Comparison and evaluation of CA 15-3, c-erbB-2, ER and PR tumor markers in pleural fluid cytology from metastatic breast cancer as a diagnostic tool

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ABSTRACT

This study has been conducted to determine the diagnostic value of CA 15-3 in detecting metastatic breast carcinoma in pleural fluid. The material studied consisted of 115 malignant pleural effusions from invasive breast cancer patients. Expressions of CA 15-3, c-erbB-2, estrogen receptor (ER) and progesterone receptor (PR) were studied using immunocytochemistry. Of the cases studied, 115 were evaluated for CA 15-3, 70 for c-erbB-2, 100 for ER and 97 for PR. The 94% of the breast cancer cases studied showed a positive reaction with CA 15-3, while 31% for c-erbB-2, 20% for ER and 9% for PR were positive ($p<0.01$). The sensitivities of CA 15-3, c-erbB-2, ER and PR were 94%, 31%, 20% and 9%, respectively, for metastatic breast carcinoma. Our results indicate that CA 15-3 has the highest sensitivity for diagnosing malignant breast carcinomas in pleural fluids. [Turk J Cancer 2009;39(2):45-50]

KEY WORDS: Pleura, metastasis, invasive breast cancer, estrogen receptors, progesterone receptors, c-erbB-2, CA 15-3, cytology, immunocytochemistry

INTRODUCTION

Breast cancer remains the most common malignancy affecting women in industrialized countries. It is estimated that 4.4 million women who have had breast cancer diagnosed within the last 5 years are alive. Because of the growing incidence rates, there would be around 1.4 million new cases in 2010 worldwide (1). Breast cancer metastasizes most frequently to axillary lymph nodes, but any organ may be involved. Metastatic spread to serosal surfaces involves primarily the pleural cavity, where breast cancer is the etiology of approximately 25% of malignant effusions (2,3). However, breast carcinoma metastasis may be infrequently found in the pericardial and peritoneal cavity as well (4). Involvement of the pleural cavity by breast carcinoma may occur at any point in time of clinical course and may be the sole manifestation of metastatic disease (5). It portends an extremely poor prognosis, with a median survival of 5 and 11 months in two series (5,6).

Despite the magnitude of the clinical problem, studies of the biology of breast cancer have focused exclusively on primary tumors and solid metastases. Consequently, the biological characteristics of breast carcinoma cells in effusions have been poorly characterized.

CA 15-3 is an antigen expressed in benign and malignant breast ductal epithelium. Antibodies against CA 15-3

have been used as possible serum markers of occult and recurrent breast carcinoma (7,8). CA 15-3 has also been studied as a serologic test for breast carcinoma in pleural fluid (9). Immunohistochemical studies using second generation CA 15-3 antibody showed promising results in terms of detecting adenocarcinoma in body cavity effusions (10).

In this study, the sensitivities of CA 15-3, c-erbB-2, estrogen receptor (ER) and progesterone receptor (PR) at the time of detection of metastatic breast carcinoma in pleural fluid were analyzed.

MATERIALS AND METHODS

Cases

The cytological files of the Hannover Cytopathology Institute were reviewed in August, 2007. The 115 pleural fluids were collected from 115 patients who were diagnosed with metastatic breast carcinoma. Their medical records were reviewed to verify that the cases were of breast carcinoma. Slides that were stained previously by routine May Grunwald Giemsa method were retrieved for each patient. The diagnosis of breast carcinoma based on cytomorphology was confirmed. The sensitivities of CA 15-3, c-erbB-2, ER and PR in pleural fluid samples for metastatic breast carcinomas were calculated.

Immunocytochemistry procedure

Cytological smears were prepared by standard cytologic method. The slides were hydrated in decreasing ethanol solutions. Endogenous peroxidase was blocked with hydrogen peroxidase for 3 minutes. The slides were rinsed in distilled water, and then incubated with the biotin blocking system (Dako) prior to application of the primary antibody. The antibodies used were as follows: CA 15-3 (1:100, clone Df-3, Dako), c-erbB-2 (1:100, polyclonal, Dako), ER (1:50, clone 1D5, Dako) and PR (1:50, polyclonal, Dako). Monoclonal antibodies were incubated with the samples for one hour. The slides were then rinsed in phosphate buffer, and then incubated for 25 minutes with the linking solution (LSAB+ kit; Dako; biotinylated antimouse, antirabbit, and antigoat). This was followed by a rinse in phosphate buffer and incubation with streptavidin peroxidase for 25 minutes. After rinsing in buffer, the slides were submerged in DAB or AEC as a chromogen for 5 minutes. The slides were counterstained with Mayer's hematoxiline. The intensity of staining was graded on the following scale: 0 (absent), 1+ (weak), 2+ (moderate), and 3+ (strong). All material was evaluated blindly by two observers. Statistical analysis was performed using the chi-squared test and the orthogonal test. They were used to determine statistical significance.

RESULTS

Immunoreactivity for CA 15-3, c-erbB-2, ER and PR were determined for neoplastic cells in a total of 115 cases of metastatic breast carcinoma from pleural fluids. Overall results are summarized in table 1.

For all metastatic breast carcinomas studied, 108 (94%) of the cases exhibited CA 15-3 immunostaining (Figure 1). The staining was predominantly cytoplasmic. Although there was membranous staining present, it was usually in the form of darker staining relative to the cytoplasmic staining. The sensitivity of CA 15-3 for these malignant breast carcinomas was 94%.

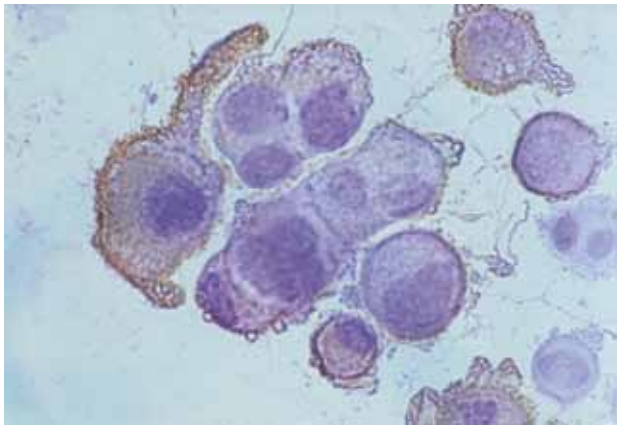

Fig 1. Immunocytochemistry of CA 15-3 showing moderate cytoplasmic and membranous staining in breast cancer cells (Pleural fluid, x600)

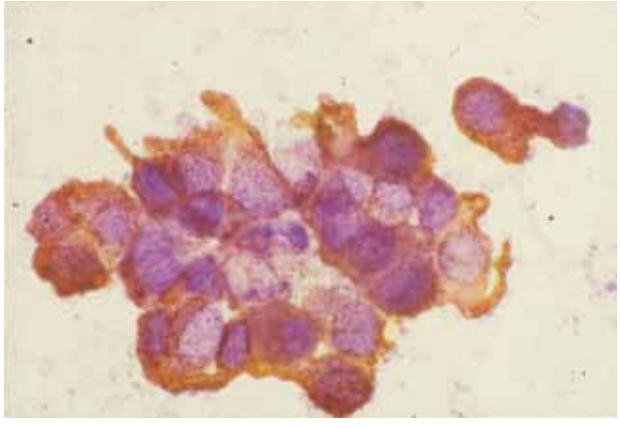

Fig 2. Immunocytochemistry of C-erbB-2 showing moderate cytoplasmic staining in breast cancer cells (Pleural fluid, x600)

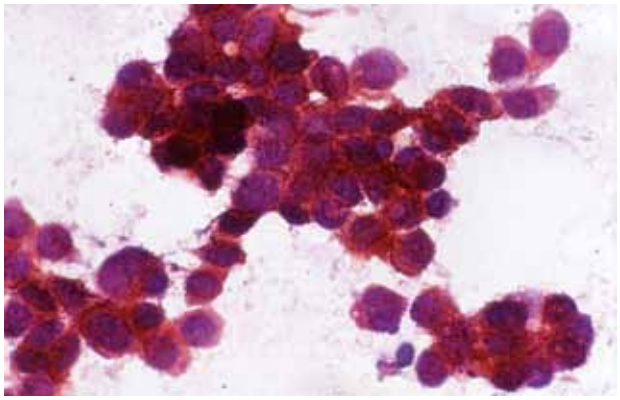

Fig 3. Immunocytochemistry of ER showing moderate cytoplasmic staining in breast cancer cells (Pleural fluid, x600)

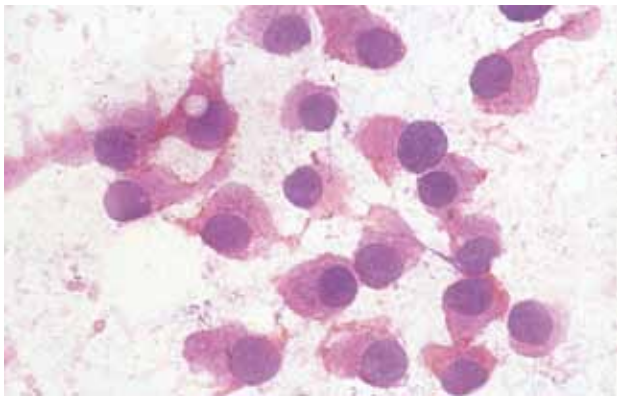

Fig 4. Immunocytochemistry of PR showing moderate cytoplasmic staining in breast cancer cells (Pleural fluid, x600)

We also examined c-erbB-2 reactivity in cytologic specimens (Figure 2) derived from metastatic breast carcinomas and detected positivity for most neoplastic cells in 15 (21%) of 70 cases, all of which exhibited a diffuse membrane staining pattern.

Of 100 cases of metastatic breast carcinoma, neoplastic cells in 20 cases (20%) exhibited nuclear reactivity for ER protein (Figure 3). Detection of PR (Figure 4) was observed in 9 (9%) of 97 cases.

There was a significant difference between CA 15-3, c-erbB-2, ER and PR expressions in metastatic breast carcinoma ($P<0.01$; Chi-squared test). The sensitivities of CA 15-3, c-erbB-2, ER and PR as a marker for metastatic breast carcinoma were 94%, 31%, 25% and 9%, respectively. CA 15-3 had the highest sensitivity of the malignant breast carcinomas studied, with a sensitivity of 94%.

DISCUSSION

Breast cancer is the most common cause of malignant pleural effusions in women, followed closely by lung adenocarcinoma. Approximately half of the patients with disseminated breast cancer develop a malignant pleural effusion at some time during their illness, while the most common tumor to initially manifest as a malignant pleural effusion is lung carcinoma (11). As with nodal and soft tissue metastases, the determination of the primary tumor site in cases of malignant effusion can be facilitated by immunoreactivity by various markers. In tissue sections, the identification of metastatic breast carcinoma has been greatly enhanced by the use of antibodies directed against ER and PR, which have been demonstrated to be useful markers for breast carcinoma (12).

Immunohistochemistry is proven to be useful in the diagnosis, classification and prognostication of neoplasms, making it an ideal tool for the study of effusions, especially in difficult cases. CA 15-3 is an antigen localized at the luminal aspect of breast epithelium. It has been used as a serum marker of occult and recurrent breast carcinoma (7,8). It has also been used in numerous studies to help diagnose malignant pleural effusions (9).

Since the early 1990s, antibodies against CA 15-3 have been developed as possible serum markers of occult or

Table 1
Staining patterns of malignant pleural effusions for CA 15-3, c-erbB-2, ER, PR

Staining	CA 15-3 n (%)	c-erbB-2 n (%)	ER n (%)	PR n (%)
Negative	7 (6)	55 (79)	80 (80)	88 (91)
Positive				
1+	25 (22)	-	11 (11)	8 (8)
2+	43 (37)	9 (12)	5 (5)	1 (1)
3+	40 (35)	6 (9)	4 (4)	-
Total	115 (100)	70 (100)	100 (100)	97 (100)

recurrent breast carcinoma (7,8). Similarly, CA 15-3 has been examined as a serologic test for breast carcinoma in pleural fluid (9). Histologic studies have centered on the specificity of CA 15-3 for breast carcinoma in metastatic carcinomas or its sensitivity for detecting micrometastases in axillary lymph nodes (13,14). These reports indicate that CA 15-3 is sensitive, but not specific, for breast carcinoma. To our knowledge, no recently published work has examined CA 15-3 as an immunochemical stain to detect carcinoma in body cavity effusions, although Szpak and coworkers (15) used their own clone to the DF3 epitope of CA 15-3 for this purpose in 1984. Given its high sensitivity for carcinomas, we evaluated a second generation CA 15-3 for its utility to detect breast carcinoma in pleural effusions.

In this study we have confirmed that CA 15-3 is a sensitive tumor marker for breast carcinoma, with a sensitivity of 94%, while Huang et al. (16) and Geraghty and coworkers (17) have reported 91% and 88% for the sensitivity of CA 15-3, respectively. Fehm et al. (18) found that positivity rate of CA 15-3 serum levels was 51% in metastatic breast cancer. Zimmerman et al. (19) reported that CA 15-3 was an immunostain with high specificity and sensitivity for breast carcinoma cases (97%) in cell block material from effusions. In conclusion, CA 15-3 shows remarkable potential in diagnosing metastatic breast carcinoma in cytologic specimens.

It is well recognized that serial radiologic studies, including bone scans, ultrasonography, and plain radiographs, are of limited value in the detection of occult metastatic breast carcinoma. Measurement of tumor marker levels is an attractive alternative screening technique for metastatic breast carcinoma, because it is noninvasive, inexpensive and relatively simple to perform. Carcinoembryonic antigen is the most widely used marker in monitoring the development and clinical course of patients with metastatic breast carcinoma (20). The poor sensitivity of this marker, however, limits its usefulness, because only 40-70% of patients with metastatic breast carcinoma have increased levels (21-24). CA 15-3 is more specific for breast cancer and is also more sensitive in patients with advanced disease (25).

Hormone receptors and c-erbB-2 are established molecular prognostic markers in breast cancer (26,27). They are often targeted with therapeutic intention in both localized and metastatic breast cancer, including in the presence of malignant effusion. Davidson and coworkers (28) reported that significantly reduced ER and PR expression was seen in effusions compared with primary tumors, with opposite findings for c-erbB-2 which was highest in effusions and lower expression in primary tumors.

Ali et al. (29) and other investigators have reported increased soluble c-erbB-2 in the plasma or serum of 20-30% of patients with metastatic breast cancer (30-32). Bozzetti et al. (33) reported that c-erbB-2 status was mostly stable in primary breast carcinoma and in the corresponding distant metastatic sites on cytologic material. We reported that 21% of breast carcinomas cases showed positive staining for c-erbB-2 in this paper. Lee et al. (34) evaluated the hormonal receptors for ER and PR for cytology cell block preparations from 96 effusion specimens. They reported that 21 (72%) were reactive for ER, 15 (52%) for PR, and 13 (45%) for both receptors. They suggested that the detection of ER and PR in metastatic adenocarcinoma from pleural effusions could distinguish breast from lung primary sites. Several studies have shown that metastatic breast cancer patients with a high disease burden have a poorer prognosis (35,36). It is difficult to quantify disease burden with imaging studies, and some patients have evaluable but not measurable disease. Pleural effusion CA 15-3 is used as a surrogate marker of disease bulk to monitor metastatic breast cancer patients undergoing treatment and for the preclinical detection of tumor recurrence. One report has shown that increased HER-2/neu is associated with both decreased response rate and shorter time of survival (37).

In this study, we evaluated c-erbB-2 status on metastases from breast carcinoma patients performed on cytologic material. Since the advent of trastuzumab, the characterization of the molecular profile in metastatic disease has become increasingly important for targeted therapy selection.

CONCLUSION

CA 15-3 is a sensitive and specific marker for metastatic breast carcinomas in pleural effusions. It shows particular potential in the setting of metastatic breast carcinoma. Further study is needed to fully assess the diagnostic value of CA 15-3 in pleural effusions.

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