THE CLINICAL SIGNIFICANCE OF IMMUNOHISTOCHEMICALLY DETECTABLE EPITHELIAL CELLS IN SENTINEL LYMPH NODE AND BONE MARROW IN BREAST CANCER

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The application of immunohistochemical techniques capable of detecting single epithelial cells in the sentinel lymph node and in the bone marrow of breast cancer patients has resulted in much confusion among treating oncologists. Many breast cancer patients, particularly those with immunohistochemically positive sentinel nodes, have been upstaged and treated as if they had significant metastatic disease. Such single cell metastases are often regarded as entirely comparable to gross metastases a million-fold or greater in size for which many decades of outcome data confirm prognostic significance.

Ten years ago an axillary micrometastasis was defined as a metastatic deposit of 2 mm or less size, identified in conventional H and E stained sections. Such metastases were relatively infrequent with standard pathologic techniques, and there was some controversy regarding their impact on disease-free survival and overall survival.

Sentinel node techniques for breast cancer are more recent. As part of the decision-making algorithm employed after the technique is well established, patients with a metastasis in the sentinel node will proceed to axillary dissection, whereas sentinel-node-negative patients require no further axillary surgery. Because of this emphasis a number of pathologic techniques were employed to maximize the finding of a metastasis in a sentinel node. These included embedding the entire node in multiple thin segments and obtaining levels of the block to increase the potential volume of the node sampled histologically. These simple techniques alone substantially increased yields of micrometastases in a sentinel node. In addition, however, immunohistochemical (IHC) techniques capable of detecting single tumor cells are now employed. As a result, a node-positive patient can be defined by one or two IHC-positive cancer cells in a lymph node. Without any well developed information about the significance of such minute IHC-positive metastases, many patients have been offered adjuvant therapy.

However, there are a number of discordant scenarios that have recently come to light that question the significance of such IHC-positive cells. A number of studies have shown a surprisingly high rate of micrometastases in axillary lymph nodes, particularly sentinel nodes, removed during mastectomy for extensive DCIS (duct carcinoma in situ). The frequency of such involvement in sentinel nodes is as high as 13% (Cox et al, 2001), yet the post-mastectomy survival for patients with DCIS alone has been established by several independent studies as 99% at 10-15 years of followup (Solin et al, 1996; Silverstein et al, 1999). Very recently, Dowlatshahi, et al, have shown a 52% positive rate for IHC-micrometastases in conventionally node-negative T1a, b patients, a group with an expected DFS of at least 90% at ten years, and greater still for subsets of low-grade invasive, occult T1a, b, conventionally No carcinomas (Tabar et al, 1999, Joensuu et al, 1999). These authors note that half of the IHC-positive, H and E - negative micrometastases comprise ten or fewer cells detected by immunohistochemistry. Clearly there is a disconnect between the pathology findings and clinical outcome in both the mastectomy patient with DCIS and the T1a, b, N0 group with IHC-positive micrometastases. Cote et al, 1999, in a recent retrospective study, noted that there seems to be an impact of tumor burden, with patients exhibiting greater than 100 IHC-positive cells doing more poorly than those with fewer. One wonders what the clinical

significance might be for a group of T1a, b, conventionally N0, carcinomas with ten or fewer cells by immunohistochemistry, a log decrease in number. Cote et al were unable to demonstrate significance for IHC-positive micrometastases in premenopausal patients, nor in their premenopausal patients in whom H and E identified occult metastases (those detected by serial levels) were identified. More recently Hansen et al (2001) were unable to demonstrate any significant differences between patients with HE-negative IHC-positive micrometastases in a prospective 5 year study of disease free survival and overall survival. The observed difference between HE and IHC-negative groups and those with HE-positive metastases less than 2 mm was 4%. In a retrospective analysis of cases initially classified as node-negative and with a mean 25 year follow-up, Reed et al (2004) found no significant difference in recurrence-free and overall survival for patients without demonstrable metastases after cytokeratin immunohistochemistry, versus those with identifiable metastases less than 0.2 mm or $\leq$ 2 mm. Only patients with metastases >2mm had a significant decrease in survival, and only those with ductal as opposed to lobular histology. Similarly Susnik et al (2004) in a retrospective reanalysis of all lymph nodes from axillary dissections of 270 pT1N0M0 patients with 15 year followup found significance for identifiable micrometastases 0.2 - 2.0 mm in size ($p = 0.04$) but not for immunohistochemically identifiable cytokeratin positive cells alone ($p = 0.31$) for distant metastases. The newly revised TNM staging system recognizes the lack of significance of IHC-positive metastases by classifying them as node-negative (Singletary et al, 2002).

Carter et al (2000) and Page and Carter (2002) have recently described aritfactual mechanisms capable of displacing benign as well as non-invasive neoplastic breast epithelium into lymphatics and resulting in micrometastatic deposits in lymph nodes. Neither type of epithelium is capable of metastatic growth but both scenarios can result in serious clinical misinterpretation resulting in adjuvant chemotherapy. Evidence supportive of epithelial displacement as a mechanism to account for a significant fraction of IHC-positive metastases (25-34%) was provided by Hansen et al (2004). Although IHC identifiable micrometastases should exhibit a cytology and pattern consistent with the invasive primary this is often difficult to establish, since such deposits are so frequently detected as single cells. For example, tubular carcinomas generally metastasize as small tubular glands, not single cells or small solid cell masses of 8 - 15 cells.

Two international consensus conferences, (2001, 2002) have concluded that IHC micrometastases and HE-identified micrometastases < 0.2 mm in size should not be used to upstage the nodal status or plan therapy, corroborating the consensus of the American College of Pathologists (Fitzgibbons et al, 1999).

These discordant findings should inject some degree of caution in utilizing the information gathered by these new technologies. All existing outcome data are based on conventional lymph node examination, however flawed and variable that may be. This fact has been lost on many colleagues who equate a grossly identifiable 3 mm metastasis to a single cluster of 3 IHC positive cells in a sentinel node.
Until these discrepancies produced by this new immunohistochemical technology are resolved, I would not recommend treating patients with DCIS post-mastectomy with an IHC-positive micrometastasis, nor those low-grade invasive carcinomas of T1a, b size with adjuvant chemotherapy who are conventionally node-negative. As a dramatic example, let us not conclude that a patient with an 8.5 mm pure tubular carcinoma and 6 IHC-positive cells in a sentinel node requires chemotherapy.

SENTINEL NODE BIOPSY FOR DCIS

The use of sentinel node biopsy in patients with DCIS has been strongly advocated by some but in my opinion is misguided. Advocates note that a significant number of biopsy diagnoses of DCIS, particularly with MIBB, will be upstaged at excision and a planned SLN as part of a segmental resection will permit appropriate staging of the patient without recourse to a separate surgical procedure. Additionally in patients at particularly high risk e.g. extensive high grade DCIS, SLN can serve as a screening procedure for the presence of occult and/or unsampled invasive foci in the resection or remaining in the breast. Finally patients who have undergone mastectomy are not candidates for a SLN after the fact (Pendas et al, 2004; Klauber-DeMore et al, 2000; McMasters et al, 2002).

In rebutting these arguments (Lagios & Silverstein, 2001) it is useful to recall certain established facts:

1. Cause specific survival at 15 years post treatment for DCIS varies from 98% (breast conservation) to 99% (post mastectomy). Therefore very few women remain at risk of dissemination or progression after treatment.

2. The frequency of truly positive axillary lymphnodes in DCIS derived from a time when standard AXN was part of a mastectomy for DCIS, hovers around 1%. Silverstein (personal communication) has reported a frequency of 0.4% among 472 patients. In contrast, histological examination of a SLN requires multiple levels and often immunohistochemistry for cytokeratin. A positive SLN in DCIS frequently reflects the presence of isolated tumor cells detected only by IHC B a condition which has no known significance for outcome and for which we have been enjoined from using to stage patients with invasive disease, let alone DCIS. The vast majority of currently reported positive SLN in DCIS reflects such scattered isolated cells.

3. In our early series of standard mastectomies for DCIS (Lagios, 1989) examined by the serial subgross technique of Robert Egan, extensive (i.e. >55 mm) DCIS was associated with a high risk (48%) of occult microscopic invasive foci being found at mastectomy. Despite this the majority of such patients will be node-negative (98.5%). Thus a SLN is a poor screen for occult invasion most of which will be foci of T1mic B T1b size.

Establishing the presence of invasion in a mastectomy or large resection by appropriately thorough histologic examination yields more clinically useful information than establishing a negative SLN status for the 99% of patients examined, and is far cheaper.

Finally two retrospective studies have employed serial sectioning and IHC to re-evaluate lymph nodes obtained in conjunction with resections for DCIS and analyzed outcome against the presence of IHC micromets in the re-evaluated lymph nodes. Neither IHC micrometastases, nor the few HE micromets had an impact on DFS in those studies (Lara et al, 2003; El-Tamer et al, 2005).

BONE MARROW INVOLVEMENT

The immunohistochemical demonstration of epithelial cells in bone marrow of breast cancer patients has also generated great interest as a possible new prognostic indicator. As both tumor size and nodal positive frequencies have decreased secondary to mammographic surveillance in recent decades, the examination and prognosis of T I node negative breast cancer patients

have become more sharply focused. The expectation would be that newer potential indicators such as microscopic bone marrow involvement by immunohistochemically positive epithelial cells might define those more likely to relapse and benefit from adjuvant intervention.

Braun et al. (2000) generated a great deal of excitement in their demonstration of a prognostic effect of IHC-positive bone marrow cells on disease-free survival and overall survival. In a prospective study of 552 new breast cancer patients with a median 38 month followup 25 percent of patients with a positive bone marrow died of breast cancer-related causes compared to only 6 percent without positive bone marrows. Overall survival rates were 68 percent for the bone marrow positive group and 93 percent for the bone marrow negative group. An analysis of pathologic features showed a relationship between increasing T size and stage and grade and the presence of bone marrow epithelial cells and to a lesser extent axillary nodal metastases.

The overall bone marrow cytokeratin positive rate was 36 percent; 93 percent were characterized by dispersed single cells; a median number of 3 positive cells per 2 million bone marrow cells occurred in patients who were classified as bone marrow positive. The median number of such cells per 2 million for Stage I breast cancer patients was 5; for Stage II, 9; and for Stage III 86. Of considerable concern, however, regarding the clinical significance of cytokeratin positive bone marrow micrometastases were the frequencies of positive bone marrows among breast cancer patients with more favorable features. Twenty-three percent of pTIa and 35 percent of pTIb breast cancer patients had bone marrow cytokeratin positive cells. The authors noted that there was no significant relationship between axillary lymph node positivity and bone marrow cytokeratin micrometastases ($P = 0.13$) but there was a relationship between the number of axillary nodes positive. Although bone marrow cytokeratin micrometastases were significantly more frequent in patients with high grade (Grade III) breast cancers, still 29 percent of low grade (Grade I) breast cancers exhibited similar marrow cells. Even among 51 patients with pTIa or b, Grade I or II, estrogen receptor-positive breast cancers, 11 (22 percent) had bone marrow cytokeratin positive micrometastases. Statistically, depending on palpability, 83 to 95 percent of such patients would be expected to be axillary lymph node negative with 10 year disease-free survival expectations in excess of 95 percent. Does the presence of such bone marrow cytokeratin micrometastases require intervention for pTIa, b N 0, ER-positive, Grade I and II breast cancers, a group which by consensus is unlikely to benefit from adjuvant therapy in any clinically significant way? Clinicians do not deal with a node negative breast cancer patient alone, but rather with the data which defines a more sharply focused subset, e.g., a T Ic, N 0, low grade, ER positive. Might not the positive correlation of bone marrow cytokeratin micrometastases in outcome reflect the 42 percent of patients at Stage II or higher in this study.

Molino and colleagues (1999) in a prospective study of 125 Stage I and II breast cancer patients, with a similar 48 month followup, were not able to demonstrate a prognostic impact of bone marrow immunohistochemical positivity on either disease-free survival or overall survival, nor an association between bone marrow immunohistochemical positivity and any of the known prognostic features, e.g., T size, nodal status, grade, estrogen receptor status, and proliferative indices, etc. They demonstrated in serial bone marrow aspirates, every 6 to 8 months changes in the frequency of positivity. The population was comparable to that of Braun et al. in terms of tumor size (T I 60 percent versus 58 percent) and axillary node positivity (45 versus 54 percent), three or more positive nodes (23 percent versus 26 percent). However, the authors speculate that the bone marrow isolated cells documented by immunohistochemistry may represent cells in transit rather than reflect true metastases.

Gebauer and colleagues (2001) report a prospective study of 393 patients who underwent bone

marrow aspiration at the time of the primary surgery, with a median 75 month followup (corrected for intercurrent deaths). Forty-two percent of bone marrows contained cytokeratin positive cells morphologically identifiable as cancer. During the follow-up period, 27 percent of all patients developed distant metastases: 35 percent among bone marrow IHC positive and 20 percent among bone marrow negative patients (P = < 0.001). However at 5 years of median followup 65 percent of the bone marrow IHC positive patients were still NED. There was no difference in distant relapse rate for node negative patients with or without bone marrow micrometastases (P = 0.8255) and in a Cox multivariate analysis bone marrow status was marginally significant for disease-free survival (P = 0.0058) but not overall survival (P = 0.0648). Tumor size and axillary nodal status were far more significant predictors for disease-free survival (P = < 0.0001 and P = 0.0018, respectively). The authors conclude that for the node negative group for whom adjuvant intervention is most problematic and in their own series who received no adjuvant therapy, there was no difference in disease-free survival or overall survival. Most of the poor outcome in bone marrow positive cases reflects patients with axillary involvement. They conclude with a caution against overstaging patients: the detection of epithelial cells alone is not a sufficient definition of growing metastatic disease that might lead to clinical consequences.

Mansi and colleagues (1999) studied 350 women undergoing primary breast cancer treatment who consented to multiple bone marrow aspirates. Micrometastases were detected by a polyclonal antibody to epithelial membrane antigen but combined with cytologic assessment of the positive cells to confirm carcinoma. The study has a median 150 month followup corrected for living patients. Bone marrow IHC micrometastases were more clearly associated with decreased relapse-free (= distant relapse and breast cancer death) and overall survivals (P = < 0.001). However, corrected for conventional prognostic features of tumor size, lymph node involvement and lymphovascular invasion in a Cox regression analysis, bone marrow micrometastases as detected by immunohistochemistry were no longer significant as an independent prognostic factor (P = -0.30). Moreover, the conventional prognostic factors exhibited larger hazard ratios (tumor size T I versus T II HR 1.97, nodes - negative versus 1-3, HR 2.02; vascular invasion HR = 1.78 and IHC bone marrow micrometastases HR = 1.09). The authors note that among a group of bone marrow positive patients whose aspirates were repeated at a time when they were NED, only two of 21 repeat bone marrows were still positive, and postulate that a proportion of bone marrow immunohistochemically detectable cells may be non viable as Carter and Page have noted for the sentinel node, and lack potential for metastatic growth.

Summary

Current consensus and limited outcome data suggest that IHC-positive scattered epithelial cells in the sentinel lymph node, even those that truly represent viable cancer cells, do not adversely impact disease free survival and should not be used to upstage patients or to recommend adjuvant therapies. Although this consensus is now officially recognized in a revision of the TNM staging classification, there are still patients including those with DCIS who are being treated for metastatic disease on the basis of IHC-positive cells in the sentinel node.

It has been recognized since the late 19th century that breast cancer cells can be recovered in the peripheral blood, therefore it should require no great extrapolation to expect their appearance in the marrow. Although the appearance of such cells identifies breast cancer patients at greater risk of recurrence, the available evidence would indicate either no or very limited independent prognostic effect. Corrected for tumor size, stage and grade, all of which are more significant prognostic indicators in multivariate analyses and more readily determinable

in current medical practice, IHC bone marrow epithelial cells provide no clinically useful information.

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