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Quantification of CD10 mRNA in Colorectal Cancer and Relationship between mRNA Expression and Liver Metastasis

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Abstract. *CD10 mRNA expression in colorectal cancer and its relationship with cancer progression and prognosis were investigated. Patients and Methods: CD10 mRNA was quantified in 167 colorectal cancer and matched normal tissue samples using real-time polymerase chain reaction (RT-PCR). The tumor to normal tissue (T/N) CD10 mRNA ratio was compared with clinicopathological factors and prognosis. Results: CD10 mRNA was overexpressed in 138 of the 167 tumors in comparison with the matched normal tissues. T/N was higher in colon, pN1/pN2, stage III and IV, and well- or moderately-differentiated adenocarcinoma than in rectum, pN0, stage I and II, and poorly-differentiated or mucinous adenocarcinoma, respectively. However, these differences were not significant. T/N was not associated with prognosis. Conclusion: CD10 mRNA showed significantly higher expression in tumor tissues than in matched normal tissues. Although CD10 mRNA was associated with invasion depth, lymph node status and TNM stage, it was not associated with prognosis.*

CD10 is a 100 kDa cell surface zinc metalloendopeptidase that was initially identified as the common acute lymphoblastic leukemia antigen. Although CD10 is commonly expressed on hematopoieteic cells and tumors, it is also expressed in a variety of normal and tumor tissues. Recently, several studies have shown an association between CD10 expression and progression of various kinds of tumors including gastric cancer (1-5), colorectal cancer (6-10), pancreatic endocrine tumor (11), ovarian cancer (12), cervical carcinoma (13), renal cell carcinoma (14, 15), prostate cancer (16), breast cancer (17), non-small cell lung cancer (18), melanoma (19, 20), nasopharyngeal carcinoma (21), oral cavity squamous cell carcinoma (22) and B-cell lymphoma (23). Therefore, CD10 is considered to play an important role in both normal and tumor tissues. We recently demonstrated that CD10 protein expression in colorectal cancer was significantly associated with liver metastasis (10). This result prompted us to examine the association between CD10 mRNA expression and liver metastasis. In the present study, CD10 mRNA in colorectal cancer tissues was quantified by real-time PCR in comparison with matched normal tissues, and the relationship between CD10 mRNA expression and clinicopathological characteristics was examined.

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Key Words: Colorectal cancer, CD10, liver metastasis, prognosis.

Patients and Methods

Patients and tissues. Tumor tissue and adjacent normal tissues (10 cm away from the tumor) were obtained from 175 patients with colorectal cancer between January 1995 and September 1996 at the National Cancer Center Hospital, Tokyo, Japan, after informed consent had been obtained. Among these, a total of 167 samples in which CD10 expression was examined using the avidin-biotin-peroxidase method with mouse monoclonal antibody 56C6 (Novocastra, Newcastle, UK) in our previous study (10) were investigated for CD10 mRNA quantification. Although in our previous study >5% staining of tumor cells had been judged as positive, in the present study we considered staining of >5% of tumor and/or stromal cells as positive, because CD10 is also expressed in stromal cells (7). Tissues had been obtained immediately after surgery and stored frozen in liquid nitrogen until RNA extraction. All surviving patients had been followed up for more than 5 years, initially at 3-month intervals for 2 years and then at 6-month intervals thereafter. Median follow-up time was 7.9 years, and no adjuvant chemotherapy was given in this period.

RNA extraction and relative mRNA quantification. Total RNA was extracted from the frozen tissues according to the procedure described by Chomczyski and Sacchi (24). Randomly primed cDNA was synthesized from 1 μg of total RNA using a High-Capacity cDNA Archive Kit in accordance with the manufacturer's instructions (Applied Biosystems, CA, USA). CD10 mRNA expression was quantified using TaqMan gene expression assay and

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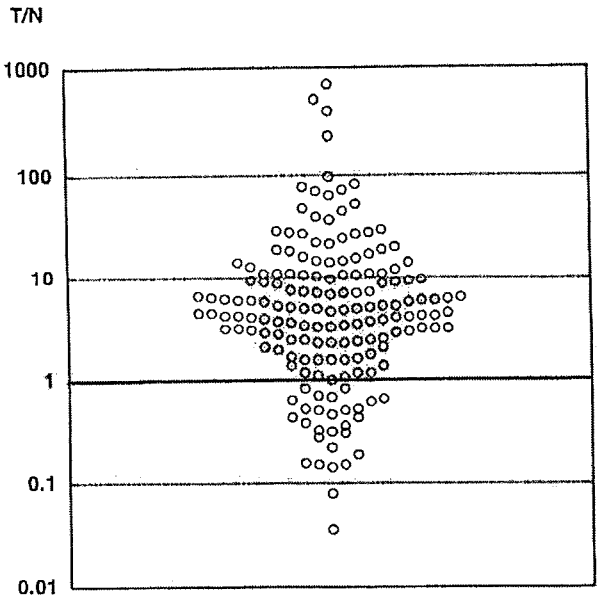

Figure 1. *Distribution of CD10 mRNA T/N. Mean T/N±standard deviation was 20.89±75.80, range was 0.036 to 709.176.*

Table I. *Patient clinicopathological characteristics and CD10 mRNA (T/N).*

Characteristic	No. (n=167)	CD10 mRNA (T/N±S.D.)	*P*
Age (yr)			
≤ 60	71	25.39±79.97	0.645
60 <	96	17.57±72.81	
Gender			
Male	99	19.77±64.88	0.881
Female	68	22.52±89.88	
Tumor site			
Colon	100	29.31±96.56	0.156
Rectum	67	8.33±14.04	
Depth of invasion (pT)			
pT1/pT2	1/27	11.98±17.37	0.333
pT3/pT4	105/34	22.68±82.66	
Lymph node status			
pN0	76	9.81±14.88	0.847
pN1/ pN2	54/37	30.14±101.11	
Stage			
I/II	19/49	9.60±15.16	0.996
III/IV	67/32	28.64±97.09	
Tumor differentiation			
Well/Moderate	70/85	22.21±78.53	0.063
Poor/Mucinous	9/3	3.86±4.30	
Lymphatic invasion			
Negative	60	19.03±66.30	0.643
Positive	107	21.94±80.92	
Venous invasion			
Negative	80	18.55±62.41	0.859
Positive	87	23.05±86.62	
CD10 protein expression			
Negative	83	5.33±5.65	0.003
Positive	84	36.64±105.35	

a 7300 Real-Time PCR System (Applied Biosystems) in accordance with the manufacturer's instructions. The CD10 mRNA level in the tumor was compared with that in matched normal mucosa after standardization against 18S rRNA as an internal control gene (25). The CD10 mRNA level was calculated using the formula: $-\Delta\Delta Ct$ (cycle threshold) (Δ Ct of tumor (CD10 Ct - 18S rRNA Ct) – Δ Ct of matched normal tissue (CD10 Ct – 18S rRNA Ct)) to the power two ($2^{-\Delta\Delta Ct}$). This value is the ratio of CD10 mRNA in the tumor relative to that in matched normal tissue (T/N).

Statistical analysis. T/N was compared statistically using Mann-Whitney *U*-test. Survival rates were calculated by the Kaplan-Meier method and survival curves were compared by the log-rank test. Data differences between groups were considered statistically significant at $p<0.05$.

Results

Patient characteristics and CD10 mRNA. The distribution of CD10 mRNA T/N is shown in Figure 1. Mean T/N±standard deviation was 20.89±75.80, and the range was 0.036 to 709.176. In 138 (83%) of the 167 tumors, T/N was more than one, which meant that CD10 was overexpressed in the tumor tissue compared with the matched normal tissue. Patient characteristics and T/N are shown in Table I. T/N was higher in colon, pN1/pN2, stage III and IV, and well or moderately-differentiated adenocarcinoma than in rectum, pN0, stage I and II, and poorly-differentiated or mucinous adenocarcinoma, respectively. However, these differences were not significant. Because CD10 protein expression had been examined in our previous study (10), T/N was compared with CD10 protein expression, and was found to be significantly associated.

Relationship between CD10 mRNA and liver metastasis. Among the 167 patients, 32 had synchronous metastasis: liver metastasis in 22 cases, peritoneal dissemination in 4, lung metastasis in one, and distant lymph node metastasis in 5. The remaining 135 patients who had no synchronous metastasis underwent curative resection. Among these patients, 41 suffered cancer recurrence, 20 of them developing liver metastasis. The relationship between CD10 mRNA and metastasis is shown in Table II. There was no significant relationship between CD10 mRNA and metastasis including liver metastasis. Because the median T/N was 4.55, the survival curves of patients with T/N ≥5 and of patients with T/N <5 were analyzed (Figure 2), but

Table II. *Relationship between CD10 mRNA and metastasis in colorectal cancer patients.*

	No. (n=167)	CD10 mRNA (T/N±S.D.)	*P*
Liver metastasis			
Negative	125	18.65±61.20	0.873
Positive	42	27.56±108.96	
All metastases			
Negative	94	20.13±69.11	0.886
Positive	73	21.87±84.12	

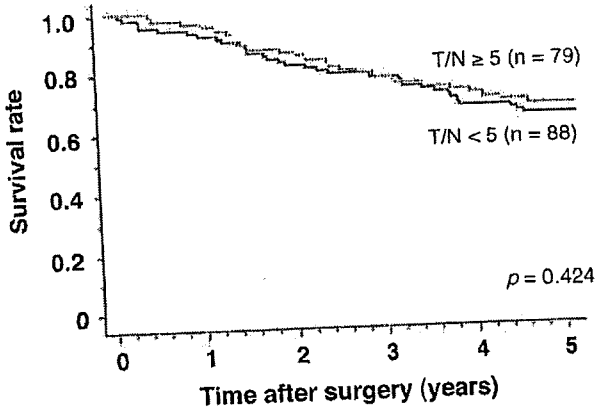

Figure 2. *Survival curves for patients with T/N≥5 or T/N<5. There was no significant difference between the groups.*

there was no significant difference between the groups. There were also no significant survival differences according to clinical stage (data not shown).

Discussion

We have recently demonstrated that CD10 protein expression in colorectal cancer cells was significantly associated with liver metastasis and that CD10 protein expression was an independent predictor of liver metastasis (10). Yao *et al.* have also demonstrated a significant association between CD10 protein expression and liver metastasis from colorectal cancer (8), and other reports have indicated a relationship between CD10 protein expression and the development and progression of colorectal cancer (7, 9). These results prompted us to examine the association between CD10 mRNA expression level and liver metastasis using real-time PCR. Although CD10 mRNA in tumor tissues was higher than that in matched normal tissues in more than 80% of colorectal cancers and was associated with tumor progression, there was no significant relationship between the level of CD10 mRNA expression and metastasis, including liver metastasis. This suggested that CD10 might play an important role in tumorigenesis and tumor progression and that measurement of CD10 mRNA in colorectal cancer tissues is not useful as a predictor of liver metastasis.

In this study, the level of CD10 mRNA was higher in pN1and pN2 tumors than in pN0 tumors, and was also higher in stage III and IV tumors than in stage I and II tumors. These facts suggested that the CD10 mRNA level was associated with tumor progression. Many previous studies have demonstrated that overexpression of CD10 protein is associated with tumor progression (1-5, 7-11, 15, 17, 19-22) and with tumor proliferation and microvascular density (11), thus indicating that CD10 plays an important role in tumor progression. Although the actual function of CD10 in tumors is not known, it is a cell surface metalloendopeptidase with structural similarity to matrix metalloprotease, and is capable of degrading a number of bioactive peptide and cytokines. Therefore, CD10 at the tumor cell surface and in the areas adjacent to tumor glands is considered to activate or inactivate tumor-related substances and to create a microenvironment that facilitates tumor cell invasion and metastasis. On the other hand, several studies have demonstrated an association between reduced expression of CD10 and tumor progression in lymphoma (23), and renal cell (14), lung (18), prostate (16), ovarian (12) and cervical cancer (13). Tumorigenesis and proliferation are inhibited (12,13), and apoptosis is induced, by CD10 (23). When CD10 expression is reduced in a tumor, loss of these functions of CD10 was considered to promote tumor progression. Because these tumors arise from tissues that normally express CD10, loss of CD10 in an advanced cancer may be explained in terms of dedifferentiation during tumorigenesis. On the other hand, CD10 expression in tumors that arise from normal tissues without CD10 expression probably represent a phenomenon acquired during tumorigenesis and is considered to be associated with tumor progression. Colorectal cancer acquires CD10 expression during tumorigenesis because it is not expressed in normal colorectal mucosa or stroma.

CD10 protein is reportedly expressed even in tumor stroma (5 , 7, 11, 17, 20-22). In colorectal cancer, CD10 protein is expressed both in tumor and stromal cells (6, 7). A similar pattern has been reported in gastric cancer (1, 5), melanoma (19, 20) and pancreatic endocrine tumor (11). CD10 expression in tumor and/or stromal cells was associated with tumor progression, except in pancreatic endocrine tumor. In the present study, because CD10 mRNA was extracted from both tumor and stroma, it was quantified in both tissues as a whole, and there was no clear relationship between CD10 mRNA expression and

liver metastasis. CD10 protein expression in tumor cells has been shown to be significantly associated with liver metastasis (8, 10). Therefore, CD10 mRNA in tumor cells might be associated with liver metastasis. Further investigation will be necessary to clarify the relationship between CD10 mRNA in tumor cells and colorectal cancer liver metastasis.

CD10 mRNA expression was higher in colon cancer than in rectal cancer. Because CD10 is expressed in the brush border of the small intestine (6), colon cancer is considered to have a higher tendency to differentiate to the small intestine than rectal cancer. In gastric cancer, phenotypic differences have been associated with prognosis and the pattern of recurrence (1, 3). These facts suggest that there are biological and oncological differences between colon and rectal cancer.

The level of CD10 mRNA was higher in well- or moderately-differentiated adenocarcinoma than in poorly differentiated or mucinous adenocarcinoma. However, CD10 mRNA expression in poorly differentiated or mucinous adenocarcinoma is still higher than in normal mucosa. On the other hand, CD10 protein is reportedly undetectable in poorly-differentiated adenocarcinoma (6, 26). This suggests that the expression of CD10 mRNA is not directly associated with protein production and that post-transcriptional regulation plays an important role in protein expression in cancer cells. This is one of the reasons why liver metastasis was associated with CD10 protein expression and not with CD10 mRNA

In conclusion, CD10 mRNA shows significantly higher expression in tumor tissue than in matched normal tissue. Although CD10 mRNA is associated with depth of invasion, lymph node status and TNM stage, it is not associated with liver metastasis, any type of metastasis, or prognosis. Therefore it seems that CD10 mRNA extracted from tumor tissues might not be useful as a predictor of liver metastasis or a prognostic marker.

Acknowledgements

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A Case of Colon Cancer Detected by Carbon-11 Choline Positron Emission Tomography/Computed Tomography: An Initial Report

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[C-11] choline positron emission tomography ([C-11] choline PET) has been expected to be one of the new PET modalities similar to [F-18] fuluorodeoxyglucose positron emission tomography (FDG-PET), which has spread worldwide as a gold standard of PET oncologic imaging. However, there has been no report on [C-11] choline PET used for detection of colorectal cancer, which is one of major targets of oncologic FDG-PET. We initiated the research to investigate the detectability of [C-11] choline PET for various tumors including colorectal cancer. This is the first report of a patient who underwent surgical resection for advanced colon cancer depicted by [C-11] choline positron emission tomography/computed tomography.

Key words: radiology-PET – diagnostic radiology – GI-colorectum-basic

INTRODUCTION

[C-11] choline positron emission tomography ([C-11] choline PET) has been expected as a new PET modality and reported to be useful for the detection of various tumors, such as brain tumor, lung cancer, esophageal cancer, prostate cancer, gynecological cancers, and bone and soft tissue sarcomas (1–6). We started the research in our institution on September 1, 2005 to clarify not only the detectability of [C-11] choline PET for various tumors but the mechanism of choline accumulation to cancer cells, which is approved by the Institutional Review Board. Here we report a successful detection of an advanced colon cancer by [C-11] choline PET, which seems to be the first case, and discuss the possibility of application of [C-11] choline PET to colorectal cancer.

CASE REPORT

A 50-year-old woman presented with melena and abdominal discomfort. The colonoscopy showed the elevated lesion in the sigmoid colon (Fig. 1). From endoscopic findings this tumor was diagnosed as type 1 advanced colon cancer with invasion into the subserosa. Pathologic diagnosis by specimen of biopsy was well-differentiated adenocarcinoma. The computed tomography scan of the thorax, abdomen and pelvis revealed the thickening in the wall of the sigmoid colon and enlarged uterus suspected of leiomyoma. No specific enlarged lymph nodes and no definite metastases including the liver were detected. A whole body [C-11] choline positron emission tomography/computed tomography (PET/CT) was performed with the written informed consent to participate in this research approved by the Institutional Review Board. Emission scans from the skull to the mid thigh were obtained starting 14 min after intravenous injection of 444MBq of [C-11] choline, which was synthesized with a commercial module essentially using the method described by Hara and Yuasa (7). [C-11] choline PET images showed abnormal prominent uptake in the middle of the abdomen (Fig. 2a and c). The maximal standardized uptake value (SUV) of this uptake was 6.97. This uptake was suspected to correspond to the sigmoid cancer. However, there was another strong accumulation close to this uptake, suspected to be physiological accumulation to the small intestine (Fig. 2c). It was not so easy to differentiate between these uptakes only by PET images. Fused PET/CT images could show clearly that one prominent accumulation corresponded to the thickening in the wall of sigmoid colon in CT images (Fig. 2d). Low abnormal uptake was observed in the pelvic space, which corresponded to myoma uteri. There was no other abnormal accumulation in

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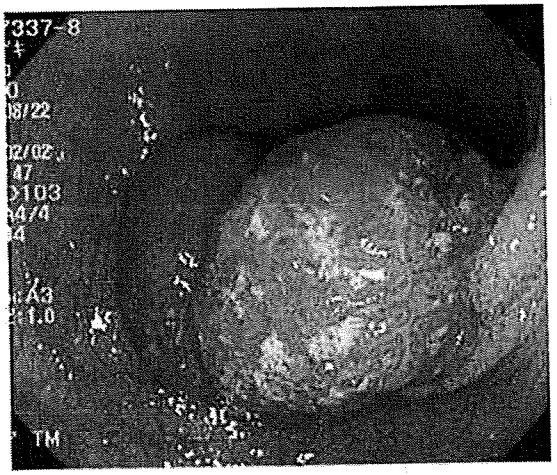

Figure 1. Endoscopic finding. Elevated lesion can be seen. Morphologic diagnosis is advanced colon cancer, type 1. Biopsy revealed well-differentiated adenocarcinoma.

the whole body (Fig. 2a). The patient underwent surgical resection of sigmoid colon and simple hysterectomy. Pathology revealed well-differentiated adenocarcinoma invading the subserosa with two metastatic lymph nodes in N1 group (2/30), which was stage IIIa according to TNM classification (8). These two metastatic lymph nodes were less than 10 mm in diameter and diagnosed as normal lymph nodes on CT images. Lymph node metastases were not detected by [C-11] choline PET. Pathology of the uterus revealed multiple leiomyomas. The patient received adjuvant chemotherapy with 5-FU and leucovorin, and was discharged 13 days after surgery.

DISCUSSION

[C-11] choline has been considered as a new PET radiopharmaceutical for tumor detection since Hara et al. reported the usefulness of [C-11] choline PET for detection of brain tumor in 1997 (1). Choline is one of the components of phosphatidylcholine, an essential element of phospholipids in the cell membrane. Malignant tumors usually exhibit a high proliferation of cells and thus are associated with increased metabolism of cell membrane components. This biochemical background will lead to an increased uptake of choline to the cancer cells (9,10). Moreover, it is assumed that, whether tumor cells are in hypoxia or in normoxia, the rate of [C-11] choline uptake in tumors is an indicator of the tumor proliferation rate, whereas in [F-18] fuluorodeoxyglucose (FDG), tumor hypoxia is closely associated with tumor uptake (11). In this view point, [C-11] choline PET might detect malignancies at an earlier stage than FDG-PET, which has spread worldwide as a gold standard of PET oncologic imaging, although further investigation is still needed. Compared with FDG-PET, [C-11] choline PET has the advantage of providing a clear image at an earlier period (5). In FDG-PET, patients have to wait for 60 min or longer after tracer injection for tumor activity to reach the peak count. With [C-11] choline, however, blood clearance is rapid and tumor activity reaches a maximum at 3–5 min after injection. The initial intense uptake remains at a nearly constant level afterwards, thus enabling the high activity ratio to remain for more than 30 min, compared with the background. Another advantage of [C-11] choline PET is the lower exposure dose, which is estimated at approximately 2.5 mSv/370 MBq in contrast with 7 mSv/370 MBq for FDG-PET (12).

[C-11] choline PET has been reported to be useful for the detection of various malignant tumors such as lung, esophageal and gynecological caners, and bone and soft sarcomas, as well as FDG-PET (1–6). Furthermore, [C-11] choline PET is reported to be superior to FDG-PET in the detection of brain tumor and prostate cancer (1,4). Ramirez de Molina et al. reported that choline kinase, which catalyzes the phosphorylation of choline, is upregulated in lung, prostate and colorectal cancers (13). Therefore, [C-11] choline is speculated to also detect colorectal cancer, which is one of the major targets of FDG-PET. However, there has been no report on [C-11] choline PET for detection of colorectal cancer. This is because it is generally accepted that [C-11] choline often accumulates in the small intestine and/or colon mucosa, in which cell turnover is very rapid. As a consequence, the various degrees of physiological uptake obscure accumulation to the tumor, which is similarly observed in FDG-PET (5). Hara reported that rectal cancer was visualized with [C-11] choline PET (14). In our case, abnormal [C-11] choline deposit to the sigmoid colon could be detected, although there was physiological accumulation to the small intestine near the cancerous lesion. There was no other significant accumulation to the colon. In our experience, physiological colon uptake of [C-11] choline tends to be lower than accumulation in the small intestine, whereas physiological colon uptake of FDG is often so much higher than small intestine uptake that the cancerous lesion cannot be depicted. That might be an advantage of [C-11] choline PET in colorectal cancer. It might be due to the considerably rapid turnover of epitherial cells in the small intestine, but the precise reason is unknown, prompting further investigation. It is sometimes confusing whether abdominal uptake is corresponding to the small intestine or the colon on [C-11] choline PET images as well as on FDG-PET images. In such cases, fused PET/CT images give great assistance in diagnosing correctly the location of abnormal uptake. Besides the detection of the primary site, staging is another important role of oncologic PET as an initial examination. There are some reports on [C-11] choline PET as a staging procedure for prostate cancer and bone and soft tissue sarcomas (15,16). In our case, lymph node metastases could not be detected by [C-11] choline PET. This was considered to be due to the

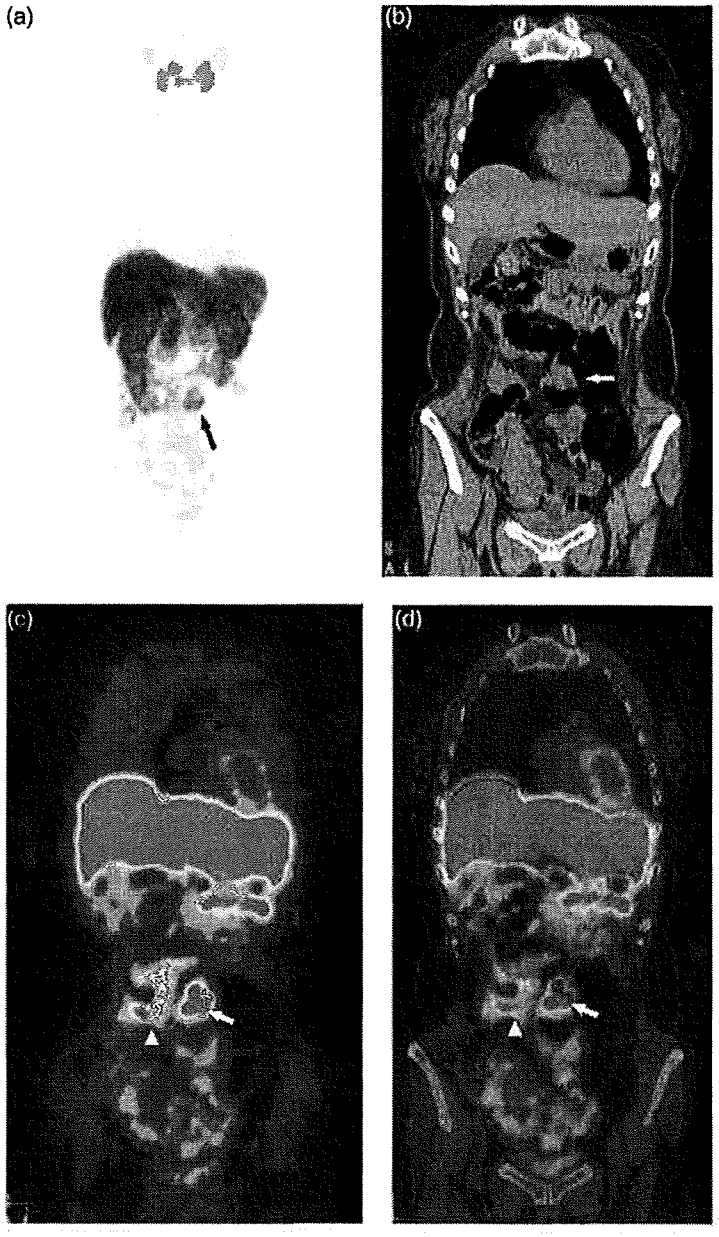

Figure 2. (a) MIP image of [C-11] choline positron emission tomography. Abnormal uptake of [C-11] choline is observed in the middle of the abdomen (arrow). There is no significant physiological uptake in the colon. (b–d) Coronal computed tomography (b), coronal positron emission tomography (c), coronal co-registered positron emission tomography/computed tomography hybrid image (d). Thickening in the wall of the sigmoid colon is observed in the computed tomography (b, arrow). Abnormal uptake of [C-11] choline is observed in the sigmoid colon corresponding to the wall thickening (c, d, arrow). Differentiation between physiological uptake in the small intestine (c, d, arrow head) and tumor uptake can be made by fused PET/CT images. PET, positron emission tomography; CT, computed tomography.

size of the lymph nodes (less than 10 mm in diameter). Hepatic metastasis, which is frequent in colon cancer, might be hardly depicted by [C-11] choline PET because [C-11] choline is observed physiologically in the liver. However, [C-11] choline PET might be useful for detecting other metastases of colorectal cancer such as brain metastasis, pulmonary metastasis and bone metastasis with low background uptake.

In summary, here we described a case of advanced colon cancer. A whole body [C-11] choline PET/CT permitted detection of the primary site. However, further studies must be performed on staging, diagnosis for recurrence and evaluation for effect of treatment to confirm the usefulness of [C-11] choline PET for colorecatal cancer.

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Conflict of interest statement

None declared.

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SURGICAL ENDOSCOPY
and Other Interventional Techniques

Wound infection after elective laparoscopic surgery for colorectal carcinoma

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Abstract
Background: The aim of this study was to evaluate various clinical parameters that would influence the occurrence of wound infection (WI) in elective laparoscopic surgery (LS) for colorectal carcinoma.
Methods: The study included 290 patients who underwent LS between June 2001 and December 2005. WI was diagnosed within 30 days of the operation, and both superficial and deep incision surgical site infection were evaluated together.
Results: Eighteen (6.2%) were diagnosed with WI. Of the infected patients, nine (50%) had WI at the extraction site, six (33%) at the port site of the drainage tube, and three (17%) at the supraumbilical incision. Following bivariate analysis, the variables of stoma creation, intraoperative hypotension, and length of operation were selected for multivariate analysis as their P values were <0.2, the predominant cutoff, and stoma creation and intraoperative hypotension were independently predictive of developing WI. Regarding the duration of postoperative hospital stay, there was no significant difference between patients with or without WI.
Conclusions: Stoma creation and intraoperative hypotension were independent risk factors for WI. The results obtained in this study should be considered in an effort to prevent WI in LS for colorectal carcinoma, although these risk factors need further evaluation.

Key words: Laparoscopic surgery — Colorectal carcinoma — Wound infection

In elective open colorectal surgery for colorectal malignancy, the most frequent complication is wound infection (WI) [14, 22]. It goes without saying that if WI occurs, hospital costs (increased length of stay, antibiotics, dressing supplies, and nursing charges) and outpatient care (outpatient follow-up, self-dressing supplies, and antibiotics) will increase, thus increasing the total medical cost. In addition, it will delay the patient's return to work, causing a social loss. For these reasons, various risk factors possibly related to the occurrence of WI have been investigated mainly in cases of elective open colorectal surgery for colorectal malignancy [16–18].

In recently reported randomized controlled trials (RCTs) that compared the oncological outcomes of conventional open surgery (OS) versus laparoscopic surgery (LS) in colorectal carcinoma cases, LS was proven to be comparable to OS, and hence the number of patients receiving LS is expected to increase in the coming years [4, 12, 13]. In RCTs comparing OS versus LS for colorectal carcinoma in terms of short-term outcome that have been reported to date, the incidence of WI was 4–14% for LS versus 5–17% for OS; one third of those reports showed a significantly lower incidence of WI for LS compared to OS, and the rest showed no difference [2, 7, 8, 12, 13, 23]. As LS involves the pneumoperitoneum with CO2 and delivery of the intestinal tract through a small incision, risk factors influencing WI occurrence in LS may be different from those in OS; however, only a few studies to date have investigated the risk factors related to the occurrence of WI in cases of LS for colorectal carcinoma.

At our institution, after having accumulated enough experience in LS for early colon carcinoma, we have gradually expanded the indications for LS. In June 2001, a single laparoscopic surgical team instructed by one surgeon (SY) began to perform LS, and postoperative management procedures were standardized, and began to expand the use of LS to include T3 colon/upper rectal carcinoma and T1-T2 middle/lower rectal carcinomas. As a consequence, the complication rate and mean length of hospitalization have been reduced [26, 27].

The purpose of this study was to evaluate various clinical parameters that would influence WI occurrence

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in LS by accumulating occurrences of WI in elective LS cases via prospective entries at a single institution and evaluating its risk factors.

Patients and methods

Between June 2001 and December 2005, we performed 290 consecutive LS for selected patients with colorectal carcinoma. As the oncological safety of LS in colorectal carcinoma patients remains to be established, candidates for radical LS were patients who were preoperatively diagnosed with T1 or T2. Additionally, we included patients who were preoperatively diagnosed with T3 but wished to undergo LS, as well as those with colon or upper rectal carcinoma for which palliative resection was considered necessary. Contraindications for LS at our institution included the following groups: tumors larger than 6 cm, a prior history of extensive adhesions, severe obesity (body mass index > 32 kg/m^2), intestinal obstruction, and patients who did not consent to LS. We defined conversion to OS as any incision greater than 7 cm, excluding cases in which the incision was enlarged due to a large specimen that could not be removed through a 7 cm incision.

Patients underwent mechanical bowel preparation with 2 liters of polyethylene glycol electrolyte solution one or two days before operation, and this decision differed according to the study period. Preoperative antimicrobial administration was given intravenously within 30 minutes before skin incision in all cases. Indications for postoperative and intraoperative repeated dosing differed according to the study period. Similarly, antimicrobial agents differed according to the study period; however, the antimicrobial agent given intraoperatively and/or postoperatively was the same antimicrobial prophylaxis given preoperatively in all patients.

Our LS techniques have previously been thoroughly described [26, 27]. During the externalized portion of the surgery, a plastic wound protector was applied to the abdominal extraction incision routinely. Wound irrigation was not performed in this series. All wounds were classified as clean-contaminated (bowel was opened without spilling the contents).

WI was diagnosed within 30 days of the operation according to the criteria of the Centers for Disease Control and Prevention (CDC) [9]. Briefly, superficial surgical site infections only involve the skin and subcutaneous tissue, whereas deep surgical site infections occur when the incisional wound involves the muscle and fascial layers but not the organ space. WI was characterized by wound erythema, cellulitis, localized pain, swelling, tenderness, or purulent or culture-positive wound discharge. All the patients visited outpatient clinic about three weeks after the day of discharge, and their wound conditions were checked. Superficial and deep incision surgical site infections were evaluated together under the umbrella term of WI [17].

Patients' theatre records were reviewed retrospectively. Other data were prospectively recorded in the divisional database. The parameters analyzed included: gender, age, body mass index, prior abdominal surgery, smoking status, ASA classification [15], preoperative hemoglobin, total protein, and hemoglobin A1c level. Pathological staging was performed according to Dukes' stage. Regarding the perioperative/operative characteristics, operative time, antimicrobial prophylaxis administration, minimum body temperature and intraoperative hypotension during surgery, intraoperative blood loss, presence or absence of a drainage tube for anastomoses, duration of drainage, stoma creation, type of anastomosis, date of preoperative mechanical bowel preparation, and postoperative hospital stay were analyzed. Stoma creation included both patients with endocolostomy who underwent laparoscopic abdominoperineal resection, and patients with covering ileostomy.

Perioperative steroid and insulin use for comorbid conditions was not evaluated because only three patients received perioperative steroid and only 10 patients required insulin, and WI did not occur in these patients. Similarly, the transfusion of cellular or plasma products was not evaluated, because only two patients without WI received transfusion in this series.

Statistical analysis was performed using Student's t-test, Fisher's exact test, the chi-square test, and the Mann-Whitney U test, as appropriate. Multivariate analysis was performed by logistic regression methods using independent variables with a P value < 0.2 by bivariate statistics. A P value of less than 0.05 was considered significant.

Results

During the study period, 290 patients underwent elective LS, and 18 (6.2%) were diagnosed with WI. Of the infected patients, nine (50%) had WI at the extraction site, six (33%) at the port site of the drainage tube, and three (17%) at the supraumbilical incision.

In bivariate analysis, patients were divided into those with or without WI and compared. Patient demographics are summarized in Table 1. There were no significant differences in baseline characteristics among groups.

Perioperative/operative results are shown in Table 2. All procedures were completed laparoscopically in this series, and we consider our exclusion criteria for LS (extensive adhesions and severe obesity) contributed to this lower conversion rate. Only stoma creation was significantly associated with the development of WI. However, there was a trend toward developing WI if the patient developed intraoperative hypotension ($P = 0.071$) or the operation exceeded 240 minutes ($P = 0.123$). Regarding the duration of postoperative hospital stay, there was no significant difference between the two groups ($P = 0.131$).

Following bivariate analysis, the variables of stoma creation, intraoperative hypotension, and length of operation were selected for multivariate analysis as their P values were < 0.2, the predominant cutoff for inclusion. Table 3 summarizes the results of multivariate analysis, and stoma creation and intraoperative hypotension were independently predictive of developing WI.

Discussion

This is the first investigation demonstrating stoma creation and intraoperative hypotension as independent risk factors for WI in nearly 300 cases of colorectal carcinoma undergoing LS. In this study, clinical parameters that have conventionally been reported as risk factors for WI were evaluated for cases of elective LS for colorectal carcinoma. As a result, we found that stoma creation and intraoperative hypotension were independent risk factors. The results obtained in this study should be considered in an effort to prevent WI in LS for colorectal carcinoma, although these risk factors need further evaluation.

The incidence of WI for LS in this study was 7.6%, which is the same as in previous reports. In this series, the indications for LS were limited as mentioned, but at our hospital, the incidence of WI for OS is more than 10%, which is higher than that for LS. Regarding the incidence of WI in LS and OS, based on the National Nosocomial Infections Surveillance System's surgical patient surveillance component protocol, CDC reported that the incidence of WI after colon surgery was significantly lower in the LS group than in the OS group [6]. In RCTs comparing OS with LS for colorectal carcinoma in terms of short-term outcome that have been reported to date, the incidence of WI was 4–14% for LS versus 5–17% for OS; one third of those reports showed a significantly lower incidence of WI for LS compared to

Table 1. Correlation between wound infection and clinicopathologic features[a]

	Wound infection		
	Positive (n = 18)	Negative (n = 272)	P value
Sex ratio (male:female)	13:5	153:119	.224
Mean age	57.9 (35–73)	61.0 (30–88)	.234
Mean body mass index (kg/m^2)	23.0 (17.3–27.9)	23.0 (15.1–32.4)	1.000
Prior abdominal surgery (percentage)	3 (16.7)	62 (22.8)	.772
Smoking status			.754
Nonsmoker	14	222	
Smoker	4	50	
ASA classification			.455
ASA I	13	165	
ASA II	5	82	
ASA III		25	
Mean preoperative hemoglobin (g/dL)	13.6 (12.2–15.4)	13.4 (8.0–16.4)	.578
Mean preoperative total protein (g/dL)	7.1 (6.5–8.1)	7.0 (5.5–8.7)	.313
Mean preoperative hemoglobin A1c (g/dL)	5.2 (4.6–5.9)	5.2 (3.1–9.5)	.917
Dukes' stage (n)			.446
A	14	185	
B	0	25	
C	3	49	
D	1	13	
Location (n)			
Colon:rectum	12:6	217:55	.229
Laparoscopic colorectal procedures (n)			
Ileocecal resection	1	28	
Right hemicolectomy	2	52	
Transverse colectomy		1	
Left hemicolectomy		2	
Descending colectomy	2	12	
Sigmoid colectomy	4	91	
Partial resection	5	30	
Anterior resection with DST	4	49	
Anterior resection with ISR-CAA	1	4	
Abdominoperineal resection		2	
Hartmann		1	
Transverse-coloplasty pouch	2	3	
Covering ileostomy	3	12	

ASA, American Society of Anesthesiologists; DST, double-stapling technique; ISR-CAA, intersphincteric rectal resection and handsewn coloanal anastomosis

OS, and the rest showed no difference [2, 7, 8, 12, 13, 23]. In addition, meta-analysis of these trials demonstrated a significantly lower incidence for LS [1]. From the results of these studies, the incidence of WI in LS is so far considered to be lower than that of OS.

At the same time, there are some reports that, when WI occurs, the treatment costs are significantly higher for OS than LS [2]. In this study, even with WI, patients undergoing LS did not require prolonged hospitalization because of smaller wounds and were able to dress their own wounds after being discharged. Even when WI occurs in LS, the maximum range of the infected area can only be 6–7 cm, which is a great advantage of LS.

To date, many studies have reported the immunological superiority of LS compared to OS [19, 25]. In our LS series, after completing manipulation of the pneumoperitoneum with CO_2, one port site is used to make a skin incision as the extraction site, through which necessary manipulations including tumor removal and anastomosis are done, and immediately thereafter, we close the wound to the fascia. Therefore, the extraction site was usually open for a total of 20–30 minutes; the procedure rarely requires more than 60 minutes. Consequently, the net length of time during which a small laparotomy wound is made is shorter in LS than in OS, and this may possibly have a favorable influence on wound healing after LS. Regarding the plastic wound protectors used to prevent port site recurrence, Kercher et al. [10] reported that a wound protector did not significantly diminish the rate of WI at the extraction site; however, it is currently unthinkable to perform LS for malignant diseases without a plastic wound protector, and the significance of plastic wound protectors in terms of WI may need to be investigated in benign diseases.

With regard to the risk factors for WI in cases of colorectal carcinoma, various parameters have been evaluated in the past [16, 17, 18]. In this study, those many parameters were evaluated, and interestingly, we found that stoma creation and intraoperative hypotension were the only independent risk factors. Although there are some reports suggesting that stoma creation or intraoperative hypotension are risk factors for WI in elective open colorectal resections [11, 17, 20], the other many parameters that have conventionally been re-

Table 2. Correlation between wound infection and perioperative/operative characteristics[a]

	Wound infection		
	Positive (n = 18)	Negative (n = 272)	P value
Length of operation			.123
≤ 240 minutes	9	186	
> 240 minutes	9	86	
Antimicrobial prophylaxis administration			
Single dosing:multiple dosing (intra- and post-operative)	11:7	176:96	.802
Intraoperative repeated dosing (LOO > 180 minutes)	2/14 (14.3%)	22/207 (10.6%)	.653
First generation: second generation	6:12	92:180	1.000
Mean minimum body temperature (°C)	35.8 (34.9–36.7)	35.9 (34.5–37.4)	.854
Intraoperative hypotension (SBP < 80mmHg)	7	54	.071
Mean operative blood loss (mL)	64 (6–250)	61 (3–217)	.839
Drainage of anastomoses	17	268	.276
Mean duration of drainage (days)	4.5 (0–8)	4.6 (0–8)	.638
Stoma creation	4	14	.019
Anastomosis			.470
Double-stapling technique	8	123	
FETE + Handsewn anastomosis	10	142	
Handsewn per anum coloanal anastomosis	1	4	
Colon preparation			.380
One day before surgery	16	210	
Two days before surgery	2	62	
Median hospital stay (days)	8 (7–23)	8 (7–21)	.131

LOO, length of operation; SBP, systoloic blood pressure; FETE, functional end-to-end anastomosis
[a] Values in parentheses are ranges unless indicated otherwise

Table 3. Multivariable analysis of factors affecting wound infection

Independent Predictors	Odds ratio	Confidence intervals	P value
Stoma creation (yes/no)	5.275	1.196–23.263	.028
Intraoperative hypotension (SBP < 80mmHg/SBP ≥ 80mmHg)	3.171	1.099–9.147	.033

SBP, systoloic blood pressure

garded as risk factors for WI were negative. Unfortunately, we were unable to evaluate matters such as the necessity of mechanical bowel preparation and the significance of preoperative oral prophylaxis, because all the patients underwent mechanical bowel preparation without oral antibiotics. In recent years, the significance of mechanical bowel preparation has been investigated in RCTs, and there are increasing reports that mechanical bowel preparation is not necessary; however many of those trials investigated cases of elective OS [3, 16]. As to whether the same result would be obtained in LS cases, further investigation is needed.

In this study, surgery was performed without oral antimicrobial prophylaxis administration in all patients. We do not administer oral antimicrobial prophylaxis in either OS or LS, for fear that clostridium difficile colitis or gastrointestinal symptoms including nausea, vomiting, abdominal pain, and diarrhea might be caused by oral antibiotics [5, 24]. In such cases, intravenous antimicrobial prophylaxis administration is required, but factors such as the type of antibiotic administered and its dosing frequency may influence the occurrence of WI. As the type of antibiotic used and its dosing frequency vary according to the study period, the influence of these factors was evaluated in this study. We found no difference in the incidence of WI between single dosing and multiple dosing as well as between the presence and absence of intraoperative repeated dosing in cases with an operative time of four hours or more. With regard to the type of antibiotic administered, no difference was seen in the incidence of WI between first and second generation. Whether these items influence the occurrence of WI in LS cases is a question that requires further investigation.

In addition, the significance of drainage tubes should also be investigated. In our study, WI at the port site through which a drainage tube was inserted accounted for one-third of all WIs. Some reports say that drainage tubes are not necessary in elective colorectal surgery [21], but we leave drainage tubes in place for 4–5 days after operation to obtain information on postoperative bleeding and postoperative peritonitis associated with anastomotic leakage. However, because of the low frequency of these complications in LS, we now remove drainage tubes as early as the second or third postoperative day. Further investigation is also required with regard to the necessity of drainage tubes in LS.

The results of this study suggest the importance of maintaining normotension intraoperatively to reduce the incidence of WI, and we must recognize that this variable needs to be further investigated [17]. Moreover, if a stoma needs to be created, the port site should be positioned away from the stoma site as far as possible, and in patients with a created stoma, the wound may need to be completely covered with plastic film or other material. On the other hand, if WI occurs, hospital costs will be reduced by opening the wound early and managing it with self-dressing gauze changes.

In conclusion, as a result of our investigation into cases of LS, we found that stoma creation and intraoperative hypotension were independent risk factors for WI. Various factors are related to the occurrence of WI. It is necessary to further investigate risk factors for WI in LS cases so that efforts can be made to effectively prevent WI by measures such as increasing the dosing frequency of antimicrobial prophylaxis only in patients at higher risk for WI.

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Diseases of the Colon & Rectum

Cancer Invasion to Auerbach's Plexus is an Important Prognostic Factor in Patients with pT3-pT4 Colorectal Cancer

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PURPOSE: By defining perineural invasion of colorectal cancer as invasion to Auerbach's plexus, we examined the usefulness of this pathologic finding as a prognostic factor. METHODS: A total of 509 consecutive patients who underwent curative surgery for pT3 or pT4 colorectal cancer between May 1997 and December 2001 were reviewed. All the surviving patients were followed for more than five years. All the pathologic findings, including perineural invasion, were described prospectively in the pathology report forms. RESULTS: Perineural invasion was detected in 132 of 509 patients (26 percent) and was significantly associated with lymph node status, lymphatic invasion, and venous invasion. Incidences of local and systemic recurrence were significantly higher in patients with perineural invasion than in those without perineural invasion. The disease-free survival of the perineural invasion-positive group was significantly poorer than that of the perineural invasion-negative group for Stages II and III colon cancer, irrespective of the use of adjuvant chemotherapy. This improved disease-free survival also was seen in patients with Stage II rectal cancer not treated with adjuvant chemotherapy. There was a nonsignificant difference in disease-free survival for Stage II rectal cancer and Stage III rectal cancer treated with chemotherapy, that of the perineural invasion-positive group being poorer. Multivariate analysis showed that lymph node status, perineural invasion, depth of invasion, and cancer site were significant prognostic factors. CONCLUSIONS: Perineural invasion defined as cancer invasion to Auerbach's plexus is an important prognostic factor for colorectal cancer. [Key words: Colorectal cancer; Perineural invasion; Auerbach's plexus; Prognostic factor]

Several reports have shown that perineural invasion (PNI) is an important prognostic factor in colorectal cancer[1–5] and rectal cancer.[6–17] Therefore, the colorectal working group of the American Joint Committee on Cancer (AJCC) prognostic factors consensus conference has classified PNI as category IIA, which means that PNI has been extensively studied biologically and/or clinically and is considered to have sufficient predictive value for outcome to be noted in pathology reports.[18] However, because many reports on PNI have been based on retrospective studies, and PNI has not been clearly defined, there is still no definitive conclusion about the degree to which PNI is a prognostic factor, especially in colon cancer. Therefore, in pathology reports compiled at the National Cancer Center Hospital from May 1997, we defined PNI as cancer invasion to Auerbach's plexus, because this feature is a prominent and easily detectable type of PNI, and

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PNI was reported prospectively. Although we had already reported that PNI was an important prognostic factor,[19] follow-up time in the previous study was short and the number of patients examined was small. In the present study, all surviving patients were followed for more than five years and the number of examined patients was larger than in our previous study. Moreover, only pT3 or pT4 tumors were examined in the present study, because PNI was not found in pT1 tumors and was rare in pT2 tumors.

PATIENTS AND METHODS

Consecutive patients who underwent curative surgery for pT3 or pT4 colorectal cancer at the National Cancer Center Hospital between May 1997 and Dec 2001 were reviewed. Synchronous or metachronous multiple cancers were excluded from the analysis. One patient who died four days after surgery because of anastomotic leakage and sepsis also was excluded. A total of 509 patients were examined. The patients were followed up at three-month intervals for two years and at six-month intervals thereafter. Tumor markers were examined at every patient visit. CT scans of the liver and lung or abdominal ultrasonography with chest x-ray were performed at least every six months. Colonoscopy was performed twice within five years after surgery. All the surviving patients were followed for more than five years. Fifty-one of 266 patients with Stage III tumors received postoperative adjuvant chemotherapy as part of a clinical trial. Adjuvant radiotherapy was not used for rectal cancer during the study period.

Pathologic Examination

All the specimens were reviewed by two pathologists (TS and YN). Perineural invasion was defined as the presence of cancer cells inside the perineurium in Auerbach's plexus adjacent to the tumor front, and the results and other pathologic findings were described prospectively in the pathology report forms.

Statistical Analysis

Statistical analysis was performed by using the chi-squared test. Survival rates were calculated by the Kaplan-Meier method and survival curves were compared by using the log-rank test. Cox proportional hazards model was used for multivariate analysis. Data differences between groups were considered statistically significant at $P<0.05$.

RESULTS

PNI and Clinicopathologic Characteristics

A representative case of PNI is shown in Figure 1. Cancer cells invaded the perineurium in Auerbach's plexus. PNI was detected in 132 of 509 patients (26 percent). PNI and clinicopathologic characteristics of the patients are shown in Table 1. PNI was significantly associated with lymph node status, lymphatic invasion, and venous invasion ($P<0.01$).

PNI in Relation to Recurrence and Survival

In colon cancer, the incidence of liver metastasis in the PNI-positive group was significantly higher than that in the PNI-negative group ($P<0.01$; Table 2). In rectal cancer, the incidences of liver and lung metastasis and local recurrence in the PNI-positive group were significantly higher than in the PNI-negative group ($P\leq0.01$). The five-year, disease-free survival rate in the PNI-positive group was 53 percent and that in the PNI-negative group was 80 percent (Fig. 2). Outcome was significantly poorer in the PNI-positive group than in the PNI-negative group ($P<0.01$). Disease-free survival rates were examined according to tumor site (colon and rectum) and Stage (Stages II and III). Disease-free survival in the PNI-positive group was significantly poorer than that in the PNI-negative group for Stage II and III colon cancer ($P=0.02$, 0.03, respectively) and Stage III rectal cancer ($P<0.01$; Table 3, Fig. 3). Although disease-free survival in the PNI-positive group also was poorer than that of the PNI-negative group for Stage II rectal cancer, the difference was not statistically significant ($P=0.21$). Because 51 of 266 patients with Stage III tumors received adjuvant chemotherapy, which is known to affect survival, the effect of adjuvant chemotherapy on disease-free survival was analyzed (Table 3). Patient survival in the PNI-positive group was poorer than that in the PNI-negative group, irrespective of whether adjuvant chemotherapy was given. Multivariate analysis of PNI, lymph node status, depth of invasion, tumor differentiation, lymphatic invasion, venous invasion, tumor site, preoperative CEA, gender, age, and adjuvant chemotherapy showed that lymph node status, PNI, depth of invasion, and tumor site were significant prognostic factors ($P<0.01$; Table 4).

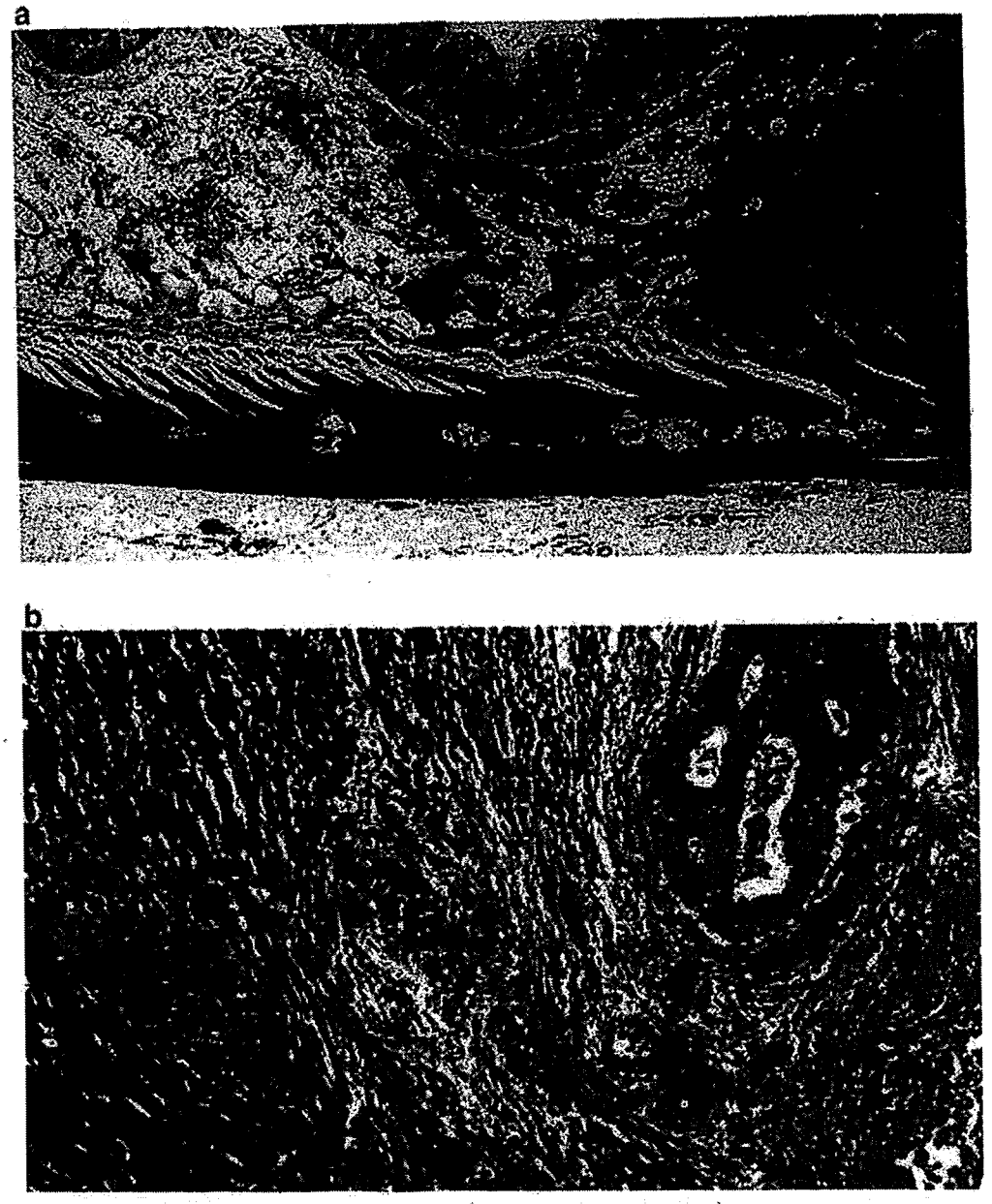

Figure 1. Representative PNI. a. Arrows shows cancer cells inside the perineurium in Auerbach's plexus. This is a case of massive PNI. b. Eighty percent of cases of PNI involve only slight invasion to Auerbach's plexus. In this case, one or two plexuses adjacent to the tumor front were invaded by cancer cells (arrow). Arrowhead shows Auerbach's plexus without cancer invasion. PNI = perineural invasion.

DISCUSSION

PNI has been reported to be a prognostic factor in colorectal cancer,[1–5] colon cancer,[20–22] and rectal cancer.[6–17] However, there is still no definitive conclusion about the degree to which PNI is a prognostic factor, especially in colon cancer, because many of the previous studies of PNI were retrospective, and PNI was not clearly defined. Although many of the reports did not define PNI, PNI was considered to be perineural cancer invasion within and outside the bowel wall in some of them,[1,6,9,12] and only extramural PNI was examined in other studies.[7,10,14] We defined PNI as cancer invasion to Auerbach's plexus, and on this basis prospectively examined more than 500 patients. Our findings clearly demonstrated that PNI was a significant prognostic factor in pT3 or pT4 colorectal cancer. Therefore, this study provides strong evidence that cancer invasion to Auerbach's plexus is a prognostic factor for colorectal cancer.

Table 1.
PNI and Clinicopathologic Characteristics of the Examined Patients

	PNI-negative (n=377)	PNI-positive (n=132)	*P* Value
Age (yr)			0.68
<60	155	57	
≥60	222	75	
Male/female ratio	225/152	75/57	0.56
Tumor site			0.16
Colon	229	71	
Rectum	148	61	
Preoperative CEA (ng/ml)			0.34
<5	257	84	
≥5	120	48	
Depth of invasion (pT)			0.08
pT3	329	107	
pT4	48	25	
Lymph node status (pN)			<0.01
pN0	209	34	
pN1	120	57	
pN2	48	41	
Tumor differentiation			0.99
Well/moderate	354	124	
Poor/mucinous	23	8	
Lymphatic invasion			<0.01
Negative	255	37	
Positive	122	95	
Venous invasion			<0.01
Negative	234	53	
Positive	143	79	

PNI = perineural invasion.

The outcome of patients with Stage II colorectal cancer with cancer invasion to Auerbach's plexus was poor, and the survival rate was similar to that of patients with Stage III colorectal cancer. Because adjuvant therapy is recommended for patients with Stage III colorectal cancer, patients with Stage II colorectal cancer with invasion to Auerbach's plexus also are thought to be candidates for such therapy. On the other hand, the outcome of patients with Stage III colon cancer without invasion to Auerbach's plexus was good, and therefore these patients may not require adjuvant chemotherapy. These findings suggest that cancer invasion to Auerbach's plexus could be used to facilitate the selection of patients with colorectal cancer for adjuvant chemotherapy. However, among patients with Stage III colon cancer without invasion to Auerbach's plexus, those who received adjuvant chemotherapy showed better survival than those who did not, although the difference was not statistically significant. Further investigations of cancer invasion to Auerbach's plexus and the need for adjuvant chemotherapy are necessary.

Cancer invasion to Auerbach's plexus is a significant prognostic factor even in patients with colon cancer. Only three studies have examined the relationship between PNI and the prognosis of colon cancer patients.[20–22] These demonstrated that PNI was associated with recurrence and poor survival, although multivariate analysis showed that this association was not significant. Therefore, our study is the first to report a significant association between PNI and survival of colon cancer patients based on multivariate analysis.

Although cancer invasion to Auerbach's plexus is a significant prognostic factor in patients with rectal cancer, the difference in disease-free survival between the PNI-positive group and the PNI-negative group was not statistically significant for Stage II rectal cancer. This may have been the result of the small number of patients with Stage II rectal cancer included in this study (n=89), and thus any apparent difference would have had low statistical power. Because the difference in five-year, disease-free survival rate between the groups was large (14 percent in this study) and the hazard ratio between the survival curves seemed to be constant over time, statistical significance may have been achieved by analyzing a larger number of patients with Stage II rectal cancer.

Table 2.
Pattern of Recurrence

	PNI-negative	PNI-positive	*P* Value
Colon	n=229	n=71	
Liver	12 (5.2)	14 (19.7)	<0.01
Lung	9 (3.9)	5 (7)	0.28
Peritoneum	6 (2.6)	2 (2.8)	0.93
Local	1 (0.4)	0	0.58
Others	5 (2.2)	1 (1.4)	0.68
Rectum	n=148	n=61	
Liver	9 (6.1)	13 (21.3)	<0.01
Lung	22 (14.9)	18 (29.5)	0.01
Peritoneum	0	1 (1.6)	0.12
Local	4 (2.7)	8 (13.1)	<0.01
Others	10 (6.7)	3 (4.9)	0.62

PNI = perineural invasion.
Data are numbers with percentages in parentheses unless otherwise indicated.

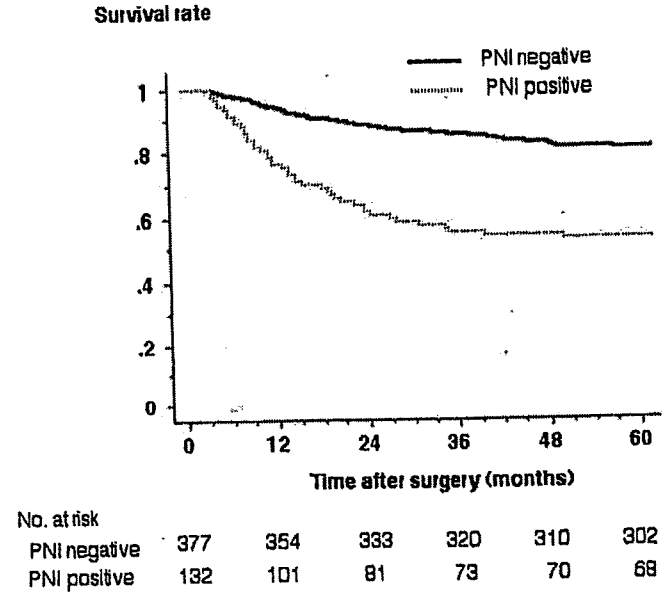

Figure 2. Disease-free survival curves according to PNI status. The prognosis of the PNI-positive group was significantly poorer than that of the PNI-negative group ($P<0.01$). PNI = perineural invasion.

In this study, the incidence of PNI in pT3 or pT4 colorectal cancer was 26 percent. The reported incidence of PNI has differed among previous studies, and in patients with advanced cancer, the incidence has ranged between 14 and 50 percent.[7–11,14] These differences are thought to have been the result of the different definitions of PNI employed. Therefore, a clear definition of PNI is very important for clinical use, and we consider our present definition to be a candidate.

Table 3.
Five-Year Disease-Free Survival Rate According to Tumor Site and Stage

	PNI negative	PNI-positive	*P* Value
Colon			
Stage II	94 (134)	80 (20)	0.02
Stage III	75.8 (95)	52.9 (51)	0.03
Adjuvant chemotherapy +	93.3 (15)	61.5 (13)	0.04
Adjuvant chemotherapy −	72.5 (80)	50 (38)	0.01
Rectum			
Stage II	78.7 (75)	64.3 (14)	0.21
Stage III	63 (73)	38.3 (47)	<0.01
Adjuvant chemotherapy +	71.4 (14)	44.4 (9)	0.08
Adjuvant chemotherapy −	61 (59)	36.8 (38)	0.01

PNI = perineural invasion.
Data are percentages with numbers in parentheses unless otherwise indicated.

Immunohistochemical evaluation can be used to confirm the presence of PNI.[23] Use of an antibody against S-100 protein showed that the incidence of PNI was 70 percent, which was more than four times the incidence revealed by routine staining. This PNI positivity rate was very high, and patients with a poor prognosis were not selected using that method and immunohistochemistry was not always used for routine pathology because of the labor, time, and cost involved.

Venous invasion and lymphatic invasion are considered to be poor prognostic factors in patients with colorectal cancer.[18] In our study, venous invasion and lymphatic invasion were significant prognostic factors in univariate analysis but were not significant in multivariate analysis, and cancer invasion to Auerbach's plexus was selected as an indicator of poor prognosis. Our data suggest that cancer invasion to Auerbach's plexus is considered to be a more important prognostic factor than venous and lymphatic invasion.

Although many molecular markers for colorectal cancer have been studied, and some, such as p53 and DCC, have been considered to indicate prognosis, some of the evidence is conflicting,[24] and these markers are still not used in routine pathology. Moreover, these techniques are labor-intensive, time-consuming, and expensive. Because PNI can be easily detected by routine pathologic examination, it is easy to add this simple finding to pathology reports.

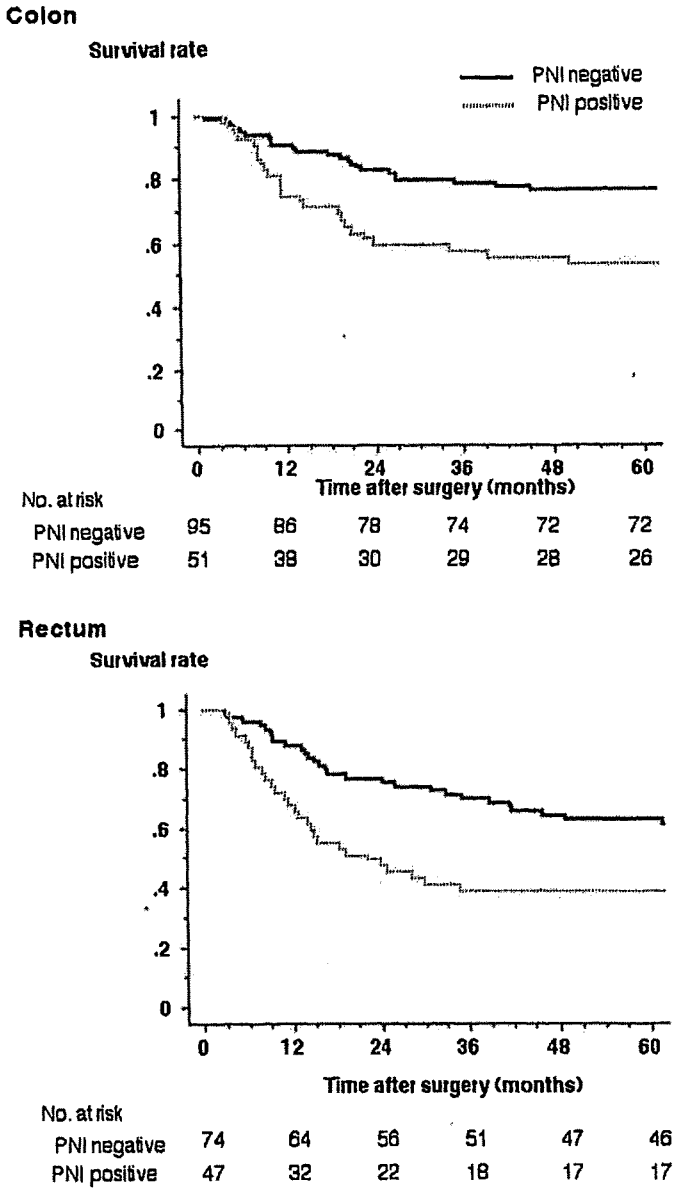

Figure 3. Disease-free survival curves of Stage III patients according to PNI status and cancer site. For both colon and rectal cancer, disease-free survival in the PNI-positive group was significantly poorer than that in the PNI-negative group ($P=0.03$ and $P<0.01$, respectively). PNI = perineural invasion.

Table 4.
Multivariate Analysis of the Prognostic Factors

Prognostic Factors	*P* Value	Hazards Ratio (CI)
Lymph node status (pN0/pN1, 2)	<0.0001	0.37 (0.25–0.57)
Tumor (colon/rectum)	<0.0001	0.44 (0.3–0.64)
PNI (negative/positive)	<0.0001	0.47 (0.32–0.68)
Depth of invasion (pT3/pT4)	0.0004	0.44 (0.28–0.69)

PNI = perineural invasion; CI = confidence interval.

Several reports have indicated that PNI is associated with local recurrence of rectal cancer.[6,9,10,14,25] Our study also showed that local recurrence of rectal cancer was significantly associated with invasion to Auerbach's plexus, and that such invasion was significantly associated with liver metastasis in colon cancer and with liver and lung metastasis in rectal cancer. These results suggest that cancer invasion to Auerbach's plexus is an important factor not only for local recurrence but also distant metastasis.

The PNI grading system has been used in our pathology reports. Slight invasion to Auerbach's plexus is classified as PNI1, massive invasion as PNI3, and