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CEA-immunoscintigraphy with ^{99}m-Technetium Correlates with Tumour Cell Differentiation in Colorectal Cancer

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The clinical usefulness of immunoscintigraphy with the monoclonal anti-CEA (carcinoembryonic antigen) antibody BW431/26, directly labelled with ^{99}m-Technetium in targeting colorectal carcinomas was investigated in 43 patients. In addition, tumour cell grading and CEA-expression were examined immunohistochemically. Best imaging results were obtained in pelvic tumour lesions (sensitivity 80%). Tumour grading correlated with radioimmunoimaging, well differentiated tumours being detectable at a higher rate ($P = 0.09$). Immunoscintigraphy preceded the findings of conventional diagnostic methods in 3 patients. In 4 cases immunoscintigraphy was decisive for patients management. Therefore, immunoscintigraphy with ^{99}m-Technetium is valuable in directing patients management if conventional diagnostic methods remain undecisive.
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INTRODUCTION

IMMUNOSCINTIGRAPHY ALLOWS specific imaging of cancer with radiolabelled antibodies directed against tumour associated antigens. The widespread occurrence of carcinoembryonic antigen (CEA) on solid tumours and the low level of expression on normal tissues makes this antigen an attractive target for radioimmunoimaging. Goldenberg and co-workers used a radiolabelled polyclonal anti-CEA antibody in patients with colorectal carcinomas in 1978 [1]. In 1981 Mach *et al.* applied a monoconal antibody Mab 17-1A [2].

^{99}m-Technetium is an ideal imaging agent for clinical routine in respect to prompt availability, low cost, and the method of direct labelling [3, 4].

In order to assess the clinical role of radioimaging with ^{99}m-Technetium labelled monoclonal anti-CEA antibodies in the diagnosis of patients with colorectal cancer, 43 patients with primary tumours, suspected recurrences or metastases were evaluated as part of a phase-III study. The influence of tumour cell differentiation and CEA-expression on the quality of radioimaging was investigated.

PATIENTS AND MATERIALS

The monoclonal IgG1 antibody BW431/26 (Behringwerke AG/Germany) [5] was directly labelled with ^{99}m-Technetium [6]. Two milligrams of antibody labelled with 1100 MBq were injected according to previous phase I studies [3].

The study design was accepted by the local ethics committee. 43 patients, 22 women and 21 men, ages 27–80 years, with rising serum CEA level or clinically suspected tumour lesions of colorectal carcinoma were included. A medical history, physical examination and determination of the CEA-serum level were performed before immunoscintigraphy. HAMA (human anti-mouse antibody) specificity was determined by an enzyme linked immunosorbent assay (ELISA) before immunoscintigraphy and 1 and 2 months later.

Four–6 and 22–26 h after injection of the antibody whole body planar images and SPECT (single photon emission computed tomography) of the pelvic and abdominal region were acquired (Double head cameras, Picker/U.S.A.). Data obtained on a 64 × 64 matrix (SPECT) or a 128 × 128 (Planar images) were reconstructed by a Metz and a Ramp filter (Picker/U.S.A.).

The scans were interpreted by two observers without knowledge of the disease status. Immunoscintigraphic findings were correlated with at least two conventional diagnostic methods or confirmed by histology. In cases remaining unresolved the final diagnosis was determined by a 12 month clinical follow-up. In 31 patients tumour tissue was examined immunohistochemically for tumour grading and CEA expression.

RESULTS

Forty five of 66 tumour lesions were detected by immunoscintigraphy in 43 patients, resulting in an overall sensitivity of 68%. Table 1 summarises the radioimaging results on a tumour site basis. The sensitivity was 80% for tumour lesions in the colon or pelvic region and 61% in the liver showing a specificity of 78% and 75%, respectively. All conventional diagnostic methods revealed 63 of 66 lesions.

Thirty six tumour lesions were measured *in situ*, by computed tomography and ultrasound. The smallest lesion detected was 1.5 cm. Immunoscintigraphy was able to disclose 10 out of 12 primary tumours which were also detectable by endoscopy and computed tomography.

Immunoscintigraphy provided valuable information in clinically suspected tumours and negative or uncertain baseline diagnostics. The immunoscintigraphic detection was the decisive diagnostic result for 4 patients with metastases or local recurrences. In 3 cases immunoscintigraphy preceded the findings of conventional diagnostic methods (Fig. 1). In another 10 patients immunoscintigraphy indicated no tumour lesions when

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Table 1. *Analysis of imaging results in* 43 *patients given* ^{99}*m-Tc-*431/26

	True positive	True negative	False positive	False negative
Primary tumour/local recurrence	20	7	2	5
Liver	17	6	2	11*
Lymph nodes	6	0	0	2
Peritoneal seedings	0	0	0	2
Bone	2	0	0	1

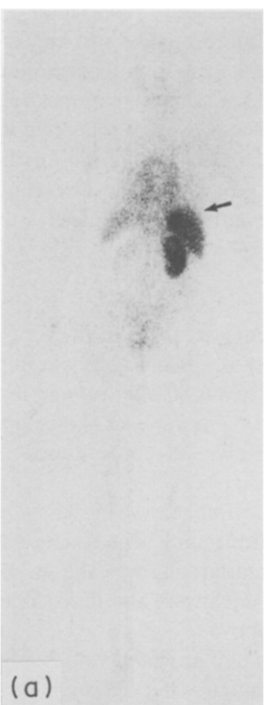
* including three cold lesions.

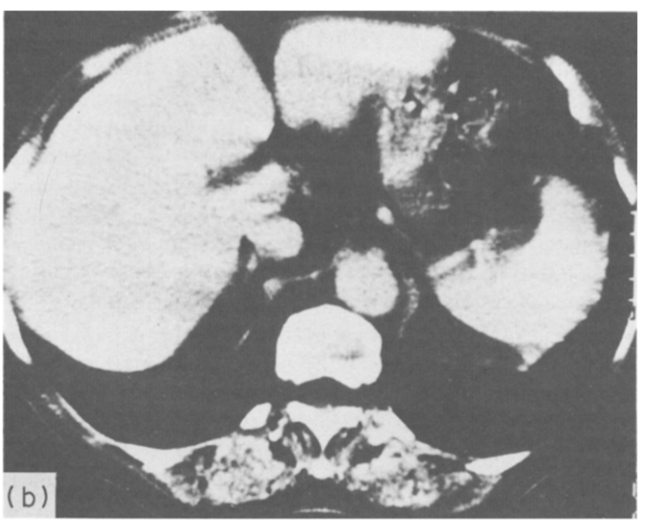
clinically suspected together with negative or unclear findings by conventional diagnostic methods. Here a tumour was also clinically and radiologically excluded in an observation period of 12 months.

After the injection of the antibody BW431/26 no adverse reaction was observed. 8 out of 43 patients however developed HAMA within 2 months.

Tumour detection was independent of CEA serum levels. The sensitivity was 64% in patients with a CEA-level below 10 ng/ml (n = 22) versus 66% in those with a CEA-level more than 10 ng/ml (n = 21).

In 31 patients 36 tumour lesions were confirmed by histology. The tumour cell morphology was graded in well to moderately and poorly to undifferentiated tumours. There was a correlation between grading and radioimmunodetection. Sensitivity of imaging reached 71% in tumour tissue of well or moderate differentiation (n = 21) but only 40% in poorly or undifferentiated tumours (n = 15) (P = 0.09).





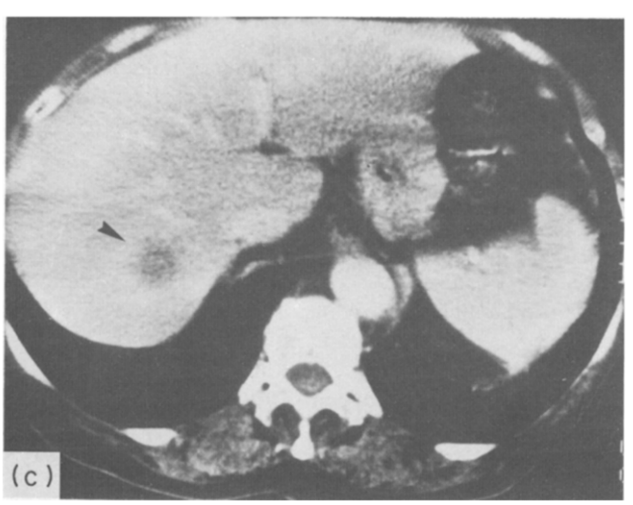


Fig. 1. (a) Posterior planar view 22 h after injection of ^{99}m-Tc-BW 431/26 in a 69 year old woman with a resected rectal carcinoma. Radioimmunoimaging detects a previously unknown metastasis in the left liver lobe (arrow). (b) The corresponding transverse CT scan did not detect any lesion. (c) CT scan 6 weeks after immunoscintigraphy reveals a solitary liver metastasis (arrow). The CT scans were kindly provided by Prof. Dr. M. Thelen, Director of the Department of Radiology, Mainz.

34 of 36 tumour tissues were CEA positive. Only 19 of 34 CEA-positive tumours were detected immunoscintigraphically.

DISCUSSION

In this study we investigated the clinical usefulness of immunoscintigraphy with ^{99}m-Technetium in targeting colorectal tumours and related tumour cell differentation and CEA-expression to the immunoscintigraphic results. Patients with clinically suspected colorectal cancer or rising serum CEA-level were studied.

Primary and metastatic lesions were identified by imaging with the ^{99}m-Technetium-labelled monoclonal antibody BW431/26. The overall detection rate was 68%. Immunoscintigraphy detected 10 out of 13 local recurrences including three tumours, that had been falsely diagnosed as fibrosis by computed tomography (CT). CT is considered to detect colorectal tumours accurately, but in local recurrences it often cannot differentiate tumour from fibrosis [7]. Immunoscintigraphy was able to detect liver metastases with a sensitivity of 61%, comparable to a CT-detection rate of 68% [8]. Only liver metastases showing accumulation of the radiolabelled antibody were classified true positive. We could correlate 'cold' lesion with necrotic tumour masses in 3/5 patients. Those tumour lesions were confirmed by surgery.

The best imaging results were obtained in primary tumours, where 10 out of 12 lesions were found. These results correlate well with CT, which was successful in staging primary tumours in 83% [8].

Radioimmunoimaging of primary tumour lesions did not provide additional information to the routine use of endoscopic examination, ultrasound and computed tomography. Therefore, we do not recommend immunoscintigraphy for the staging of primary tumours. Nevertheless, CEA-immunoscintigraphy with BW431/26 proves to be a clinically useful method, in particular if conventional diagnostic evaluations remain undecisive [3, 9]. In 4 patients a therapeutic decision was based on immunoscintigraphic results. In 3 cases immunoscintigraphic detection of liver metastases even preceded CT-findings. In 10 patients negative findings, together with clinical examination and conventional diagnostic methods, led to the exclusion of tumour lesions, which was confirmed by clinical and radiological follow-up for 1 year and more. In clinical routine the main advantage of a ^{99}m-Technetium labelled antibody in contrast to 111-indium is prompt availability and low costs [9, 10].

Besides exploring the clinical usefulness of immunoscintigraphy, we identified parameters that influence radioimmunoimaging. For the first time a correlation between radioimmunoimaging and tumour cell grading was demonstrated. Well or moderately differentiated tumours were imaged with a significant higher detection rate than poorly or non-differentiated tumours. These findings might be crucial for future therapeutic trials. Comparable to previous studies 94% of tumour tissue stained CEA-positive with BW431/26 and there was no correlation between tumour grading and CEA expression [11].

In conclusion, immunoscintigraphy should be introduced in the clinical course of colorectal cancer diagnosis if conventional diagnostic findings remain undecisive.

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