Anticarcinoembryonic Antigen Immunoscintigraphy with a $^{99m}$Tc-Fab’ Fragment (Immu 4™) in Primary and Recurrent Colorectal Cancer

A Prospective Study

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Forty-seven patients were submitted to 68 radioimmunoscintigraphic investigations for primary or recurrent colorectal cancer. Immunoscintigraphy with Immu-4™ correctly detected 28 primary colorectal cancers of 29 and 12 of 12 recurrent colorectal cancers. Overall accuracy was 93.75 percent in primary and 91.6 percent in recurrent colorectal cancer. Immunoscintigraphy had a decisive influence on treatment planning in every third primary colorectal cancer patient and was by far superior to CT scan in the detection of early recurrences, especially in patients with a history of abdominoperineal or low anterior resection. Immu-4™ scintigraphy is a safe and convenient diagnostic approach to colorectal cancer. Because radioactivity is acceptably low and the method is absolutely free of side effects, there are no objections to the repeated use of immunoscintigraphy which provides important information in primary diagnosis as well as in the follow-up of colorectal cancer patients. [Key words: Colorectal cancer; Anticarcinoembryonic antigen antibodies; Radioimmunoscintigraphy]

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In primary colorectal cancer (PCRC) diagnosis can be ascertained with an accuracy of about 95 percent by means of endoscopy, endosonography, barium enema, and CT scan. Among these, endoscopy and CT scan are the most frequently recommended preoperative diagnostic studies. CT scan, which has provided a fairly accurate imaging modality (its accuracy being 48–74 percent), is of low sensitivity. At the time of initial staging, 70 percent of patients appear to have evidence of only local disease. Of this group, up to one-half will develop recurrence or evidence of metastases, both suggesting that initial staging might have been inaccurate. And while only 16 to 25 percent of patients with PCRC have synchronous liver secondaries at the time of laparotomy, these figures also may underestimate the true incidence. The inability to identify all deposits of cancer in patients with PCRC can lead to selection of inappropriate therapy.

In the follow-up period of patients with a previous history of colorectal cancer, the main problem is the time lag and the key issue in recurrent colorectal carcinoma (RCRC) is its early detection. In these cases CT scan is not really reliable, as we already demonstrated in a previous series: 32 patients, in all of whom second-look surgery revealed pelvic recurrences, had been submitted to CT scan that had proved true positive in only 12 of them. Ten cases had been diagnosed false negative and in the remaining 12 no unequivocal differentiation could have been made between recurrences and scar tissue.

Serum carcinoembryonic antigen (CEA) testing proved to be of little value either. In a series of 200 patients, its sensitivity was 64 percent, its specificity was 69 percent. The development of recurrence or metastases frequently occurs prior to elevation of serum CEA, or the CEA elevation is followed by recurrence or metastases that are surgically unresectable at the time of detection. On the other hand, tissue CEA is to be found in the apical portions of tumor cells and in the glandular lumen of almost every colorectal carcinoma. This fact, along with the vital need for an additional tool...
in the diagnostic armamentarium of PCRC and RCRC, was the rationale for this prospective study.

Immunoscintigraphy (IS) is based on the principle that a specific radioactive-labeled antibody recognizes a defined epitope and is bound to this antigen. Since Koehler and Milstein introduced the hybridoma technique in 1975, various murine monoclonal antibodies have been manufactured. The clinical breakthrough, however, did not arise before Goldenberg et al. focused their effort on the Fab' fragment. This fragment, an immunoglobulin of the IgG1 fraction that had its Fc portion removed, is highly capable for targeting CEA epitopes on the tumor surface. But, because of its lack of antigenity, it causes neither human antimouse antibody response nor any allergic reactions of unpredictable nature. The smaller molecular weight of the Fab' fragment compared with the intact murine antibody allows the fragment to leave the intravascular space and target the tumor earlier.

99mTc, an isotope with a short physical half-life and high photon abundance, can be administered at high doses and allows early imaging with a gamma camera. It is very suitable for use in conjunction with a Fab' fragment, the half-life of which is short, too.

**PATIENTS AND METHODS**

The Immu-4™ kit (Immunomedics Inc., Warren, NJ) is an instant, one-step, one-vial method that is convenient and simple for routine use. Disclosure of tumor sites is possible within two to five hours.

After labeling with 925 MBq of 99mTc, 1 mg of the Fab' fragment is slowly injected intravenously. Because the Fab' fragment, despite its murine origin, has no antigen properties, we never had to administer any antiallergic drugs like cortisone, antihistamines, or others. Imaging is performed with an Elscint Apex 409 AG (Elscint, Haifa, Israel) rotating gamma camera with whole-body option (Fig. 1). A whole-body scan is taken after 100 minutes (Fig. 2), followed by single-photon emission-computerized tomography (SPECT) of the abdominopelvic and liver region 2 and 6 hours after injection, respectively. The SPECT images are acquired in a 64 × 64 matrix in 6° steps by continuous rotation over 360°. The resulting 60 planar projections are reconstructed to transverse slices with a filtered backprojection (Hanning filter) method. An attenuation correction method is used. From these corrected transverse slices coronal slices are interpolated from anterior to posterior and sagittal slices from right to left.

While planar imaging requires a high target to nontarget ratio for reliable diagnosis, a ratio of 1.5:1 is clearly enough for SPECT imaging. Although CEA is not a tumor specific but only a tumor-associated antigen, which is just more abundant in tumors than in normal tissues, this ratio can easily be obtained, so that background subtraction is not mandatory.

In this study every uptake was considered pathologic, if it was detectable in at least two planes and in two consecutive sections, thus representing a lesion of at least 2.0 cm in diameter. After gamma camera studies, all patients underwent a biopsy or surgical procedure for diagnosis and consecutive treatment. For serum CEA testing we used a CEA
imunoradiometric assay (SORIN, Inc. Saluggia, Italy).

A total of 68 immunoscintigraphic investigations were performed in 47 patients, aged 39 to 89 years (mean, 67.2 years). Thirty-two patients were evaluated for primary carcinoma, another 15 for recurrent disease only. Among the 32 PCRC patients, 6 had two additional examinations in their follow-up period and another 3 of them had three IS taken during their follow-up. So 32 investigations were performed with regard to primary tumors, while 36 IS were targeted at recurrent or metastatic disease.

RESULTS

Primary Colorectal Cancer

Among the 32 PCRC patients IS detected a tumor in 29 cases (distribution: rectum 11, sigmoid 8 (Fig. 3), descending colon 4, transverse colon 2, ascending colon (Fig. 4), and cecum 4). In one patient the enhanced antibody uptake simulating a tumor in the ascending colon could not be verified, so this result had to be considered false positive. All of the remaining 28 tumors were adenocarcinomas.

In one patient endoscopy and surgery revealed a poorly differentiated mass in the transverse colon, unidentified by IS, which was to be considered false negative. In the remaining two IS-negative patients, microscopy of the resected specimen showed inflammatory disease of the sigmoid colon.

While serum CEA levels were elevated in only 12 patients (sensitivity, 41.4 percent), antibody imaging identified at least one surgically confirmed cancerous lesion in 28/29 patients. Twenty-eight correct positive and 2 correct negative findings, 1 false positive, and 1 false negative result out of 32 cases represent a sensitivity of 96.5 percent (28/29) and an accuracy of 93.75 percent (30/32).

We also assessed the impact of antibody imaging on patient management. In every third PCRC patient antibody scans detected previously occult lesions or confirmed strictly localized disease, thus influencing therapeutic decision making.

In eight patients who had their primaries situated in the left hemicolon, IS suggested metastatic involvement of lymph nodes in the C2 region and we performed extended left-side hemicolectomy with transverso-rectostomy. In another two patients with cancer of the sigmoid, both aged over 75, IS excluded lymph node involvement and we performed only limited sigmoid resection as a low-risk procedure. Microscopy confirmed the N-O stage in both of them.

Recurrent Colorectal Cancer

Among 36 IS performed in order to identify recurrent or metastatic disease, 5 revealed solitary (Fig. 5) or multiple (Fig. 6) metastases to the liver, which were all confirmed by biopsy. In two patients the liver secondaries went along with IS-positive lymph node metastases, undetected by previous CT scan. In five patients with a history of abdominoperineal resection and in another two with low-anterior resection IS revealed local recur-
Figure 5. Transverse slice: solitary metastasis in the right lobe of the liver.

Figure 6. Transverse slice: multiple metastases in both liver lobes.

Figure 7. Recurrent rectal cancer. A. transverse slice: recurrent tumor in the presacral cavity, no lymph nodes involved. B. Sagittal para-median slice: three foci representing the urinary bladder, the recurrent tumor, and a bulk of lymph nodes along the internal iliac artery (from left to right).

In the strict sense of the word. In another two patients after anterior resection, the antibody accumulated in the distended urinary bladder, which was misinterpreted as a local recurrence. Twelve correct positive, 21 correct negative, and 3 false positive findings represent an overall accuracy of 91.6 percent (33/36) in RCRC, which is superior to the results we could obtain by means of CT scan and/or endoscopy. In comparison to CT scans, IS detected more lesions in extrahepatic areas (i.e., lymph nodes), whereas CT imaging detected more liver lesions.

DISCUSSION

An ideal diagnostic approach for colorectal cancer would combine high sensitivity and specificity...
for primary or locally recurring colorectal tumors with the ability to detect accurately regional and distant metastases. This would include the detection of tumor deposits in lymph nodes and other soft tissues in the abdomen and retroperitoneal area that are commonly missed by currently available diagnostic tests. Any new diagnostic modality must be able to compete with others in terms of sensitivity, specificity, safety, and ease of use. In evaluating IS, the question has to be whether or not antibody imaging has an impact on patient management, i.e., whether this technique provides any additional information that could not be obtained by means of other diagnostic approaches.

Our previous experiences with a $^{99m}$Tc-labeled intact monoclonal antibody (BW 431/26; Behring-Werke, Marburg, FRG) indicated that the high sensitivity and specificity of IS may eventually make this method a standard procedure in the diagnosis of colorectal cancer. At that time we concluded that IS in PCRC does not furnish additional data above those obtained with conventional diagnostic procedures, and we suggested that IS only be used in the follow-up of colorectal cancer patients. In this recent prospective study, it becomes evident that IS also influences therapeutic decision making in every third PCRC patient. It is not yet clear whether or not our patients benefit from a more accurate presurgical staging in terms of recurrence rate and survival. Further clinical trials are necessary to test this hypothesis. But there is already a strong body of evidence that stage-adjusted treatment planning helps to avoid overtreatment without sacrificing radicality.

One might object that false positive results can occur, forbidding the use of IS in the diagnosis of PCRC. In our opinion, the decision for laparotomy must not be based on IS alone, but on IS in conjunction with endoscopy. But, if laparotomy is decided, IS can provide additional information regarding lymph glands and therefore may determine the extent of resection and lymphadenectomy.

In the follow-up of colorectal cancer patients IS is, according to our results, superior to every other diagnostic approach. We strongly recommend its use for the detection of local recurrences after rectal cancer. As a matter of fact, these recurrences are usually situated in the perirectal space, while the anastomosis is seldom involved (among our clientele in less than 5 percent). These extrarectal recurrences cannot be identified by means of endoscopy. Endosonography and CT scan are often inconclusive in differentiating between cicatricial and cancer tissue. In many cases the decision can only be made from the course of disease, which means an unacceptable delay in the diagnosis of a recurrence. In patients with a history of abdominoperineal resection IS seems to be the only reliable diagnostic approach to early local recurrence, as our findings suggest. From this personal experience we recommend second-look surgery in every patient with suspicious CT findings, even when serum CEA levels are not elevated! False positive results in this situation were always because of urine activity in patients with cicatricial dislocation of the bladder after rectal surgery. Urine catheterization immediately before IS is therefore mandatory.

With regard to the detection of liver metastases, IS still seems to be inferior to CT scan, although its accuracy could be markedly improved since the introduction of the Fab' fragment instead of the intact antibody. The repeated use of the latter induced the production of human anti-mouse antibodies which accumulate in the liver, so reducing the information gained from a second or third investigation.

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