Cellular biomarkers may predict tumor cell behavior in breast cancer. One of the most paradoxical biomarker in breast cancer is cathepsin D. A number of studies has implicated cathepsin D in promoting tumor growth as a proteolitic enzyme (1). Others reported that the expression of cathepsin D was regulated by estrogen in breast carcinomas, with the functional integrity of the estrogen response pathway (2). Accordingly, the positive association between cathepsin D and steroid hormone receptor status is confusing, suggesting that they should provide the opposite prognostic as well as predictive information.

Our recent study (3) showed statistically significant direct correlation between cathepsin D expression and estrogen (ER) and progesterone receptor (PR) status. Baseline i.e. nonestrogen-regulated cathepsin D expression (<28.0 pmol/mg) was found in patients with ER-negative, PR-negative status and node-negative status, or carcinomas less than 2 cm.

In the current study, our aim was to assess cathepsin D status, estrogen-regulated vs. nonestrogen-regulated in relation to ER and PR status, and classical clinicopathological features of breast carcinomas.

PATIENTS AND METHODS

Patients. This study was performed with a group of 152 consecutive patients with primary operable breast cancer. None of the patients had received preoperative radiotherapy, chemotherapy or endocrine therapy. The median age of patients was 56 years (range 24 to 78 years). A patient was considered to be premenopausal when the menstrual cycles still persisted or postmenopausal if menstruation had ceased at least six months before. The patients were categorized in younger age group (up to 45), middle-aged (between 45 and 59) and older age (60 or more).

Histological features. Histological specimens were reviewed and then classified according to histology type (4), TN-stages, i.e. tumor size and the presence or absence of regional lymph node metastases (5) and histology grade (6). According to histology type two groups were obtained: invasive ductal (IDC, n = 85) and invasive lobular carcinomas (ILC, n = 59). The remaining carcinomas - rare, mixed or unclassifiable - were not considered in histology type analysis. Further, out of these patients, 85 were found to have regional lymph node metastases, 53 were free of lymph node metastases and 14 were undefined. According to the size of tumors, 67 were smaller than 2 cm (pT1), 85 were equal or greater than 2 cm (pT2). Carcinomas were graded as follows: grade I (n = 22), grade II (n = 102) and grade III (n = 27). A single carcinoma was not graded.

Steroid hormone receptor assay. Breast carcinoma samples were pulverized and homogenized with phosphate buffer. Low-salt extract, cytosol, was prepared by centrifugation at 100,000 g for 1h at 4°C. ER and PR were determined by analysis standardised under recommendation of EORTC (7). Quality assessment was confirmed (8) as recently recommended (9). The cut-off for the classification of positive receptor status was 10 fmol/mg for ER and 20 fmol/mg for PR. Cytosol protein concentration was determined by the Lowry method (10).

Cathepsin D assay. Cathepsin D in breast carcinoma cytosol was determined by a solid-phase immunoradiometric assay IRMA (ELSA - CathD, Cis Biointernational, Gif-sur-Yvette, France), based on two monoclonal antibodies D7E3 and M1G8, which detect the total amount of cathepsin D (52-kd, 48-kd and 34-kd). The first antibody (D7E3) was coated on the solid phase, acting as a "catcher", while the latter (M1G8), radiolabeled with 125I, was used as the "tracer". In this way, by forming a sandwich the cathepsin D molecules (anti-gen) are identified in the cytosol sample of breast carcinoma. The procedure was carried out exactly as described by the manufacturer. The cut-off for the classification of positive cathepsin D status was 28.0 pmol/mg as estrogen-regulated cathepsin D expression (3).
Statistical methods. Nonparametric statistical methods were applied to analyze the data - the Chi-square test to assess the difference in frequencies of events between subgroups, and the Spearman rank correlation test to assess the correlation between the proportion of carcinomas, classified as cathepsin D-positive, and the cut-off values used. The limit of significance was set at p<0.05.

RESULTS

Cathepsin D IRMA validation

Figure 1 shows the relative frequency distribution of cathepsin D values found in 152 breast carcinoma samples. In the majority of breast carcinomas cathepsin D was detectable, although at very low quantitative levels and in many carcinomas the values varied, but all samples contained cytosol cathepsin D protein (range 15.4-261.6; median 43.2 pmol/mg). Using IRMA to determine cathepsin D expression in this study and in previous studies (11-19), an indirect relationship was found between the percentage of cathepsin D-positive cases and the cut-off value used (Figure 2).

ER status and PR status, and cathepsin D status

Using the biochemical method, we found 96 carcinomas (63%) with ER-positive status and 70 carcinomas (46%) with PR-positive status. One hundred and fourteen (75%) carcinomas contained estrogen-regulated cathepsin D expression, i.e. cathepsin D-positive status. Table 1 displays relationships between these findings. Fifty-six (53%) of the 105 cathepsin D-positive carcinomas displayed PR positive status. A Chi-square analysis indicates a high likelihood of an association between the cathepsin D status and PR status, p=0.007. This association might be more clearly defined within the segregation of the carcinomas on the basis of their ER status. Among the patients aged between 45 and 59 years and over 59 years, 76% (42/55) and 89% (54/65) were cathepsin D-positive, in contrast to 56% (18/32) of those patients younger than 45 years. A homogenous distribution of the cathepsin D status with changing of menopausal status was found, p=0.1 (Table 2).

Cathepsin D status and clinicopathological features

Cathepsin D status was correlated to clinical features, i.e. breast carcinoma-host features and breast carcinoma features. Significant association between cathepsin D positivity and patient age was noted, p=0.02. Among the patients aged between 45 and 59 years and over 59 years, 76% (42/55) and 89% (54/65) were cathepsin D-positive, in contrast to 56% (18/32) of those patients younger than 45 years. A homogenous distribution of the cathepsin D status with changing of menopausal status was found, p=0.1 (Table 2).

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positive in lymph node-negative subgroup. Of the grade II, 76% (78/102) and grade III, 85% (23/27) were cathepsin D-positive compared to 55% (12/22) cathepsin D-positive in grade I subgroup (Table 3).

DISCUSSION

The expression of cathepsin D may be determined by immunohistochemical or immunoradiometric methods. The immunohistochemical method indicates the special localization of cathepsin D, thus permitting the assessment of the intratumoral heterogeneity, but does not allow its precise quantification. On the contrary, with the immunoradiometric method, which is performed on the same cytosol samples prepared routinely for steroid hormone receptor determination, it is possible to quantify cathepsin D expression. A brief review of the literature indicates that the obtained distribution of cathepsin D values found in our study (Figure 1) is in accordance with previously reported results (11,12,14,17). Also, in our, as well as in those studies using immunoradiometric methods (11-19), there is an indirect relationship between percentage of cathepsin D-positive cases and the cut-off value used (Figure 2). Therefore, the differences in "positivity" of cathepsin D expression simply reflect the varying cut-off values used.

The simultaneous determination of ER and PR status and cathepsin D status, defined as estrogen-regulated vs. nonestrogen-regulated cathepsin D expression, suggested an additional functional heterogeneity in ER-positive and in ER-negative breast carcinomas.

According to the literature data on the association of cathepsin D and clinicopathological features of breast cancer it is clear that no consensus has been reached so far (13,17,20,21).

Regarding the age and menopausal status as the most important classical prognostic/predictive breast carcinoma-host features, a general agreement on a lack of association between age and cathepsin D status is evident (17,20,21). Our present data show that a statistically significant higher frequency of cathepsin D-positive status exists in middle-aged and/or older patients, than in younger patients. Further, our data showed a lack of association between menopausal status and cathepsin D status, which is in accordance with the results of Gion et al. (17), but at variance with the results of Thorpe et al. (20).

Regarding the most important classical prognostic/predictive features of breast cancer - lymph node status and tumor size - there is a general agreement on an existence of association in the former and the lack of association in the latter case. We also found significantly higher frequency of cathepsin D-positive status in node-positive, than in node-negative carcinomas, and no association between tumor size and cathepsin D status. Further, we found significantly higher frequency of cathepsin D-positive status in histological grade II and III, than in grade I carcinomas, which is in accordance with some reports (17, 20) but contradictory to others (13,21). The lack of association between histologic type of breast carcinoma and cathepsin D status, found in our study, is in agreement with the study by Gion et al. (17).

CONCLUSION

Classification of tumors according to cathepsin D status within ER and PR status should be regularly applied in considering the association between cathepsin D status and clinicopathological features of breast cancers.

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REFERENCES