Report

Comparison of monoclonal antibodies for the detection of occult breast carcinoma metastases in bone marrow

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Key words: bone marrow, breast carcinoma, cytokeratin, epithelial antigens, immunohistochemistry, monoclonal antibodies, micrometastasis

Summary

Twenty percent (n = 6) of Stage III or IV breast cancer patients (n = 30) had bone marrow metastases detected in bilateral bone marrow biopsy/aspiration preparations using standard histologic preparations. Each metastasis was also detected by four separate monoclonal antibodies (MAbs) which recognize breast carcinoma associated antigens (DF3, anti-EMA, HMFG-2, and CAM5.2). These MAbs were then utilized to stain other bone marrow preparations (n = 81) to determine their utility for the detection of micrometastatic breast carcinoma. MAbs HMFG-2, anti-EMA, and DF3 were each strongly reactive with bone marrows containing histologically-evident metastatic breast carcinoma (18/18). These anti-epithelial membrane antigen MAbs, however, were also reactive with rare plasma cells and immature cells (as well as cell clusters) in some of the control bone marrow samples tested, including those from normal patients and patients with hematologic disorders. They also reacted with some of the preparations from patients with leukemia and lymphoma, and with uninvolved marrows from patients with non-epithelial malignancies. The anti-keratin MAb CAM5.2, in contrast, reacted with 83% (15/18) breast cancer metastases and failed to stain any cells in the various categories of control marrow preparations. These data suggested that MAb CAM5.2 might be utilized to immunohistochemically differentiate micrometastatic breast carcinoma from immature myeloid or erythroid elements.

Each MAb was then reacted with histologically uninvolved marrow preparations from the remaining 24 of 30 breast cancer patients in an attempt to identify occult breast carcinoma metastases. While MAbs HMFG-2, DF3, and anti-EMA demonstrated reactive cells in some of these marrows, this reactivity was similar to that seen with control preparations. MAb CAM5.2, in contrast, was negative with all specimens. These data suggest that MAb CAM5.2 may be a useful immunologic probe for the detection and confirmation of metastatic breast carcinoma in bone marrow, while more caution must be employed in the interpretation of results obtained using MAbs anti-EMA, DF3, and HMFG-2.

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Introduction

Prognosis among breast cancer patients is critically dependent on the presence or absence of metastases; the two year survival drops to 50% regardless of therapy once overt metastases have appeared [1, 2]. Breast cancer may metastasize (a) by local extension, (b) along mammary ducts, or (c) via lymphatics and vascular channels [1]. Regional metastases to lymph nodes are the most common, followed by distant metastases to bone, lung, liver, pleura, and adrenal glands, in order of decreasing frequency [1, 3, 4].

Bone marrow metastases have been reported (although the data were not presented) in 57% of patients at first relapse [5]. In addition, histologically identifiable metastatic breast carcinoma has been demonstrated in 44 to 77% of patients who died of disseminated disease at autopsy [1]. Metastatic lesions to bone, when advanced, are not usually difficult to recognize using radiologic and/or biopsy techniques. In light of these data, some regard breast carcinoma as a systemic disease and have hypothesized that micrometastases to bone marrow and other organs occur very early in the majority of patients [5-7]. It has also been suggested that bone marrow micrometastases are difficult to detect using standard histologic stains and criteria, and that immunologic probes should be utilized. These hypotheses are largely based on several studies by one group of investigators utilizing anti-epithelial membrane antigen (EMA) heteroantisera which stained single cells and cell clusters in histologically unremarkable bone marrow and solid organs [5, 6, 8-10]. If, in fact, micrometastatic disease is detectable using immunohistologic probes, the correct diagnosis is of urgent clinical importance, since early therapy for systemic disease may enhance patient survival. These findings are also of critical concern in patients with Stage III or IV breast cancer who are being considered for autologous bone marrow transplants and high dose chemotherapeutic protocols. In these immunosuppressed patients, reinjection of breast cancer cells could result in early treatment failures and a poor clinical outcome. The value of immunohistochemical screening of pre-transplant bone marrows as well as which antibodies should be utilized are relevant clinical issues and deserve confirmation by an independent investigation with rigid controls; hence this study.

Antibodies reactive with breast carcinoma can be separated, based on the immunogen utilized to produce the polyclonal or monoclonal immunoglobulin response, into five broad categories:

1. membrane-enriched extracts of breast carcinomas,
2. milk fat globule membranes,
3. mammary carcinoma cell lines,
4. intermediate filaments, and
5. surface receptors.

Of those MAbs described in the literature, however, most recognize breast cancer associated determinants which have also been detected in other benign or malignant cell types.

Polyclonal and monoclonal anti-epithelial milk fat globule membrane antibodies (anti-EMA) [5, 8-12], anti-keratin MAb LE61 [11], as well as anti-carcinoembryonic antigen (CEA) MAb 11.285.14 [11] have been previously used to detect breast cancer metastases in bone marrow. These studies have suggested that the diagnostic recognition of metastatic breast cancer cells could be increased using antibody preparations and immunohistochemical techniques, yielding detection in 12% of cases with anti-EMA heteroantisera [8] and up to 28% using a combination of multiple biopsy sites and anti-EMA antisera [5]. In each of these studies, however, the specificity and sensitivity of each immunologic probe was not defined using control bone marrow biopsies from patients with benign hematologic disorders or other neoplastic disease states. These controls are of critical importance because women with a history of breast cancer may demonstrate a variety of benign hematopoietic disorders including anemia of chronic disease, anemias secondary to vitamin deficiencies, chemotherapeutic and/or radiation effects. In addition, Pinkus et al. [12] reported that antigens recognized by a commercially available anti-EMA MAb (Dako Corp., Santa Barbara, CA) were present in non-epithelial tissues (malignant mesotheliomas, histiocytic lymphomas, and T cell lymphomas, as well as neoplastic and non-neoplastic plasma cells).