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A Preliminary Report on the Usefulness of Monoclonal Antibodies to CA 15-3 and MCA in the Detection of Micrometastases in Axillary Lymph Nodes Draining Primary Breast Carcinoma

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The most critical factor affecting survival in patients with breast carcinoma is axillary nodal involvement. Monoclonal antibodies raised aginst specific human mammary tumour associated antigens may increase detection of micrometastases. This preliminary report examines two of these antigens; CA15-3 antigen and mucin-like carcinoma associated antigen (MCA) in the detection of such deposits. Specimens from 39 stage 1 (node negative) breast carcinoma patients were assessed. Two further 'negative' sections were stained with antisera to CA15-3 and MCA antigens. Micrometastases were detected in 5 patients and in each, MCA and CA15-3 identified the same micrometastases. 3 of these patients had disease progression compared with 3/34 of the remaining patients. The use of monoclonal antibodies to CA15-3 and MCA significantly ($P < 0.05$) increases detection rates of micrometastases and this is associated with significantly worse disease-free survival rates ($P < 0.001$).
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INTRODUCTION

THE MOST critical factor affecting patient survival in breast carcinoma is the presence or absence of axillary node metastases [1–3] and a decision to use adjuvant chemotherapy frequently hinges on the number of metastatic lymph nodes [4]. Use of such criteria presupposes that such nodes can be reliably detected by conventional histological methods. Nodal metastases, especially when < 2 mm in diameter (micrometastases) are frequently not detected using conventional histological methodology. This must be of concern because of the association between occult metastases and subsequent disease recurrence. Serial sectioning of lymph nodes has increased detection rates by between 10 and 33% [5–8]. 15–20% of patients with stage 1 breast carcinoma succumb within 5 years of initial presentation, and another 10% die in the following 5 years. Initial assessment of axillary nodal status may be inadequate and as a consequence such patients with undetected occult metastases inappropriately receive no adjuvant treatment.

Serial sectioning to increase yields has been performed [5, 6, 9] but is costly, time consuming, and ultimately impractical. A means of circumventing this, yet improving observer accuracy is to utilise immunocytochemical methods, labelling tumour specific antigens with monoclonal antibodies by indirect immunohistochemical staining. Initial work by Sloane *et al.* [10] was unrewarding. They examined 'negative' lymph nodes using a polyclonal antiserum directed against epithelial membrane antigen (EMA), a glycoprotein found on the surface of all human breast cancer cells. They failed to detect metastases not seen by routine histological assessment. Wells *et al.* [7] subsequently demonstrated that by staining sections with monoclonal antibodies directed against human milk fat globule antigen (E29 and HMFG2), and cytokeratins extracted from human epidermis (KL1), previously undetected micrometastases in 15% of patients were uncovered. No information regarding clinical outcome in these patients was provided.

Two new antigens, CA15-3 and MCA have shown particular promise as serum markers of disease progression in patients with breast carcinoma [11, 12]. They are both noted to be sensitive markers for breast carcinoma [13–15] and should be effective in the assessment of axillary nodes draining a breast carcinoma.

The aims of this study were: to determine whether immunocytochemical evaluation of axillary lymph nodes in stage 1 mammary carcinoma offers any advantage over standard histological methods in detecting occult metastases, and to assess whether detected metastases affect disease free survival rates in this group.

MATERIALS AND METHODS

Material from 39 patients with stage 1 breast carcinoma was retrieved from the files of the Department of Pathology, University College Hospital, Galway. These patients had simple mastectomy with axillary clearance to the level of the axillary vein without adjuvant therapy. In all cases axillary nodes had been stained with haematoxylin and eosin, and declared free of metastatic tumour by a consultant pathologist (JC). Paraffin-embedded tissue blocks of the primary tumour and draining lymph nodes were retrieved. Two new sections were cut from tumour and node prepared for staining with monoclonal antibodies in conjunction with positive and negative controls.

Two distinct monoclonal antibodies were tested for this investigation, CA15-3 (gift of International-Cis Ltd, Paris), and Mab-b12 (Roche Diagnostics, Berne).

Each slide was incubated with normal rabbit serum as a 'blocking' antibody (Dakopatts code no.x902). The primary antibody, CA15-3 or Mab b-12, was then applied followed by

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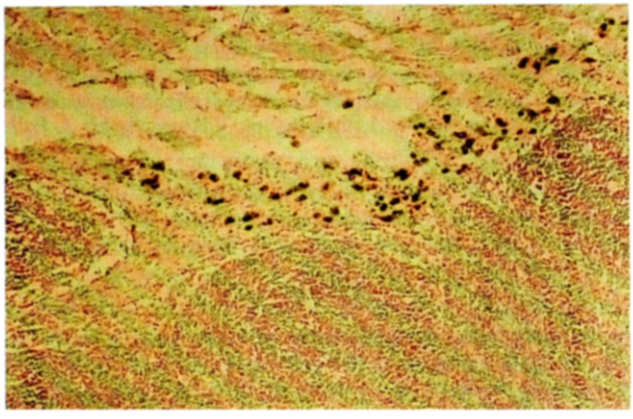

Fig. 1. Area from "negative" axillary lymph node in patient with stage 1 breast carcinoma on restaining with monoclonal antibodies, in this case Mab b-12, demonstrating clearly the presence of a tumour deposit (brown cells).

biotinylated rabbit anti-mouse immunoglobulins (Dakopatts code no.E354) diluted 1:200 with tris-buffered saline (TBS) which served as a link between the primary antibody and the immunoperoxidase-labelled avidin, the ABComplex (Dakopatts code no.K355). The substrate solution was then applied, in this case a 3,3 diaminobenzidine tetrahydrochloride (DAB) solution. Slides were then washed with distilled water, counterstained with haematoxylin and finally mounted with an aqueous base mounting medium. A positive and negative control slide were included with each batch of slides.

The slides were assessed 'blindly' by two pathologists (JC and SE) for the presence of tumour deposits. Hospital files were retrieved and the presentation and follow-up of the patients studied including: age, menopausal status, histological type of tumour, clinical status of patient and duration of follow-up.

Data was analysed using the log rank test and life table methodology [16, 17].

RESULTS

39 primary breast carcinomas were examined in this study. The histological classifications of these tumours were as follows: 35 were infiltrating ductal carcinomas, and 4 were invasive lobular carcinomas. The mean tumour size was 2.4 cm (range 1–6 cm). The mean age of our patient group was 51.3 years (range 34–70 years) of whom 19 were premenopausal. Overall, the mean duration of follow-up was 3.6 years (range 0.8–14 years). A positive reaction was defined as a cell associated definite brown colour consistent with oxidation of the diaminobenzidine substrate. Subtle blushes were considered negative. 38 of the primary breast tumours expressed the CA15-3 and the MCA epitopes. A single poorly differentiated ductal carcinoma in a 39 year old showed no immunoreactivity even following restaining. This patient remains well at 3 years post mastectomy.

A total of 227 lymph nodes were examined from 39 patients, representing a mean of 5.8 nodes per patient (range 2–15). Further sections of the node were prepared and stained individually with either Mab-b12 or CA15-3. A node was deemed positive if a cell or cluster of cells within the node were found to show the characteristic cytoplasmic staining of malignant cells.

Lymph node metastases were found in 5 patients (a total of 8 metastatic nodes found). The total number of nodes examined in this group was 40 (mean 8, range 4–13). These tumour deposits were all in the subcapsular sinus of the lymph node indicating true lymphatic spread (see Fig. 1). All cell clusters discovered were small deposits and would have been extremely difficult to detect on routine histological staining. In 5 patients Mab-b12 and CA15-3 identified the same deposits. The 5 patients in whom micrometastases were found had primary ductal carcinomas with a mean diameter of 2.7 cm (range 1–4 cm). Their mean age was 51 years with a mean follow-up duration of 3.2 years (range 10 months–6 years). 3 of these 5 patients went on to develop progressive disease. The remaining 2 patients with micrometastatic disease remain well, 10 and 18 months post-mastectomy.

Of the remaining 34 patients (31 with invasive carcinoma) 3 patients have developed evidence of disease recurrence. One patient had local recurrence, 2 patients progressed to disseminated disease and one of these has subsequently died. A total of 187 nodes were examined in this group (mean 6, range 2–15).

Those patients in whom micrometastatic mammary cancer was demonstrated, had a significantly worse outcome at follow-up than those without metastatic disease (log rank test $P < 0.001$).

DISCUSSION

This preliminary report confirms the fallibility of routine histological methodology in determination of nodal status of patients with breast cancer. The use of immunohistological techniques increases detection rates of axillary micrometastases, identifying a subgroup of stage 1 patients who may have a significantly increased risk of disease recurrence.

The pattern of staining was predominantly apical in benign disease and cytoplasmic in cancerous cells, confirming the findings of previously cited investigators [13, 15, 18]. 20% of breast carcinoma patients with negative axillary lymph nodes later die of malignancy. The earliest attempt to determine the fallibility of routine histological methods in the evaluation of axillary lymph nodes in breast cancer was that of Saphir and Amronin [5]. They examined alternate serial sections of nodes from 30 patients in whom routine study had failed to detect metastatic disease, and found 10 nodes to be positive by this extended method. The presence of tumour deposits, whether macro or micro, is an ominous prognostic indicator in breast carcinoma, and if a policy of systemic therapy is to be instituted in such patients, there is a clear role for any technique which aids in their detection.

This present work is the first study to describe the use of MCA and CA15-3 antigens in the detection of micrometastases. Detection rates were increased by 12.8%, with both monoclonals detecting disease in the same lymph nodes. Furthermore we demonstrated in our group of patients that this correlated with a worse disease free survival rate. Our findings would suggest a potential role for immunohistochemical techniques in the routine assessment of axillary lymph nodes in patients with breast carcinoma, as the technique clearly identifies an at risk subset.

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Factors Influencing the Risk of Local Recurrence in the Breast

John M. Kurtz

This paper reviews what is currently known regarding possible factors influencing the risk of local failure in the breast after conservative surgery and radiation therapy for clinical stage I and II breast cancer. The best established features correlating with increased risk are young age at time of primary therapy, and the presence of an extensive intraductal component within the primary tumour. The interactions of tumour- and treatment-related factors is complex, but adequacy of surgical excision, quality of radiation therapy technique, and use of systemic therapy all appear to contribute to risk reduction.
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INTRODUCTION

TUMOUR GROWTH in the conservatively operated and radically irradiated breast represents a relatively new oncological event, whose clinical and pathological characteristics only began to be investigated and defined during the past decade. In contrast with local recurrence in the skin flaps after total mastectomy, failure in the conserved breast arises from progression of residual cancer adjacent to the excised primary or from the appearance of separate cancer foci elsewhere in the breast. Intramammary recurrences are generally considered to have a more protracted time course and a more favourable prognosis [1–3]. There is thus little reason to expect that the factors influencing the incidence of relapse in the breast are necessarily the same as those which correlate with the risk of local failure following primary radical surgery.

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Many factors have been proposed in the literature as playing a significant role in determining local recurrence risk. Assessment of the relative merits of individual factors is made difficult by the marked inhomogeneities within the published series: variations in patient selection, surgical and radiotherapeutic techniques, use of systemic treatments, and type of pathological evaluation. For clarity and simplicity of discussion, the present paper will consider separately those factors which are patient-related, those which relate to characteristics of the primary tumour, and those which are to a great extent treatment-related.

DEFINITION OF LOCAL RECURRENCE

Considered here is the clinical appearance of progressive cancer within the parenchyma or skin of the breast, occurring at some time after macroscopically complete primary tumour excision and standard megavoltage whole-breast radiotherapy. The results of excision alone without radiotherapy will not be discussed, as the risk factors for local failure in this setting have not yet been defined. As the available data is restricted to clinical