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Usefulness of CA 15-3 for breast or ovarian primary sites in metastatic adenocarcinoma of pleural fluid

Serpil OĞUZTÜZÜN[1], Murat KILIÇ[1], Nisa TANDOĞAN[1], Latif ÖZTÜRK[2]

[1]Department of Biology, Kırıkkale University, 71450 Yahşihan, Kırıkkale - TURKEY

[2]Department of Economics, Kırıkkale University, 71450 Yahşihan, Kırıkkale - TURKEY

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Abstract: This study was conducted to evaluate the diagnostic value of CA 15-3, mammaglobin, c-erbB-2, estrogen receptor (ER), progesterone receptor (PR), CK 20, and CA 125 for detecting metastatic breast and ovarian carcinoma in pleural fluids. The material under study consisted of 26 pleural effusions from invasive breast and ovarian cancer patients from the Hannover Cytopathology Institute. Expressions of these markers were studied using immunocytochemistry. All of the breast cancer cases studied showed a positive reaction with CA 15-3, while only 36% with mammaglobin, 21% with c-erbB-2, 36% with ER, and 7% with PR were positive. The sensitivities of CK 20, CA 15-3, and CA 125 as markers for metastatic ovarian carcinoma were 43%, 88%, and 55%, respectively. Our results indicate that CA 15-3 has a higher sensitivity than other tumor markers for diagnosing metastatic breast and ovarian carcinoma in pleural fluids.

Key words: Pleura, metastasis, breast cancer, ovarian cancer, CA 15-3, cytology

Plevra sıvısında metastatik meme ve yumurtalık adenokanserinin teşhisinde CA 15-3'ün kullanımı

Özet: Bu çalışmada, plevra sıvısında metastatik meme ve yumurtalık adenokanserinin teşhisinde CA 15-3, mammaglobin, c-erbB-2, estrogen reseptör (ER), progesteron reseptör (PR), CK 20 ve CA 125'i değerlendirilmektedir. Almanya Hannover Sitoloji Kliniği'nden 26 metastatik meme ve yumurtalık adenokanser hastalarından alınan plevra sıvısı örneklerinde tümör belirleyicilerinin ekspresyonları immünositokimya yöntemiyle çalışılmıştır. Meme kanser vakalarında, CA 15-3 % 100 pozitifken, sadece mammaglobin % 36, c-erbB-2 % 21, ER % 36, PR % 7 pozitiftir. CK 20, CA 15-3 ve CA 125'in metastatik yumurtalık adenokanserinde tümör belirleyici olarak duyarlılıkları sırasıyla % 43, % 88 ve % 55'dir. Sonuçlarımız CA 15-3'ün diğer belirleyicilere kıyasla plevra sıvısında metastatik meme ve yumurtalık adenokanserinin teşhisinde yüksek duyarlılıkta olduğunu göstermiştir.

Anahtar sözcükler: Plevra, metastaz, meme kanseri, yumurtalık kanseri, CA 15-3, sitoloji

Introduction

Malignant pleural fluids are considered to be uncommon clinical manifestations of adenocarcinoma (1-3). Elucidating the origin of these malignant neoplasms can pose a considerable diagnostic challenge to both clinicians and pathologists, which may often have therapeutic consequences for the patient. Immunocytochemical analysis has become a standard practice in the evaluation of nodal and soft tissue metastasis, and a number of highly effective

tissue-specific tumor markers are available. When used in specific panels, immunocytochemical studies can be extremely useful for the determination of tumor type, particularly in cases with an unknown primary site. These markers can also be used in the identification of primary tumors in patients with malignant effusions from pleural fluids.

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 (4, 5). CA 15-3 has also been studied as a serologic test for breast carcinoma in pleural fluid (6). There are few publications on the expression of CA 15-3 in ovarian cancer from pleural fluids. Immunocytochemical studies using a second-generation CA 15-3 antibody showed promising results in terms of detecting adenocarcinoma in body cavity effusions (7).

In this immunocytochemical study, we examined the sensitivity of CA 15-3 as a marker for metastatic breast and ovarian adenocarcinomas in cytologic material prepared from pleural fluid specimens. Furthermore, we compared CA 15-3 with 4 breast cancer markers, namely mammaglobin, c-erbB-2, estrogen receptor (ER), and progesterone receptor (PR), and with 2 ovarian cancer markers, CK 20 and CA 125.

Materials and methods

Cases

All cytology specimens used in this study were obtained from the cytological files of the Hannover Cytopathology Institute and reviewed 3-15 August 2009. The 26 pleural fluids were collected from 14 patients diagnosed with metastatic breast carcinoma and 12 patients diagnosed with metastatic ovarian carcinoma. The medical records were reviewed to verify that the cases were of breast and ovarian carcinoma. Slides that were previously stained using the routine May-Grünwald-Giemsa method were retrieved for each patient. The diagnosis of breast and ovarian carcinoma based on cytomorphology was confirmed. The sensitivities of CA 15-3, mammaglobin, c-erbB-2, ER, and PR in pleural fluid samples of the metastatic breast carcinomas and the sensitivities of CK 20, CA 15-3, and CA 125 for metastatic ovarian carcinomas were calculated.

Immunocytochemistry procedure

Cytological smears were prepared by the standard cytologic method. The previously used immunocytochemical staining procedure was followed (8). The slides were hydrated in decreasing ethanol solutions. Endogenous peroxidase was blocked with hydrogen peroxidase for 3 min. The slides were rinsed in water and then incubated with a biotin blocking system (Dako) prior to the application of the primary antibody. The antibodies used were as follows: Mammaglobin (1:100, clone Df-3, DAKO), CA 15-3 (1:100, clone Df-3, DAKO), c-erbB-2 (1:100, polyclonal, DAKO), ER (1:50, clone 1D5, DAKO), PR (1:50, polyclonal, DAKO), CK 20 (1:400, clone IT-Ks 20.8, DAKO), and CA 125 (1:100, clone M 11, DAKO). Monoclonal antibodies were incubated with the samples for 1 h. The slides were then rinsed in buffer and incubated for 25 min with the linking solution (LSAB+ kit, Dako; biotinylated antimouse, antirabbit, and antigoat). This was followed by a rinse in buffer and incubation with streptavidin peroxidase for 25 min. After being rinsed in buffer, the slides were submerged in 3-amino-9-ethylcarbazole (AEC) for 5 min. The slides were counterstained with Mayer's hematoxylin. Only cytoplasmic staining was regarded as a positive result. The intensity of staining was graded on the following scale: 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong). All material was blindly evaluated by 2 observers.

Statistical analysis

A statistical analysis was performed using the chi-squared test. A P-value of less than 0.05 was considered to be statistically significant. Statistical analyses were performed with SPSS.

Results and discussion

Immunoreactivity for CA 15-3, mammaglobin, c-erbB-2, ER, and PR was determined for neoplastic cells in a total of 14 cases of metastatic breast carcinoma from pleural fluids. Overall results are summarized in Table 1.

For all breast carcinomas studied, 14 (100%) of cases exhibited CA 15-3 immunostaining (Figure 1). The sensitivity of CA 15-3 for these malignant breast carcinomas was 100%.

Table 1. Staining patterns of malignant pleural effusions for CA 15-3, mammaglobin, c-erbB-2, ER, and PR in metastatic breast carcinoma.

Tumor markers	n	Negative n (%)	Positive n (%)	1+ (%)	2+ (%)	3+ (%)
CA 15-3	14	0 (0)	14 (100)	1 (7)	7 (50)	6 (43)
Mammaglobin	14	9 (64)	5 (36)	3 (22)	2 (14)	0 (0)
c-erbB-2	14	11 (79)	3 (21)	2 (14)	1 (7)	0 (0)
ER	14	9 (64)	5 (36)	3 (22)	1 (7)	1 (7)
PR	14	13 (83)	1 (7)	1 (7)	0 (0)	0 (0)

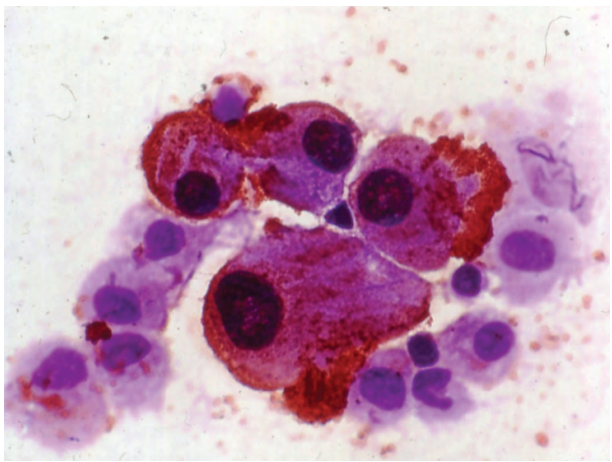

Figure 1. Immunocytochemistry of CA 15-3 showing moderate cytoplasmic and membranous staining in breast cancer cells (pleural fluid, ×600).

We also examined c-erbB-2 and mammaglobin reactivities in cytologic specimens derived from metastatic breast carcinomas and detected positivity for most neoplastic cells in 21% and 36% of cases, respectively.

Out of 14 cases of metastatic breast carcinoma, neoplastic cells in 5 cases (36%) exhibited nuclear reactivity for ER protein. Detection of PR was observed in 1 (7%) of 14 cases.

There was a significant difference between CA 15-3, mammaglobin, c-erbB-2, ER, and PR expressions in metastatic breast carcinoma ($P = 0.000 < 0.05$). The sensitivities of CA 15-3, mammaglobin, c-erbB-2, ER, and PR as markers for metastatic breast carcinoma were 100%, 36%, 21%, 36%, and 7%, respectively (Table 2).

Table 3 shows the staining patterns of metastatic ovarian carcinomas from pleural effusions for CK 20, CA 15-3, and CA 125. For all metastatic ovarian carcinomas studied, 7 (88%) of the cases exhibited CA 15-3, 6 (54%) of the cases exhibited CA 125, and 6 (42%) of the cases exhibited CK 20.

Table 2. Statistical values of metastatic breast carcinoma.

Tumor markers	Sensitivity = TP/(TP + FN) × 100
CA 15-3	100.00
c-erbB-2	21.43
Mammaglobin	35.71
ER	35.71
PR	7.14

Sensitivities were calculated using the following formula: (sensitivity = true positive/(true positive + false negative) × 100).

Table 3. Staining patterns of malignant pleural effusions for CK 20, CA 15-3, and CA 125 in metastatic ovarian carcinoma.

Tumor markers	n	Negative n (%)	Positive n (%)	1+ (%)	2+ (%)	3+ (%)
CK 20	14	8 (58)	6 (42)	3 (21)	0 (0)	3 (21)
CA 15-3	8	1 (12)	7 (88)	1 (12)	3 (38)	3 (38)
CA 125	11	5 (46)	6 (54)	0 (0)	3 (27)	3 (27)

There was a significant difference between CA 15-3, CK 20, and CA 125 expressions in metastatic ovarian carcinoma ($P = 0.028 < 0.05$). The statistical values are given in Table 4. The sensitivities of CK 20, CA 15-3, and CA 125 as markers for metastatic ovarian carcinoma were 43%, 88%, and 55% respectively. CA 15-3 had the highest sensitivity among the malignant ovarian carcinomas studied, with a sensitivity of 88% (Figure 2).

Table 4. Statistical values of metastatic ovarian carcinoma.

Tumor markers	Sensitivity = TP/(TP + FN) × 100
CK 20	42.86
CA 15-3	87.50
CA 125	54.54

Sensitivities were calculated using the following formula: (sensitivity = true positive/(true positive + false negative) × 100).

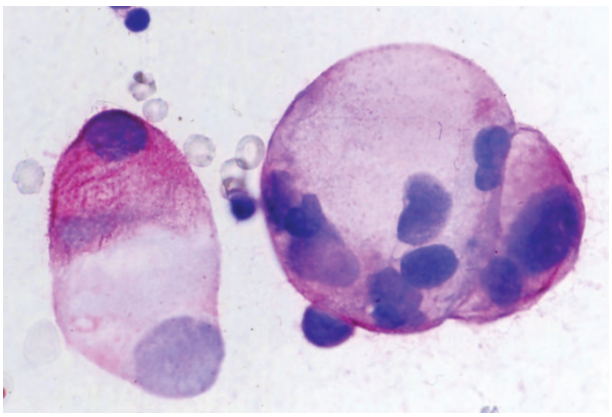

Figure 2. Immunocytochemistry of CA 15-3 showing moderate cytoplasmic staining in ovarian cancer cells (pleural fluid, ×600).

To identify the origin of a metastatic malignant neoplasm in pleural effusions based on morphology alone is a diagnostic challenge even for the most experienced cytopathologist. Immunocytochemistry (ICC) can be helpful and is often used to determine the origin of a primary tumor. For cytology specimens, the task can be even more challenging. The specimen is usually scarce and lacks good architectural features, thus requiring careful selection of ICC studies when at all possible. A cytology specimen, however, is usually the first diagnostic modality for a clinical inquiry into a potential metastatic carcinoma. Determining the origin of a primary tumor will undoubtedly provide invaluable information for further treatment of patients. To better distinguish mammary and ovarian carcinomas from other carcinomas in pleural fluid effusion cytology, more specific markers would be useful.

Since the early 1990s, antibodies against CA 15-3 have been developed as possible serum markers of occult or recurrent breast carcinoma (4,5). Similarly, CA 15-3 has been examined as a serologic test for breast carcinoma in pleural fluid (6). Histologic studies have centered on the specificity of CA 15-3 for breast carcinoma in metastatic carcinomas (9) or its sensitivity for detecting micrometastases in axillary lymph nodes (10). These reports indicate that CA 15-3 is sensitive, but not specific, for breast carcinoma. Oğuztüzün et al. (11) examined CA 15-3 (87% sensitivity) as an immunochemical stain to detect metastatic breast carcinoma in pleural fluid, although Szpak et al. used their own clone of the DF3 epitope of CA 15-3 for this purpose (12). Given its high sensitivity for carcinomas, we evaluated a second generation CA 15-3 for its utility in detecting breast carcinoma in pleura effusions.

In this study, we confirmed that CA 15-3 is a sensitive tumor marker for breast and ovarian carcinomas, with a sensitivity of 100% and 87.5%, respectively. In comparison, Huang et al. (13) and Geraghty et al. (14) reported 91% and 88% sensitivity of CA 15-3, respectively. Fehm et al. (15) found that the positivity rate of CA 15-3 serum levels was 51% in metastatic breast cancer. Zimmerman et al. (7) reported that CA 15-3 was immunostained with high specificity and sensitivity for breast carcinoma cases (97%) in cell block material from effusions.

We combined CA 15-3 with 4 breast cancer markers in this study. We found the sensitivities of CA 15-3, mammaglobin, c-erbB-2, ER, and PR as markers for metastatic breast carcinoma to be 100%, 36%, 21%, 36%, and 7% respectively. CA 15-3 had the highest sensitivity of the malignant breast carcinomas studied, with a sensitivity of 100%.

CA 15-3 is elevated in approximately 70% of epithelial ovarian cancer patients (16,17),

predominantly in those with advanced disease. Jakob et al. (18) found that 26% of the patients had elevated serum CA 15-3 values. We found that 7 (88%) metastatic ovarian carcinomas were positive for CA 15-3. Moreover, 42% and 54% of the patients had elevated CK 20 and CA 125 expressions. The sensitivity values of CK 20, CA 15-3, and CA 125 for the detection of ovarian cancer were found to be 42.86%, 87.50%, and 54.54%, respectively.

In conclusion, CA 15-3 is a sensitive marker in diagnosing metastatic breast and ovarian carcinomas in cytologic specimens. Further study will be needed to fully assess the diagnostic value of CA 15-3 in pleural effusions.

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Corresponding author:

Serpil OĞUZTÜZÜN

Department of Biology,

Kırıkkale University,

71450 Yahşihan, Kırıkkale - TURKEY

E-mail: soguztuzun@yahoo.com

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