis: The primary endpoints were tumor recurrence and the overall survival. Statistical correlations between micrometastasis and the pathological variables were

Table 2. The immunohistochemical staining and the re-do H-E staining findings

case	H-E re-exam	Re-do H-E	IHC*	Size of OTCs†		Recurrence
1-a	−	+	+	1.4 mm		
1-b	−	+	+	3.7 mm	Tumor deposits →LNs‡ metastasis	
2	−	+	+	3.3 mm		Peritoneal
3	−	+	+	263 μm		
4	+	+	+	4.1 mm	False negative	
5-a	−	+	+	258 μm		
5-b	−	+	+	3.0 mm		
6	+	+	+	1.7 mm	False negative	
7	−	+	+	1.0 mm		
8-a	−	+	+	800 μm		Liver
8-b	−	+	+	510 μm		
8-c	−	+	+	3.7 mm		

*immunohistochemistry, †occult tumor cells, ‡lymph nodes.

assessed using the chi-square test and Fisher's exact test, and the relationships between the pathologic variables and survival were estimated using the Kaplan-Meier method. The survival differences between the groups were analyzed using the log-rank test. The Cox proportional hazards regression model was used to assess the clinicopathologic factors that could independently influence survival. P values <0.05 were considered to be statistically significant.

RESULTS

1) H-E re-staining and the CK20-positive LNs

2852 LNs from 160 patients were examined after staining them with anti-CK20 monoclonal antibody. Cytokeratin 20-positive neoplastic cells were found in 12 of the 2852 LNs (0.4%) from 8 of the 160 patients (5.0%). In addition, all the corresponding H-E re-stained sections confirmed the presence of these neoplastic cells. Micrometastases ($\geq$0.2 mm but $<$2.0 mm) were detected in 7 of the LNs, whereas isolated tumor cells ($<$0.2 mm) were not found. Macrometastases ($>$2 mm) were found in 5 of the LNs. A retrospective review of the original histologic slides revealed that neoplastic cells were present in 2 of the 12 CK20-positive LNs at the original evaluations (Table 2).

2) Relationship between the CK20-positive LNs and the clinicopathologic factors

The clinicopathologic characteristics of the patients with or without CK20-positive LNs are listed in Table 3. None of the variables that were evaluated was found to be significantly different between those patients with or those patients without CK20-positive LNs.

3) The Overall Survival and the Disease-Free Survival

During the observation period, 20 patients developed tumor recurrence and 23 patients died after surgery. Nine of these 23 patients succumbed to diseases other than colon cancer; therefore, the survival analysis was performed on the remaining

Table 3. Clinicopathologic characteristics of the patients and the presence of CK20-positive tumor cells in their LNs

Variable	Cases	CK20-negative LNs		CK20-positive LNs		p
		n	%	n	%	
Gender						0.713
Male	103	97	94.2	6	5.8	
Female	57	55	96.5	2	3.5	
Tumor site						0.284
Proximal colon	75	73	97.3	2	2.7	
Distal colon	85	79	92.9	6	7.1	
Gross morphology						0.152
Exophytic	84	82	97.6	2	2.4	
Nonexophytic	76	70	92.1	6	7.9	
Tumor size						0.297
≤5 cm	88	82	93.2	6	6.8	
>5 cm	72	70	97.2	2	2.8	
Histological type						1.0
Mucinous	5	5	100	0	0	
Nonmucinous	155	147	94.8	8	5.2	
Tumor grade						1.0
Low	148	140	94.6	8	5.4	
High	12	12	100	0	0	
T stage						0.282
T1-2	34	34	100	0	0	
T3	123	115	93.5	8	6.5	
T4	3	3	100	0	0	
Lymphatic invasion						0.343
Absent	136	130	95.6	6	4.4	
Present	24	22	91.7	2	8.3	
Recurrence						0.262
Yes	20	18	90.0	2	10.0	
No	140	134	95.7	6	4.3	
LN retrieved						0.706
<12	50	47	94.0	3	6.0	
≥12	110	105	95.5	5	4.5	

CK20: monoclonal, anti-human cytokeratin 20.

Table 4. Univariate analysis of survival for the stage I and II colon cancer patients

Variable	5-year disease-free survival (%)	p	5-year overall survival (%)	p
Gender				
Male	84.1	0.83	93.4	0.27
Female	84.7		91.1	
Tumor site				
Proximal colon	86.9	0.4	92.9	0.34
Distal colon	81.6		92.6	
Gross morphology				
Exophytic	88.5	0.24	96.4	0.14
Nonexophytic	79.6		88.3	
Tumor size				
≤5 cm	82.5	0.62	89.3	0.065
>5 cm	81.5	97.0		
Histotype				
Mucinous	75.0	0.33	75.0	0.36
Nonmucinous	84.6		93.3	
Tumor grade				
Low	83.8	0.75	92.9	0.89
High	90.0		90.9	
T stage				
T1-2	96.4	0.057	96.7	0.59
T3-4	80.7		91.7	
Lymphatic invasion				
Absent	87.2	0.24	94.5	**0.007**
Present	75.0		82.1	
CK20				
Negative	85.3	0.98	93.1	0.59
Positive	80.0		91.7	
LN* retrieved				
<12	76.3	**0.029**	83.5	**0.004**
≥12	87.6		97.0	

*lymph node.

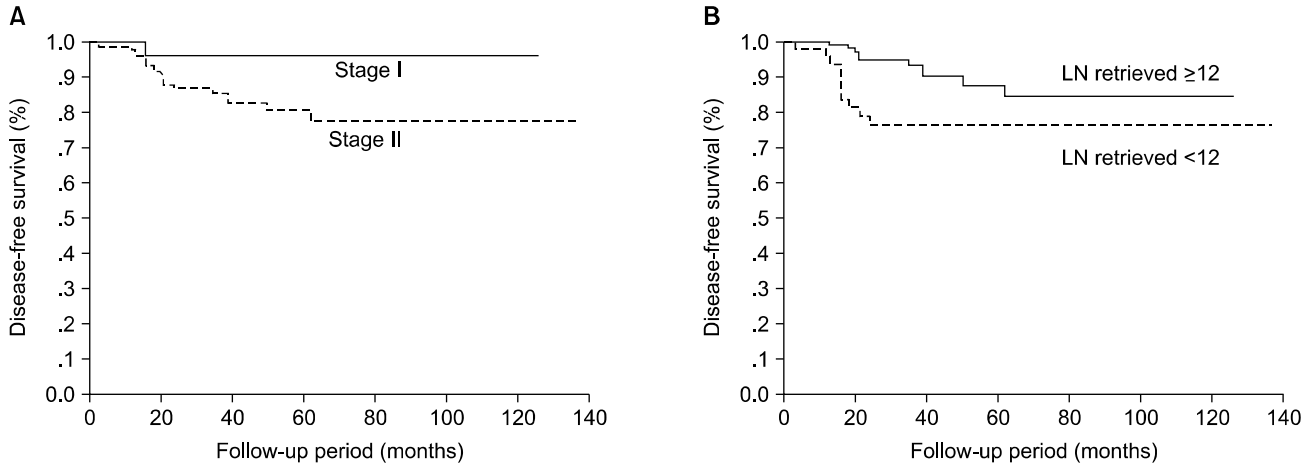

Fig. 1. Disease-free survival according to (A) the stage (stage I vs. stage II) and (B) the number of retrieved LNs (<12 vs. ≥12). Both parameters were found to be significantly associated with disease-free survival.

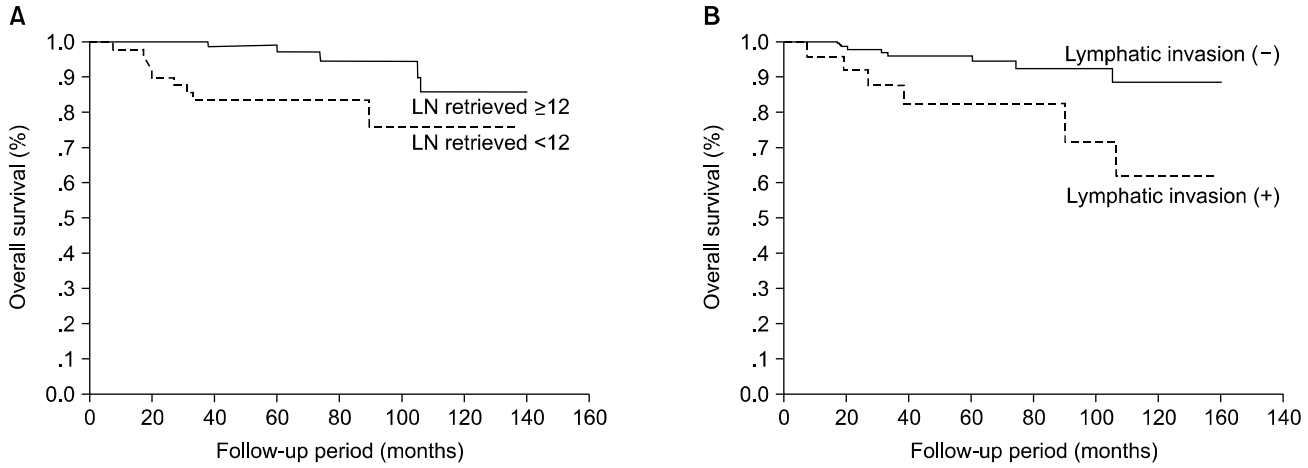

Fig. 2. Overall survival according to (A) the number of retrieved LNs (<12 vs. ≥12) and (B) lymphatic invasion (absent vs. present). Both parameters were found to be significantly associated with disease-free survival.

14 cases. These 14 patients more often had hematogenous metastasis (n=14) than local recurrence (n=6). The sites of recurrence were as follows: six hepatic metastases, four pulmonary metastases, four peritoneal metastases and six local recurrences (anastomotic or local lymphatic).

Univariate analysis was utilized to evaluate the impacts of CK20-positive staining in the LNs and the various clinicopathologic parameters on the prognosis of patients with node-negative colon cancer (Table 4). Less than twelve retrieved LNs and the presence of lymphatic invasion were found to be significantly associated with poor overall survival (p=0.004 and 0.007, respectively). Moreover, retrieval of less than twelve LNs was found to be significantly associated with shorter disease-free survival (p=0.029). CK20-positive staining in LNs and the other clinicopathologic parameters were not found to have a significant impact on the overall survival or disease-free survival. Multivariate Cox regression analysis revealed that the stage (stage I vs. stage II) and LN retrieval (<12 vs. ≥12) were the significant prognostic factors of disease-free survival (p=0.011 and 0.015, respectively) (Fig. 1). For overall survival, LN retrieval (<12 vs. ≥12) and lymphatic invasion were retained as significant covariates (p=0.012 and 0.031, respectively) (Table 5) (Fig. 2). The five-year disease-free and overall

Table 5. Multivariate Cox proportional hazards analysis of the survival factors for stage I and II colon cancer

Variable	Comparison	HR* (95% CI†)	p value
Disease-free survival			
Stage	Stage I vs. stage II	7.045 (0.933~53.193)	0.011
LN retrieved	<12 vs. ≥12	0.327 (0.136~0.790)	0.015
Overall survival			
LN retrieved	<12 vs. ≥12	0.253 (0.084~0.757)	0.012
Lymphatic invasion	Absent vs. present	3.454 (1.187~10.047)	0.031

*hazard ratio, †confidence interval.

survival rates were 87.5% and 94.9%, respectively.

DISCUSSION

The terms "micrometastasis" and "macrometastasis" were originally defined to be metastatic deposits of breast carcinoma cells that measured less than or greater than 2 mm, respectively (14). However, the 6th edition of the AJCC (American Joint Committee on Cancer) Cancer Staging Manual has recently recommended that minute amounts of metastatic tumor cells should be classified as micrometastases or isolated tumor cells (ITCs) based on their dimensions, i.e., ITCs are classified as single tumor cells or cell clusters measuring <0.2 mm, and micrometastases are classified as clusters of cells measuring >0.2 mm, but <2.0 mm (15). Moreover, although the presence of LN micrometastases in CRC patients has previously been investigated, no consensus has been reached regarding its prognostic significance. The previously reported results are barely comparable because they differ with regard to the clinicopathologic parameters and the methodologies that were used, and the previous studies usually included both colon and rectal cancer, which do not have the same prognosis.

Special techniques such as IHC or reverse transcriptase polymerase chain reaction (RT-PCR) can be used to identify the micrometastases within LNs that are not detectable by conventional H-E staining. Several studies have employed IHC with using monoclonal antibodies directed against carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA) or cytokeratin (CK). O'Brien et al. (16) reported that although LN micrometastases may be more easily detected by performing IHC for CEA, the screening of H-E stained sections by a competent pathologist appeared to be equally sensitive. Davidson et al. (6) found that among 200 LNs reported as being free of tumor deposits by H-E, micrometastasis was only found in one LN by performing IHC for CEA and EMA, and these researchers suggested that performing routine IHC of the LN sections in conjunction with H-E was unhelpful. Adell et al. (4) re-examined regional LNs with using monoclonal antibodies against CK, and they detected micrometastases in 39% of the patients, but these patients' outcomes were not significantly different from those patients with negative LNs. In contrast, Greenson et al. (7) reported that CK staining revealed occult metastases in 5.8% of the examined nodes, and they found that CK-positive cells within the LNs were correlated with a significantly poorer prognosis. Consequently, they recommended CK staining of the pericolic LNs in patients with Dukes' B colorectal carcinoma. Moreover, Sasaki et al. (17) detected LN micrometastases in 38% of the Dukes' A and B patients with recurrent disease, which was significantly greater than in those patients without recurrent disease (13%).

In the present study, occult cancer cells in the previously considered negative LNs were detected by H-E staining in 5.0% of the patients, and these patients were consequently restaged as pN1. However, the disease-free and overall survival rates of the restaged pN1 patients on univariate analysis were not significantly different from those of the pN0 patients. Significant correlations between the presence of occult tumor cells and survival have been reported by some studies (7,8,12), whereas others have failed to demonstrate this correlation (4-6,9-11,13). However, the previous studies that used only 1 section per lymph node detected micrometastases at rates ranging from 4% to 31% (5-7), whereas other studies that used 2 or more sections per lymph node showed higher rates that ranged from 76% to 100% (17,18). Noura et al. (11) demonstrated that the rate of detecting LN micrometastases increases as the slice number is increased from 1 to 2 to 5.

However, the prognostic significance of the LN micrometastases that are detected by IHC in colorectal cancer patients is controversial. In contrast, based on the findings obtained with using molecular genetic techniques, some investigators have suggested there is a positive correlation between the presence of micrometastases and a poor prognosis in patients with node-negative colorectal cancer (19-21). RT-PCR has the advantage of allowing large numbers of LNs to be processed. However, it is unclear whether RT-PCR is more useful for detecting of micrometastases than IHC, and furthermore, its prognostic significance has not been established (22).

In the present study, all the occult tumor cells detected on IHC were confirmed by performing examinations of the corresponding H-E re-stained sections. As many as 70% of the tumor-positive LNs have been reported to contain metastases of <0.5 cm in diameter (23), and these metastases can not be seen or felt by a surgeon or a pathologist, and they might also be missed during conventional pathologic dissection and H-E staining. Using multiple step sections for IHC can improve the staging accuracy, but this process is labor intensive and the cost of examining the slides corresponding to a whole LN is prohibitive.

However, the microscopic examinations involving only one histologic section of a LN are clearly inadequate for the detection of micrometastases and also possibly for the detection of macrometastases. In the present study, macrometastases were detected in 5 of the LNs with occult tumor cells.

It is also important to recognize that patients may be at a high risk of recurrence regardless of micrometastatic involvement. A poorly differentiated tumor, lymphatic and venous invasion, extension to adjacent organs and inadequate regional LN retrieval all have adverse effects, and those patients with such features should be recommended for adjuvant treatment (24). The National Cancer Institute recommends that a minimum of 12 LNs should be assessed when staging a patient with colorectal cancer (25). In the present study, 20 (12.5%) patients developed disease recurrence and 14 (8.8%) patients finally succumbed to colon cancer. Retrieval of less than 12 LNs and the presence of lymphatic invasion were significantly associated with poor overall survival. Furthermore, retrieval of fewer than 12 LNs was found to be significantly associated with shorter disease-free survival.

CONCLUSION

In the present study, we examined the tumor-negative LNs in patients with stage I and II colon cancer. IHC staining was performed, and the predictive values of the clinicopathologic parameters were investigated. CK20-positive neoplastic cells were found in 5.0% of the patients, but these were not found

to have a significant effect on the disease-free survival or overall survival. However, all the occult tumor cells detected by IHC were confirmed in the corresponding H-E re-stained sections. We estimate that including one more section on conventional H-E staining would upstage pN0 disease to pN1 disease in 5% of the node-negative colon cancer patients. Furthermore, LN retrieval and lymphatic invasion were found to have adverse effects on survival. We recommend that surgeons and pathologists seriously consider examining an adequate number of LNs and a sufficient number of cut sections in patients suffering with stage 1 and 2 colon cancer.

REFERENCES

1. Park JG. Cancer Incidence in Korea 1999～2001. Goyang: National Cancer Center; 2005.
2. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin. 2007;57:43-66.
3. Chen SL, Bilchik AJ. More extensive nodal dissection improves survival for stages I to III of colon cancer: a population-based study. Ann Surg. 2006;244:602-10.
4. Adell G, Boeryd B, Franlund B, Sjodahl R, Hakansson L. Occurrence and prognostic importance of micrometastases in regional lymph nodes in Dukes' B colorectal carcinoma: an immunohistochemical study. Eur J Surg. 1996;162:637-42.
5. Choi HJ, Choi YY, Hong SH. Incidence and prognostic implications of isolated tumor cells in lymph nodes from patients with Dukes B colorectal carcinoma. Dis Colon Rectum. 2002;45:750-5.
6. Davidson BR, Sams VR, Styles J, Deane C, Boulos PB. Detection of occult nodal metastases in patients with colorectal carcinoma. Cancer. 1990;65:967-70.
7. Greenson JK, Isenhart CE, Rice R, Mojzisik C, Houchens D, Martin EW Jr. Identification of occult micrometastases in pericolic lymph nodes of Duke's B colorectal cancer patients using monoclonal antibodies against cytokeratin and CC49. Correlation with long-term survival. Cancer. 1994;73:563-9.
8. Isaka N, Nozue M, Doy M, Fukao K. Prognostic significance of perirectal lymph node micrometastases in Dukes' B rectal carcinoma: an immunohistochemical study by CAM5.2. Clin Cancer Res. 1999;5:2065-8.
9. Kronberg U, Lopez-Kostner F, Soto G, Zuniga A, Wistuba I, Miranda V, et al. Detection of lymphatic micrometastases in patients with stages I and II colorectal cancer: impact on five-year survival. Dis Colon Rectum. 2004;47:1151-7.
10. Nakanishi Y, Ochiai A, Yamauchi Y, Moriya Y, Yoshimura K, Hirohashi S. Clinical implications of lymph node micrometastases in patients with colorectal cancers. A case control study. Oncology. 1999;57:276-80.
11. Noura S, Yamamoto H, Miyake Y, Kim B, Takayama O, Seshimo I, et al. Immunohistochemical assessment of localization and frequency of micrometastases in lymph nodes of colorectal cancer. Clin Cancer Res. 2002;8:759-67.
12. Rosenberg R, Friederichs J, Gertler R, Hoos A, Mueller J, Nahrig J, et al. Prognostic evaluation and review of immunohistochemically detected disseminated tumor cells in peritumoral lymph nodes of patients with pN0 colorectal cancer. Int J Colorectal Dis. 2004;19:430-7.
13. Tschmelitsch J, Klimstra DS, Cohen AM. Lymph node micrometastases do not predict relapse in stage II colon cancer. Ann Surg Oncol. 2000;7:601-8.
14. Huvos AG, Hutter RV, Berg JW. Significance of axillary macrometastases and micrometastases in mammary cancer. Ann Surg. 1971;173:44-6.
15. Greene FL, Page DL, Fleming ID, Fritz A, Balch CM, Haller DG, et al. AJCC Cancer Staging Manual, 6th ed. New York: Springer-Verlag; 2002. p. 226-8.
16. O'Brien MJ, Zamcheck N, Burke B, Kirkham SE, Saravis CA, Gottlieb LS. Immunocytochemical localization of carcinoembryonic antigen in benign and malignant colorectal tissues. Assessment of diagnostic value. Am J Clin Pathol. 1981;75:283-90.
17. Sasaki M, Watanabe H, Jass JR, Ajioka Y, Kobayashi M, Matsuda K, et al. Occult lymph node metastases detected by cytokeratin immunohistochemistry predict recurrence in "node-negative" colorectal cancer. J Gastroenterol. 1997;32:758-64.
18. Yasuda K, Adachi Y, Shiraishi N, Yamaguchi K, Hirabayashi Y, Kitano S. Pattern of lymph node micrometastasis and prognosis of patients with colorectal cancer. Ann Surg Oncol. 2001;8:300-4.
19. Liefers GJ, Cleton-Jansen AM, van de Velde CJ, Hermans J, van Krieken JH, Cornelisse CJ, et al. Micrometastases and survival in stage II colorectal cancer. N Engl J Med. 1998;339:223-8.
20. Noura S, Yamamoto H, Ohnishi T, Masuda N, Matsumoto T, Takayama O, et al. Comparative detection of lymph node micrometastases of stage II colorectal cancer by reverse transcriptase polymerase chain reaction and immunohistochemistry. J Clin Oncol. 2002;20:4232-41.
21. Rosenberg R, Hoos A, Mueller J, Baier P, Stricker D, Werner M, et al. Prognostic significance of cytokeratin-20 reverse transcriptase polymerase chain reaction in lymph nodes of node-negative colorectal cancer patients. J Clin Oncol. 2002;20:1049-55.
22. Bilchik AJ, Hoon DS, Saha S, Turner RR, Wiese D, DiNome M, et al. Prognostic impact of micrometastases in colon cancer: interim results of a prospective multicenter trial. Ann Surg. 2007;246:568-75.
23. Rodriguez-Bigas MA, Maamoun S, Weber TK, Penetrante RB, Blumenson LE, Petrelli NJ. Clinical significance of colorectal cancer: metastases in lymph nodes <5 mm in size. Ann Surg Oncol. 1996;3:124-30.
24. Benson AB 3rd, Schrag D, Somerfield MR, Cohen AM, Figueredo AT, Flynn PJ, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol. 2004;22:3408-19.
25. Nelson H, Petrelli N, Carlin A, Couture J, Fleshman J, Guillem J, et al. Guidelines 2000 for colon and rectal cancer surgery. J Natl Cancer Inst. 2001;93:583-96.