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Expression of p53 and Ki-67 immunoreactivity in breast cancer patients

ABSTRACT

The aim of this study is to investigate the association between p53, Ki-67 expression and hormone receptor status as well as clinicopathological parameters in breast cancer patients. The study group consists of 146 breast cancer patients (stage I to stage III, according to the postoperative TNM classification of UICC, 1997 guideline system) who underwent radical mastectomy with axillary lymphadenectomy between January 1998 and March 2000. The expression of p53, Ki-67, estrogen receptor (ER) and progesterone receptor (PgR) has been evaluated by using standard immunoperoxidase technique. The scoring system has been performed for determination the results of p53 and Ki-67 immunoreactivity. The semiquantitative ER-ICA and PR-ICA scoring system has been used for assessment of staining for ER and PgR. The results from p53 and Ki-67 expression were correlated to hormone receptor status, and to clinicopathological parameters. Statistical significance was determined with χ^2 test. Strong correlation was found between the values of p53 and Ki-67 ($p < 0.00001$), p53 and ER ($p = 0.0004$), Ki-67 and ER ($p = 0.0006$) as well as ER and PgR ($p < 0.0001$). p53 and Ki-67 expressed additional correlation to the age of the patients ($p = 0.01$). No correlation was found between p53, Ki-67, ER as well as PgR and lymph node involvement or the stage of the disease. The preliminary results of our study suggest that determination of p53 and Ki-67 expression associated to hormone receptor status and some clinicopathological parameters could be helpful in standardizing the protocols for further treatment of breast cancer patients. However, additional investigations with long term follow-up of the patients are needed to clarify the prognostic significance of p53 and Ki-67 immunoreactivity.

KEYWORDS: Breast Neoplasms; Ki-67 Antigen; Protein p53; Receptors, Eestrogen; Receptors, Progesterone

INTRODUCTION

Breast cancer is a common disease with a variable clinical course. Numerous prognostic factors have been proposed in different studies in order to help the selection of patients who should receive additional therapy. Proliferative rate determined by Ki-67 antibody expression has been frequently reported as an important prognostic marker in breast cancer patients (1). The prognostic value of p53 immunohistochemical expression in breast cancer is still controversial, although many reports have shown that it may be an

indicator of poor prognosis (2,3). The aim of this study is to investigate the association between p53, Ki-67 expression and hormone receptor status as well as clinicopathological parameters in breast cancer patients.

PATIENTS, MATERIALS AND METHODS

The study group consists of 146 breast cancer patients (stage I to stage III, according to the postoperative TNM classification of UICC, 1997 guideline system) who underwent radical mastectomy with axillary lymphadenectomy between January 1998 and March 2000. Routinely-processed, formalin-fixed, paraffin-embedded samples have been examined and treated with standard streptavidin-biotin-peroxidase complex technique for assessment of p53 and Ki-67 immunoreactivity. Standard peroxidase-antiperoxidase (PAP) method has been used for immunohistochemical localization of estrogen receptor (ER) and progesterone receptor (PgR). p53 and Ki-67 expression was determined by counting the positively stained nuclei in at least 100 cells of primary tumor tissue samples. Tumors were scored as 0 (fewer than 10% positive cells in 10X HPF), 1 (11-20%), 2 (21-50%) or 3 (51-100%). Tumors were classified as p53 positive if at least 10% of tumor cells exhibited distinct nuclear immunostaining (cut-off point, score 1-3), (5,6) and Ki-67 positive if at least 20% of tumor cells were stained intensively (cut-off point, score 2-3) (1,6). ER and PgR were scored in a semiquantitative fashion (ER-ICA and PR-ICA), incorporating the intensity and the percent of positively stained cells designated as HSCORE (4). The results from p53 and Ki-67 expression were correlated to hormone receptor status and clinicopathological parameters (age, tumor size, histopathologic grade, lymph node involvement, histologic type of the tumor and the stage of the disease). Statistical significance was determined with χ^2 test.

RESULTS

The mean age of the patients was 46.93 years (range, 29-85). Half of the patients were in the age group under 50 years and the other half in the age group over 50 years old. 16 (11%) patients were in stage I, 95 (65%) in stage II, and 35 (24%) in stage III of the disease. Ductal carcinoma was diagnosed in 91 (62%) and lobular carcinoma was found in 55 (38%) patients. Lymph nodes involvement was present in 90 (62%) patients. p53 was positive in 58 (39.7%) breast carcinomas, and strongly correlated to tumor size ($p = 0.0002$) and to age of the patients ($p = 0.01$), (Table 1). Ki-67 was positive in 46 (31.5%) breast carcinomas and strongly correlated to p53 expression ($p < 0.00001$), tumor size ($p = 0.001$), histological type of tumor ($p = 0.007$) and to age of the patients ($p = 0.01$). The ER was positive in 93 (63.7%) and PgR was positive in 72 (49.3%) breast carcinomas. Expression of ER was in strong correlation to p53 ($p = 0.0004$), Ki-67 ($p = 0.0006$), PgR ($p < 0.00001$), as well as to histologic grade ($p = 0.019$) and histologic type of the tumor (ductal vs. lobular carcinoma, $p = 0.00002$), while PgR was strongly correlated only to histologic type of the tumor ($p = 0.0007$).

No correlation was found between p53, Ki-67, ER as well as PgR and lymph node involvement or the stage of the disease.

DISCUSSION

The preliminary results of our investigation of the prognostic significance of p53 and Ki-67 expression in breast carcinomas are consistent to the observations of other authors reported in similar studies (1-3). We have determined an association of p53 and Ki-67 expression to hormone receptor status as well as to tumor size, histopathologic grade and the age of the patients, that is in concordance to the results of other studies (6,7). Nevertheless, the lack of association between p53 or Ki-67 and lymph node involvement or the stage of the disease was reported only in few other studies (7). However, additional investigations with long term follow-up of the breast cancer patients are needed to clarify the prognostic significance of p53 and Ki-67 immunoreac-

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The manuscript was received: 15. 02. 2000.

Accepted for publication: 14. 04. 2000.

Table 1. Correlation between p53, Ki-67 and hormone receptor status

| | p53 | | Ki-67 | | ER | | PgR | |
|-------|-----|-----------|----------|----|-----------|----|-----|----|
| | + | - | + | - | + | - | + | - |
| p53 | + | | 32 | 26 | 27 | 31 | 26 | 32 |
| | - | | 14 | 74 | 66 | 22 | 46 | 42 |
| Ki-67 | + | | | | 22 | 24 | 21 | 25 |
| | - | p<0.00001 | | | 71 | 29 | 51 | 49 |
| ER | + | | | | | | 66 | 27 |
| | - | p=0.0004 | p=0.0006 | | | | 6 | 47 |
| PgR | + | | | | | | | |
| | - | NS | NS | | p<0.00001 | | | |
| | | 58 | 88 | 46 | 100 | 93 | 53 | 72 |
| | | | | | | | | 74 |

tivity.

CONCLUSION

The preliminary results of our study suggest that determination of p53 and Ki-67 expression associated to hormone receptor status and some clinico-pathological parameters could be helpful in standardizing the protocols for further treatment of breast cancer patients. However, additional investigations with long term follow-up of the patients are needed to clarify the prognostic significance of p53 and Ki-67 immunoreactivity.

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Immunocytochemical staining of cells in pleural effusions from patients with breast carcinomas

ABSTRACT

In order to assess the value of immunocytochemical staining as a method of discriminating between reactive mesothelial cells and malignant epithelial cells in pleural fluids, we have studied the reactions of a few markers on cells harvested from pleural effusions due to breast carcinomas. A total of 20 specimens of malignant pleural effusions formed the basis of this study. The antibodies used were raised against epithelial membrane antigen (EMA), carcino-embryonic antigen (CEA) and cytokeratin (CAM 52). Malignant epithelial cells in all cases demonstrated a strong peripheral membrane-type reaction for EMA and CEA, while mesothelial cells were negative. In only 40% neoplastic cells exhibited moderate or strong diffuse cytoplasmic reaction with CAM 52, while in all cases mesothelial cells demonstrated strong positive reaction for cytokeratin. EMA and CEA, in contrast to CAM 52, are specific markers for differentiating carcinoma cells from mesothelial cells in pleural effusions due to breast carcinoma.

KEYWORDS: Breast Neoplasms; Pleural Effusion, Malignant; Immunohistochemistr

INTRODUCTION

The diagnosis of seorus effusions remains one of the most difficult tasks in diagnostic cytology. The main problem is the differentiation of neoplastic cells from reactive mesothelial cells. The latter, which occur either singly or arranged in small clusters, have an epithelial appearance and often show large and hyperchromatic nuclei, related to their hyperplastic nature. Neoplastic cells, especially breast cancer cells, can look benign and do not always show highly atypical nuclei. This study was carried out in order to determine the usefulness of immunocytochemical analysis of pleural effusions due to breast carcinomas in discriminating between reactive mesothelial and malignant epithelial cells.

MATERIALS AND METHODS

A total of 20 specimens of malignant pleural effusions, which had been sent to the Institute of Pathology in Niš, formed the basis of this study. All samples in this study came from patients who had been documented as having primary breast carcinoma. After cytocentrifugation of the fluids, slides were fixed in acetone for 10 min. Immunocytochemical staining was per-

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The manuscript was received: 13. 03. 2000.

Accepted for publication: 17. 04. 2000.