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Adenocarcinoma of the Lower Third of the Rectum: Metastases in Lymph Nodes Smaller Than 5 mm and Occult Micrometastases; Preliminary Results on Early Tumor Recurrence

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Background: The number of examined lymph nodes and metastases in lymph nodes smaller than 5 mm (small lymph nodes) are a determining factor in the stage of rectal cancer although the clinical significance of occult micrometastases is controversial. We are reporting our preliminary results on the identification and prognostic utility of metastases in small lymph nodes and occult micrometastases.

Methods: We searched small metastatic lymph nodes in 101 cases of adenocarcinoma of the lower third of the rectum. We used the manual technique to dissect mesorectal fat and occult micrometastases in the lymph nodes of 52 Dukes' A and B patients, using a pool of anticytokeratin antibodies.

Results: Forty-five percent of the metastatic lymph nodes were smaller than 5 mm in diameter and determined the Dukes' stage in 15 (30.6%) of 49 Dukes' C patients. Occult micrometastases were found in 21 (40.4%) patients: five recurred but vascular invasion, positive distal margin of the rectum, and positive circumferential margin of the mesorectum were present.

Conclusions: Small metastatic lymph nodes, vascular invasion, positive distal margin of the rectum, and positive circumferential margin of the mesorectum were found to be more important than occult micrometastases in predicting early recurrence of rectal cancer.

Key Words: Occult micrometastases—Lymph nodes—Serial sectioning—Rectal cancer.

Detection of metastases in the regional lymph nodes is the most important prognostic factor of rectal cancer. The accuracy in identifying the pathologic stage depends on the number of examined lymph nodes and a careful search for metastases in lymph nodes smaller than 5 mm (small lymph nodes).[1,2] There is no agreement whether or not the occult micrometastases detected in the negative lymph nodes, by immunocytochemical staining[3–10] or molecular techniques,[11] really affect the patient outcome.[5–7,9–11] So, it is questionable if the search for occult micrometastases justifies the use of expensive techniques when many lymph nodes are collected and examined using conventional methods.

The purpose of this study was to evaluate the importance of occult micrometastases and metastases in small lymph nodes in modifying the pathologic stage and in predicting early tumor recurrence in a consecutive series of 101 patients (operated on for adenocarcinoma of the lower third of the rectum).

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METHODS

Patients and Methods

One hundred one consecutive cases of primary adenocarcinoma of the lower third of the rectum were treated by curative total rectal resection and coloanal anastomo-

sis between November 1991 and December 1996 at the Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan. All the surgical specimens were examined by the same pathologist (S Andreola) after fixation in 10% buffered formalin for 24 hours; the regional lymph nodes were searched using a manual technique without previous fat clearing.[2] The metastatic lymph nodes were measured on the histologic slides. Histopathologic examination of the surgical specimen included: the distal resection margin of the rectum, the circumferential resection margin of the mesorectum, and the search for neoplastic vascular invasion. The original hematoxylin- and eosin-stained slides from 19 Dukes' A and 33 Dukes' B patients were reviewed to ascertain the absence of lymph node metastases. Three new sections were cut at different levels from the lymph nodes and were immunostained with a pool of antibodies against different types of cytokeratins (CK) consisting of the following antisera: 35 beta H 11 (Dako, 1:100); 34 beta E 12 (Dako, 1:200); CAM 5.2 (Becton Dickinson, 1:20); AE1 and AE3 (Hybritech, 1:1500); and KL1 (Immunotech, 1:400). This study only considered cases in which immunostain allowed the morphologic evaluation of cellular details. Independence between the CK status and tumor recurrence of Dukes' A and B patients was tested by χ^2 or Fisher's exact test. The incidence of benign epithelial inclusions in paraintestinal lymph nodes was investigated in a series of 523 negative lymph nodes from 11 patients operated on for familial polyposis coli without associated adenocarcinomas. Three sections were cut from each lymph node and immunostained with the same pool of antibodies.

RESULTS

Nineteen (18.8%) patients were Dukes' A, 33 patients (32.7%) were Dukes' B, and 49 patients (48.5%) were Dukes' C. The average number of lymph nodes per patient was 42.3 (range, 10–98; SD, 16.9). The mean number of harvested lymph nodes was 33.9 in Dukes' A patients (range, 10–64; SD, 14.1), 47 in Dukes' B (range, 20–98; SD, 18.9), and 42.4 in Dukes' C (range, 15–83; SD, 15.5). Metastases were found in 278 lymph nodes, corresponding to 6.5% of all lymph nodes; 45.3% of the metastatic lymph nodes were smaller than 5 mm in diameter (small lymph nodes). Seven (14.3%) patients had metastases only in small lymph nodes, and eight patients (16.3%) were classified as pN2 (pathologic node) instead of pN1 because of the presence of metastases in lymph nodes smaller than 5 mm.

Micrometastases consisting of individual or small clusters of neoplastic cells were present in 27 lymph nodes from 21 (40.4%) of 52 patients: 6 (31.5%) of 19 were Dukes' A and 15 (45.4%) of 33 were Dukes' B. Three patients had multiple positive lymph nodes: one patient had two, one had three, and one had four positive lymph nodes. The frequency of the nodal micrometastases ranged between 2.2% and 10% in Dukes' A tumors and between 1.1% and 9% in Dukes' B tumors. The diameter of the lymph nodes containing micrometastases was <5 mm in 17 (63%) of 27 lymph nodes. The mean diameter of tumors in patients with occult micrometastases was 47.8 mm and 35.0 mm in patients without micrometastases ($P = .02$, Student's t-test). Vascular neoplastic invasion was found in 1 of 6 Dukes' A patients and in 3 (20%) of 15 Dukes' B patients.

The circumferential resection margin of the mesorectum was positive in one Dukes' B patient. The distal resection margin of the rectum was positive in one Dukes' A patient.

No statistically significant association was found between CK status and tumor recurrence in both Dukes' stage A patients ($P = .544$, Fisher's exact test) and in Dukes' stage B patients ($P = .626$, Fisher's exact test). No CK-reactive cells were found in 523 lymph nodes from 11 patients operated on for familial polyposis coli.

Follow-Up

The median follow-up for the 52 patients is 40 months (range, 1–80 months); the patients who are still alive have been followed for at least 24 months. Clinicopathologic data for patients with CK-reactive micrometastases are shown in Table 1. One Dukes' A patient died of nonneoplastic disease; one experienced local recurrence and is alive with no evidence of disease after a second surgical treatment: the distal resection margin of the surgical specimen was microscopically positive for this patient. Four patients are alive with no evidence of disease. Four (26%) of 15 Dukes' B patients experienced tumor recurrence: neoplastic blood vessel emboli was found in three patients, two developed lung metastases and one developed liver metastases. One died after pelvic recurrence; the circumferential resection margin of the mesorectum was infiltrated by the tumor in this patient.

Thirty-one patients had no occult micrometastases: two developed local recurrence and one developed liver metastases. Neoplastic invasion of the blood vessels was found in two patients; the distal resection margin of the surgical specimen was positive in one patient.

Three (43%) of seven Dukes' C patients with metastases in small lymph nodes developed distant metastases: two died of disease, one is alive with lung and liver metastases, and four are alive with no evidence of disease. Six (75%) of eight patients classified pN2 (because

TABLE 1. *Local and distant recurrence in patients with CK+ occult metastases*

Dukes'	Rec.	Local rec.	Dist. Met.	Follow-up	Pathol.
A: 6 pts*	1	1		NED	RDM +
B: 15 pts	4	1		DOD	Mes M +
			1 liver	DOD	BVI +
			1 lung	AWD	BVI +
			1 lung	NED	BVI +

* One patient died of nonneoplastic disease without tumor recurrence.

Pts: number of patients; Rec.: number of patients with local or distant tumor recurrence; Local rec.: local recurrence; Dist. met.: site of distant metastases; Pathol.: prognostic pathologic factors; NED: no evidence of disease; AWD: alive with disease; DOD: dead of disease; RDM +: positive distal resection margin of the rectum; Mes M +: positive resection margin of the mesorectum; BVI +: neoplastic emboli in the blood vessels.

of the presence of metastases in small lymph nodes) experienced local recurrence (two patients) or distant metastases (four patients): five (62%) died of disease, one (13%) is alive with disease, and two (25%) are alive with no evidence of disease.

DISCUSSION

In this series, the presence of single or small groups of CK-reactive cells in the lymph node sinus, so-called occult micrometastases, was restricted to the lymph nodes collected from the specimens with adenocarcinoma of the rectum; no CK-reactive cells were found in the lymph nodes from familial polyposis coli. Moreover, the cells' morphology could be sufficiently verified in the immunostained slide to differentiate them from benign epithelial inclusions (which can be found in the abdominal lymph nodes but show benign morphologic appearance and are generally located in the capsule or in the trabeculae instead of the lymph node sinus).[12–15]

Many reports have, in turn, sustained[5,11] or denied[6,7,9,10] the significance of the prognostic relevance of occult micrometastases. Occult micrometastases have been searched through the use of different histopathologic techniques. Serial sectioning and hematoxylin and eosin stain is the cheapest method; however, small clusters or isolated tumor cells in the peripheral sinus of the lymph node may be missed or misinterpreted as benign histiocytes. Immunocytochemical staining for tumor-specific antigens has been widely employed for the detection of occult micrometastases; carcinoembryogenic antigen,[6,8] epithelial membrane antigen,[8] tumor-associated glycoprotein-72,[5] and different types of cytokeratin[3–7,9,25] have also been used. Molecular pathology methods, such as polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR, have been proposed for the detection of occult micrometastases.[7,26–28] These techniques are not available in every surgical pathology laboratory, but, according to Yamamoto,[29] these super-sensitive methods can detect tumor DNA from degraded and nonviable tumor cells.

The clinical meaning of occult micrometastases is even more disputed. Some reports sustain that occult micrometastases correlate with a significantly poorer prognosis;[5,7] other reports have not found statistical differences in survival between patients in whom occult micrometastases were detected and patients in whom they were not detected.[6,7,9,10,30] In our patients, occult micrometastases did not seem to influence the clinical outcome, and local or distant tumor recurrences were associated with the presence of neoplastic emboli in the blood vessels and neoplastic infiltration (of circumferential resection margin of the mesorectum and of distal resection margin of the surgical specimen). Neoplastic blood vessel invasion and positive distal resection margin of the rectum were also found in 3 of 31 patients, with no detectable occult micrometastases, who experienced local or distant tumor recurrence. Similarly, in Greenson's[5] report, four of six patients with occult micrometastases, who died of disease, had neoplastic emboli in the blood or lymphatic vessels. These data suggest that the studies on the clinical importance of occult micrometastases must not underestimate the possibility of extranodal spread of the tumor, such as the invasion of the blood and lymphatic vessels, the nerve branches, and the circumferential resection margin of the mesorectum, which can be demonstrated only by extensive sampling of the tumor and can be easily lost during the routine examination of the surgical specimen. In our study we sampled up to 80% of the tumor tissue per case. Even if such extensive sampling is expensive and time-consuming, our results suggest that the presence of extranodal neoplastic spread—instead of occult micrometastases—may determine the risk of tumor progression. Moreover, the search for occult micrometastases is reliable only if an adequate number of lymph nodes have been examined because the significance of occult micrometastases may be overestimated due to pathologic understaging of the

nodal status. In fact, it is well known that the accuracy of the pathologic stage depends on the number of collected lymph nodes,[16–22] particularly in patients with no nodal metastases. Caplin[23] has recently shown that Dukes' stage B patients with up to six examined lymph nodes had a poorer prognosis than those with seven or more examined lymph nodes.

Because the clinical meaning of single or small tumor cell groups found in the lymph nodes is not yet clear, we think that the recently proposed term of "isolated or disseminated tumor cells" could be a more appropriate one.[24] At the moment, we agree with other previous studies, which sustain that occult micrometastases do not influence the clinical outcome but, instead, represent the neoplastic diffusion controlled and destroyed by the host's immunologic system.[6,7] Unfortunately, the group we studied was relatively small; larger series are needed to confirm these results. Multicenter studies could better evaluate the clinical meaning of occult micrometastases and, above all, the possible role of adjuvant chemotherapy in patients in whom micrometastases are detected by cytokeratin stain.

The second result of our study concerns the clinical importance of metastases in lymph nodes with a diameter smaller than five millimeters that are routinely detected by the standard histologic hematoxylin and eosin stain. Metastases in small lymph nodes turned out to be the determinant in the staging of 15 (30.6%) of 49 Dukes' C patients; they also identified two groups of patients with a high risk of tumor recurrence. Three of seven patients classified as pN1 (because of the presence of metastases only in lymph nodes smaller than 5 mm) and six of eight patients classified pN2 (because of the presence of metastases in small lymph nodes) developed local or distant recurrence. In the first group, metastases represented neoplastic dissemination, which arrested and proliferated in the lymph nodes and showed clinical importance notwithstanding their very small size. In the second group, the more precise pathologic stage allowed a correct evaluation for the outcome of patients who would have been classified as pN1 if only large metastatic lymph nodes had been examined.

In conclusion, we think that immunostaining negative lymph nodes or using other more expensive techniques to search for nodal micrometastases is time-consuming and unnecessary in the routine work of a surgical pathology laboratory, provided that a large number of lymph nodes have been examined. At the moment, the occult micrometastases represent an interesting biological problem, suggesting an interaction between the host's immunologic system and neoplastic diffusion, but their impact on staging and treatment of patients is questionable. On the contrary, the accurate examination of the mesorectum for the collection of the lymph nodes (particularly for the identification of metastases in lymph nodes smaller than 5 mm), the search for emboli in blood vessels, and the search for neoplastic invasion of the circumferential resection margin of the mesorectum (performed by the routine histologic stains) are determining factors for the pathologic stage and the prognosis of rectal cancer.

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