Immunolymphoscintigraphy with $^{99m}$Tc-labeled Monoclonal Antibody (BW 431/26) Reacting with Carcinoembryonic Antigen in Breast Cancer

Kalevi J. A. Kairemo

Department of Clinical Chemistry, Division of Nuclear Medicine, Helsinki University Central Hospital, Finland, Haartmaninkatu 4, SF-00290 Helsinki, Finland

Abstract

In breast cancer, the most interesting sites showing the regional spread of the disease are the axillary and internal mammary lymph nodes. Monoclonal antibodies are specific in detecting tumor metastases. The aim of this study is to present a simple method whereby the immunolymphoscintigraphic approach is introduced whether bimanually or parasternally.

Twenty consecutive female breast cancer patients were imaged with $^{99m}$Tc-labeled monoclonal intact IgG1 antibody (BW 431/26, Marburg, Federal Republic of Germany), which reacts with carcinoembryonic antigen. The labeling yield was in 10 cases over 98%. The patients had a suspicion of scar recurrence, skin metastases, or palpable lymph node afflictions. The patients were imaged twice after bimanual s.c. injections (at 2–3 h and 20–22 h); the bimanual injections were given into first and fourth interdigital interstitial spaces and in one case parasternally following Sappey's lines.

A total of 105 lesions in 18 patients were detected. Twenty-five lesions in 18 patients were verified cytologically or histologically. Sensitivity of morphological data was 84%. In supraclavicular and axillary lymph node regions the sensitivity was 90% and the specificity 88% compared to other findings. Most of the undetected lesions were skeletal. The carcinoembryonic antigen concentration in serum had no correlation with the findings. The human anti-murine antibodies showed in two patients of 15 elevated responses.

This immunolymphoscintigraphy method could be of clinical importance because it enables detection of both regional and systemic lesions in a common type of cancer. The method is sensitive, except for bone lesions, and might be applied for screening purposes in selected patients.

Introduction

Monoclonal antibodies have shown their importance in the screening of cancer (1). Various i.v. injected radiolabeled monoclonal antibodies have been used in detecting breast cancer lesions with iodine or indium label (2–5). Internal mammary and axillary lymphoscintigraphy with radiocolloids is widely used in detecting lymph nodal breast cancer affliction in axillary and mammary regions (6); the problem is specificity and furthermore the kinetics of the tracer. This approach has even been applied three dimensionally in breast cancer patients (7).

Monoclonal antibodies have been administered into the lymphatics for therapeutic purposes and for detection of the kinetics of lymphatic tracers (8). There are several reports of applying this type of approach in animal studies: pulmonary and mediastinal lymph nodes in pulmonary cancer of dogs (9); colorectal carcinoma metastases in mice (10); and in hepatomas of guinea pigs (8). There is evidence that the kinetics is dependent of the size of the antibody fragment (11) and the distribution is dependent on the administered dose (12).

The first trials in radioimmunodetection with polyclonal antibodies in the lymphatics of breast cancer were at the end of the 1970s (13). In breast cancer the immunolymphoscintigraphic approach has been used by several authors with $^{125I}$ and $^{123I}$ label (14–16), but thus far there are no clinical studies with $^{99m}$Tc-labeled antibodies.

The immunolymphoscintigraphy technique is much simpler than lymphography when trying to discover lymphatic involvement in malignant lymphatic infiltration. In breast cancer the most important sites are the axillary and internal mammary lymph nodes. Serum carcinoembryonic antigen concentration has been helpful in predicting recurrent disease at an early stage (17–20). With high serum CEA$^2$ concentration the survival time is reduced (19, 20).

The aim of this study was to present a simple and specific method for detecting especially axillary lymph node affliction in the staging of breast cancer. The tracer used in this study was a monoclonal antibody reacting with CEA and it was labeled with $^{99m}$Tc. Technetium is suitable for lymphatic administration, because there is generally no technetium retention in the lymph nodes.

Materials and Methods

Patients. Twenty consecutive female breast cancer patients (33–78 years; average, 55) who underwent regular staging and clinical follow-up program were investigated. All patients had a suspicion of cutaneous or axillary lymph node metastases or scar recurrence. Afterwards the lesions were examined clinically, radiologically, and cytologically/histologically when it was ethically and therapeutically indicated.

Radioantibody. The monoclonal antibody (BW 431/26) was provided by Behringwerke (Marburg, Federal Republic of Germany). This IgG1 subclass antibody was labeled with $^{99m}$Tc using the stannous reduction technique. The antibody component vial (I) contained 2.0 mg monoclonal intact antibody and 2.0 mg phosphate buffer at pH 7.2. The vial (II) of stannous salt component contained 2.7 mg 1,1,3,3-propanetetraphosphonic acid$\cdot$4H$_2$O as the tetrasodium salt and 0.12 mg tin(II) chloride$\cdot$2H$_2$O. The content of vial II was dissolved in 5 ml of physiological saline solution during about 1 min. One ml of this solution was introduced into vial I for a total dissolvement within 2 min. Approximately 1.5–3 ml of eluate from a $^{99m}$Tc generator (Ultratechnekow FM, Mallinckrodt Diagnostica B.V., Petten, Holland) with a radioactivity of 350–650 MBq/ml was introduced in a shielded vial of the antibody solution. The incubation time at room temperature was at least 10 min. The labeling yield was measured in 10 cases by thin layer chromatography (ITLC SG; Gelman Sciences, Ann Arbor, MI) and the radioactivity was measured after an elution time of 5 min with a gamma counter (Compugamma 1282; Wallac, Turku, Finland).

Antibody Characteristics. The labeling measured in 10 cases was over 98%. The labeling yield tested by the manufacturer was 95.2 ± 2.6% (SD) and the immunoreactivity was 80–85% (21). The stability by the separation by column chromatography (Bio-Gel P-10, 16 × 230 mm; eluent, 0.9% NaCl solution) after 3 and 6 h was such that 96.3 and 95.3% of $^{99m}$Tc was bound to the monoclonal antibody (21).

Injections. The total patient dose was 25–30 mCi (925–1110 MBq) per 2 mg of the antibody. One-half of this amount was injected s.c. This amount of radioantibody was divided into four equal doses with an approximate volume of 0.25–0.40 ml. The injections were bimanual and equal doses were injected s.c. into the first and fourth interdigital interstitial spaces and in one case parasternally following Sappey's lines. The patients were imaged twice after s.c. injections (2–3 and 20–22 h). After the first imaging one-half of the radioantibody dose was injected i.v.

1 Presented at the "Second Conference on Radioimmunodetection and Radioimmunotherapy of Cancer," September 8–10, 1988, Princeton, NJ. This study was funded by grants from the Cancer Institute of Finland and Instrumentarium Science Foundation.

2 The abbreviations used are: CEA, carcinoembryonic antigen; HAMA, human anti-murine antibody.
Table 1 Data on immunolymphoscintigraphy, serum CEA concentrations, radiology, histology/cytology, and clinical findings in female breast cancer patients

<table>
<thead>
<tr>
<th>Patient/age (yr)</th>
<th>CEA (ng/ml)</th>
<th>Lesions in immunolymphoscintigraphy</th>
<th>Radiology</th>
<th>Histology/cytology</th>
<th>Clinical</th>
<th>No. of lesions detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA/45</td>
<td>35.0</td>
<td>Neg.*</td>
<td>BS:lesions</td>
<td>+/C</td>
<td>Ax Inn</td>
<td>0</td>
</tr>
<tr>
<td>MR/49</td>
<td>b 3</td>
<td>Ax Inn, scar</td>
<td>BS:lesions</td>
<td>+/H</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>EM/58</td>
<td>b 3</td>
<td>Scar</td>
<td>BS:lesions</td>
<td>+/C</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>MF/48</td>
<td>b 3</td>
<td>Bone, ax Inn, abdomen</td>
<td>BS:lesions</td>
<td>+/C</td>
<td>lap/H</td>
<td>6</td>
</tr>
<tr>
<td>AA/78</td>
<td>81.0</td>
<td>Bone, scar, supr Inn</td>
<td>BS:neg.</td>
<td>+/C</td>
<td>-/H</td>
<td>3</td>
</tr>
<tr>
<td>DN/66</td>
<td>b 3</td>
<td>Bone, mamma gl</td>
<td>BS:lesions, mammogr:pos.</td>
<td>+/H</td>
<td>C</td>
<td>4</td>
</tr>
<tr>
<td>TT/46</td>
<td>b 3</td>
<td>Bone and supr Inn</td>
<td>BS:lesions, mammogr:pos.</td>
<td>+/H</td>
<td>Scar</td>
<td>0</td>
</tr>
<tr>
<td>AS/76</td>
<td>12.5</td>
<td>Neg.</td>
<td>CT:pos.</td>
<td>+/C</td>
<td>C</td>
<td>6</td>
</tr>
<tr>
<td>OB/33</td>
<td>b 3</td>
<td>Neg.</td>
<td>BS:lesions</td>
<td>+/H</td>
<td>Scar</td>
<td>0</td>
</tr>
<tr>
<td>ME/65</td>
<td>b 3</td>
<td>Ax and supr Inn, lung</td>
<td>BS:lesions, mammogr:pos.</td>
<td>+/C</td>
<td>C</td>
<td>5</td>
</tr>
<tr>
<td>LA/38</td>
<td>b 3</td>
<td>Mamm gl</td>
<td>BS:lesions, mammogr:pos.</td>
<td>+/H</td>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>AP/59</td>
<td>4.2</td>
<td>Bone, ax Inn, scar</td>
<td>BS:lesions, mammogr:pos.</td>
<td>+/H</td>
<td>C</td>
<td>7</td>
</tr>
<tr>
<td>KV/57</td>
<td>4.9</td>
<td>Ax Inn, bone, liver</td>
<td>BS:lesions, mammogr:pos.</td>
<td>+/H</td>
<td>C</td>
<td>5</td>
</tr>
<tr>
<td>SP/56</td>
<td>b 3</td>
<td>Ax Inn, mamma gl</td>
<td>BS:lesions</td>
<td>+/H</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>SW/36</td>
<td>8.4</td>
<td>Bone, ax Inn, mamma gl, liver</td>
<td>BS:lesions</td>
<td>+/H</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>MA/65</td>
<td>6.7</td>
<td>Bone, ax Inn</td>
<td>BS:lesions</td>
<td>+/H</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>AP/49</td>
<td>4.3</td>
<td>Brain, ax and ing Inn</td>
<td>BS:lesions</td>
<td>+/C</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>ET/73</td>
<td>12.3</td>
<td>Bone, mamma gl, ax and supr Inn</td>
<td>BS:lesions</td>
<td>+/C</td>
<td>+</td>
<td>14</td>
</tr>
<tr>
<td>AF/62</td>
<td>133.0</td>
<td>Bone, mamma gl, ax and supr Inn</td>
<td>BS:lesions</td>
<td>+/C</td>
<td>+</td>
<td>23</td>
</tr>
</tbody>
</table>

Neg., negative; BS, bone scintigraphy; CT, computed X-ray roentgenography; rtg, X-ray roentgenography; US, ultrasonography; b, below; ax, axillary; ing, inguinal; sup, supraclavicular; Inn, lymph node; C, cytology; H, histology; lap, laparotomy; mets, metastases; mamm gl, mammary gland; mammogr, mammography; pos., positive.

Fig. 1. Anterior spot images of head (A), thorax (B), and pelvis (C). Ultrasonography of right groin (D). Brain metastasis was confirmed by computer-assisted tomography. Lymph nodal involvement in right axillary and inguinal region were confirmed by needle biopsy. Inguinal lymph node was detected also by ultrasonography (diameter, 15 mm) (AP/49).

Imaging Protocol. In the first phase the axillary and thoracic regions were imaged using a General Electric 400 T Maxi gamma camera. In each scintigram 250,000-400,000 counts were collected. In the second study the whole body was scanned with a speed of 16 cm/min using a Siemens Scintiview II gamma camera. Low energy general purpose collimators were used. In the affected sites and axillary and thoracic...
IMMUNOLYMPHOSCINTIGRAPHY IN BREAST CANCER WITH Tc

Fig. 2. Whole body scan showing bone metastasis in the left humerus, confirmed by bone biopsy (A). Large liver metastases, visualized as defects in immunoscintigram (B), were also visualized in the computer-assisted tomogram (C) (KV/57).

Histological and Cytological Procedures. Surgical procedures (biopsies) were applied in 10 patients in 14 lesions. Bone biopsy (anterior iliac crest and caput humeri) was performed in two cases. In one case laparotomy was performed because of bowel occlusion and carcinosis was observed. Needle biopsies were performed in 9 patients in 11 lesions. Totally morphological data were obtained from 18 patients with 25 lesions.

Clinical Examination. All patients had palpatory examinations in axillary, supraclavicular, neck, and inguinal regions. Whenever there was a suspicion of metastases a needle or a surgical biopsy was performed. The suspicious cutaneous lesions were examined by inspection and palpation by an experienced physician.

Serum CEA Concentration. The serum CEA concentration was measured in 19 patients 0-19 days before administering the antibody, mainly on the same day. The reference value of the radioimmunoassay method was below 2.5 ng/ml.

Human Anti-Murine Antibodies. HAMAs were measured using an enzyme immunoassay method for human IgG class anti-mouse antibodies (Enzygnost-HAMA micro; Behringwerke AG, Marburg, Federal Republic of Germany). In the first immune reaction the monoclonal antibodies were fixed on the anti-mouse layer of the microtitration plate. During the second incubation the HAMAs of the diluted patient sera bound to the fixed mouse immunoglobulins. After washing the peroxidase-conjugated anti-human IgG antibodies bound to the HAMAs. The enzyme activity was measured after washing nonbinding species and stopping the enzyme-substrate (hydrogen peroxide with 3,3',5,5'-tetramethylbenzidine) reaction with 0.5 N sulfuric acid. The color intensity was measured at 450 nm and the relative HAMA factors were measured. The blood samples were taken just before administering the antibodies and 3–6 months later.

Results

The results are presented in Table 1. The investigated patients had a total of 105 lesions. Twenty-five lesions in 18 patients were verified cytologically or histologically (needle, biopsy, surgery); 19 lesions were true positive, 3 lesions were false positive, and 3 lesions were false negative. Sensitivity was thus 84%.

In soft tissue lesions, there were 34 true positive findings compared to other diagnostic modalities, 5 negative detected by other methods, and 12 positive lesions not detected by other methods (sensitivity, 86%). The main interest was axillary and supraclavicular lymph nodes and the results were as follows.
Fig. 3. Whole body scan of patient with bone, axillary lymph node metastases (bilateral breast cancer), and peritoneal carcinosis (confirmed in laparotomy) (MF/48).

Fig. 4. Anterior spot image of thorax of a patient with bone (right humerus), lung metastases, and scar recurrence (left) (EY/57).

compared to other findings: 16 true positive, 8 false positive, 54 true negative, and 2 false negative findings. Sensitivity was thus 90% and specificity 88% (accuracy, 89%).

Fig. 5. Anterior spot images of patient with lung carcinoma (A). Chest roentgenography showing carcinoma (B).

The CEA concentration had no correlation with the findings. The paired sera measured in 15 patients showed elevated HAMA response in only two cases (Figs. 1–6).

Discussion

In the thoracic, supraclavicular, and axillary regions this method seems to be sensitive and specific. The verified undetected lesions were small: 0.6–1 cm. This study shows that with the immunolymphoscintigraphy method it is possible to improve the diagnostic value of radioimmunodetection and, because it is easy to use, it is recommended for routine use with a suitable antibody. This antibody seems to have potential even in screening of breast cancer in case of metastasized or recurrent disease with soft tissue involvement, because the smallest lesions seen in planar images were smaller than 0.7 cm.
In soft tissues there were lesions observed that were not palpable; some of these were considered false positive findings; the follow-up, however, might show some of these to be malignant. Striated growth of malignancy in patients with a plexus syndrome as a clinical sign is impossible to detect with other radiological methods whereas immunoscintigraphy at least in one case has shown positive results.

In bone lesions this method is unreliable even though suitable reference methods are lacking. Computer-assisted tomography will offer more information than other conventional radiological methods in detecting skeletal metastases in breast cancer (22). Bone scintigraphy is very unspecific (23-25) and two radiological methods whereas immunoscintigraphy at least in one case has shown positive results.

The serum CEA concentration gives no indication of existent lesions in a very important type of cancer. This method is sensitive, except for bone lesions, and might even be applied for screening purposes in selected patient groups, because there is yet no sufficiently sensitive and reliable in vitro test for this purpose.

Acknowledgments

The author is grateful to Dr. Kari Tiisanen for clinical examination of the patients and to Dr. Kristian Liewendahl for his advisory comments and reviewing of the data in doubtful cases.

References

16. Paulick, R., and Caffier, H. Elevated serum carcinoembryonic antigen and...


Immunolymphoscintigraphy with $^{99m}$Tc-labeled Monoclonal Antibody (BW 431/26) Reacting with Carcinoembryonic Antigen in Breast Cancer

Kalevi J. A. Kairemo


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/3_Supplement/949s

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/50/3_Supplement/949s.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.