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Tissue Polypeptide Antigen in Breast Cancer Cytosol: a New Effective Prognostic Indicator

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Since 1982 we have been evaluating oestrogen and progesterone receptors (PgR), cathepsin D and the cytosolic levels of the tumour marker, tissue polypeptide antigen (TPA), in 257 patients radically resected for breast cancer (follow-up 24–81 months). TPA was measured by an immunoradiometric assay previously validated for cytosol. No significant associations were found between cytosolic TPA and age, tumour size, lymph-node status, receptor status and cathepsin D. TPA+ cases showed a significantly longer disease-free survival (DFS) and overall survival (OS) than TPA−patients (log-rank $P < 0.0001$). The prognostic value of cytosolic TPA was also demonstrated after stratification by nodal status, PgR and cathepsin D. The prognostic value of TPA was independent of the other prognostic indicators, being the most powerful among the evaluated indices (Cox multivariate analysis: χ^2 15.5 for DFS, 11.4 for OS). We conclude that cytosolic TPA is a powerful additional prognostic factor in primary breast cancer. Its prognostic role should therefore be extensively evaluated.
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INTRODUCTION

IN PATIENTS with primary breast cancer, tumour marker positivity rate is low since their serum level is related to tumour spread [1, 2] and their possible prognostic role is therefore dependent upon the tumour mass [2]. Tumour markers have been studied at tissue levels in order to obtain information more closely related to the tumour phenotype. However, the prognostic role of tumour markers in the tissue has so far been the object of anecdotical studies, whose results lack clinical relevance [3, 4].

Since 1982 we have been evaluating several markers in breast cancer cytosol in order to characterise the phenotypic pattern of the tumour [5]. In a preliminary study on 108 cases we identified a highly significant relationship between tissue polypeptide antigen (TPA) in the cytosol and the prognosis of the disease [6]. TPA is a tumour marker identified by Björklund and Björklund in 1957, closely related to the keratin family [7], which is frequently positive in patients with breast cancer [8, 9]. In our preliminary study, the prognostic value of TPA was independent of axillary lymph-node status, oestrogen (ER) and progesterone receptor (PgR), clinical stage and tumour size [6]. The aim of the present investigation was to verify the prognostic value of TPA in a wider patient series.

MATERIALS AND METHODS

The study was carried out between January 1982 and December 1988. Clinical data were evaluated as of 31 December 1990 at which time the follow-up time ranged from 24 to 81 months. 257 patients from three institutions entered the study.

Inclusion criteria were as follows: (1) under 75 years of age; (2) no evidence of distant metastasis using standard staging procedures (clinical examination, blood test, bone scan, liver sonography); (3) no previous or concomitant malignancies in different organs; (4) no clinical or instrumental (liver enzymes, liver sonography) evidence of liver disease; (5) no preoperatory chemotherapy or radiotherapy. Clinical stage was assessed according to UICC criteria. Histological typing was carried out following the WHO recommendations (85.7% invasive ductal carcinoma, 4.9% invasive lobular carcinoma, 3.0% medullary carcinoma and 5.1% other types). Non-invasive carcinomas (intraductal and lobular *in situ*, 1.1%) were excluded since tissue samples were not adequate for biochemical assay. Tumour grade was assessed according to the method of Bloom and Richardson [10].

All patients were primarily treated with surgery (Patey mastectomy or quadrantectomy, axillary dissection and radiotherapy QUART). Patients without axillary metastasis had no further treatment. In the three institutions, node positive cases were treated with adjuvant chemotherapy (six courses cyclophosphamide/methotrexate/5-fluorouracil, CMF) if premenopausal or tamoxifen (30 mg daily for 3 years) if postmenopausal.

Tissue samples were collected fresh from the operatory theatre and stored in liquid nitrogen until the assay. Supercooled tissue was powdered and homogenised, and high-speed cytosol was obtained using a method previously described [5]. TPA was measured in the cytosol using a commercially available immunoradiometric assay (IRMA) kit which uses polyclonal antibodies (Sangtec Medical, Bromma, Sweden); the validation as well as the performance characteristics of the method for the assay of cytosol had been previously reported [5, 6].

ER and PgR were assayed using a radioligand binding assay set up according to the recommendations of the European Organisation for the Research and Treatment of Cancer (EORTC) [11]; a conventional +/− cut-off point of 10 fmol/mg of cytosol protein was used for both ER and PgR.

Cathepsin D was assayed with commercially available IRMA (ELSA cathepsin D, CIS Diagnostici, VC, Italy). Both steroid receptor and cathepsin D assays were monitored using intralaboratory as well as interlaboratory quality assurance programmes. Total protein concentration in the cytosol was meas-

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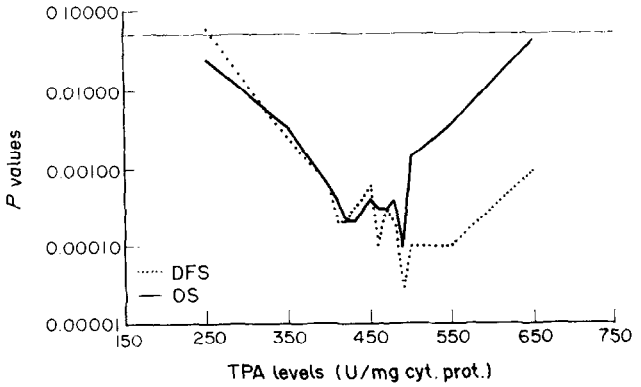

Fig. 1. Evaluation of the best cut-off value of cytosolic TPA for prediction of DFS (dotted line) and OS (solid line) (each cut-off value was plotted against its *P* value).

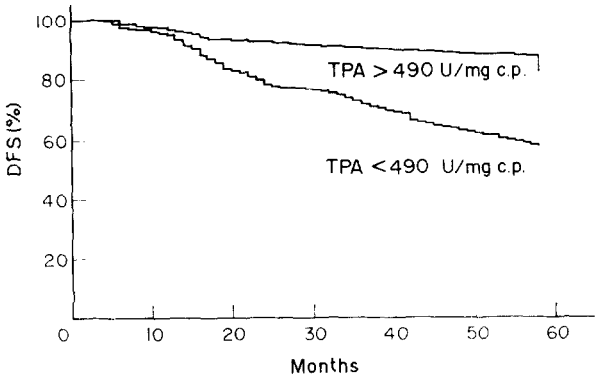

Fig. 2. Proportional hazard disease-free survival according to TPA (covariates in the model: tumour size, nodal status, cathepsin D, ER, PgR).

ured with the colorimetric Bradford's method (BIO-RAD, Anheim, California, USA). Results of TPA, ER, PgR and cathepsin D were expressed per milligram of cytosol protein.

Correlations were performed by the use of simple correlation and Mann–Whitney U test.

The disease-free survival (DFS) and the overall survival (OS) were considered as the period from mastectomy until the date of relapse or death. DFS and OS were calculated by the Kaplan–Meier product limit method. Analyses of DFS and OS were performed using the logrank univariate [12] and the Cox's multivariate methods, respectively [13]. All computations were carried out using the BMDP statistical analysis software.

RESULTS

Association between cytosol TPA and other prognostic parameters

TPA levels in breast cancer cytosol ranged between 3 and 6951 U/mg cytosol protein (median 459, interquartile range 232–742). Cytosolic TPA was not significantly different in specimens from patients with node-negative and node-positive breast cancer. No correlation was found between cytosolic TPA concentration and patient's age at diagnosis, tumour size, the number of positive lymph nodes, tumour grade, ER, PgR and cathepsin D. TPA cytosol levels were significantly higher in ER+PgR+ than in ER−PgR− cases ($P < 0.0001$) as it had been shown in a previously published study of our group [5].

Choice of the best positive/negative cut-off point

In a preliminary study we used the 95th percentile value of TPA levels found in cytosols from 62 normal breast tissue samples as +/− cut-off point [6]. The wider patient series evaluable in the present investigation allowed for the determination of the cut-off point according to the Tandon's graphic method [14]; that is, several TPA values are plotted against the *P* value of the differences of percentage of relapse and death between TPA+ and TPA− cases. As it is shown in Fig. 1, the best cut-off value for the prediction of probability of both relapse and death was 490 U/mg cytosol protein which was therefore used to categorise patients as TPA+ or TPA− (cases are TPA+ 122, TPA− 135). Note that both the graphic Tandon's method and the 95th percentile technique used in the preliminary study [6] led to comparable prognostic information when tested in the same patient series (data not shown).

Univariate analysis

The association of several possible prognostic parameters with disease-free and overall survival was first evaluated. The patient characteristics included in the analysis were: patients age and menopausal status at diagnosis, type of treatment of primary tumour (QUART, Patey mastectomy), type of adjuvant therapy (no therapy, CMF, tamoxifen), axillary status, ER, PgR, size of primary tumour, cytosolic TPA and cathepsin D. Tumour grade was not included since G1+G2/G3 ratio was significantly different in the three institutions. Age, menopausal status, type of surgery and adjuvant therapies did not significantly affect the disease outcome in the present patient series.

Parameters which showed a significant relationship with prognosis were axillary nodal status ($P < 0.0001$), tumour size ($P < 0.05$), ER ($P < 0.05$), PgR ($P < 0.02$), cathepsin D ($P < 0.01$, for OS only) and cytosol TPA ($P < 0.001$). TPA+ cases showed a significantly longer disease-free survival and overall survival than TPA−.

When cases were stratified according to lymph-node status, TPA+ cases still showed a lower probability of relapse ($P < 0.0001$) and a longer survival ($P = 0.0007$) than TPA−. The prognostic information given by TPA is still highly significant after stratification of patients according to PgR ($P = 0.0053$) and cathepsin D ($P < 0.0001$).

Multivariate analysis

The relation between TPA and other prognostic parameters was further evaluated using the Cox multivariate analysis, in which were included only parameters which showed a relation to prognosis with a *P* value lower than 0.05 in the univariate approach (Figs 1 and 2).

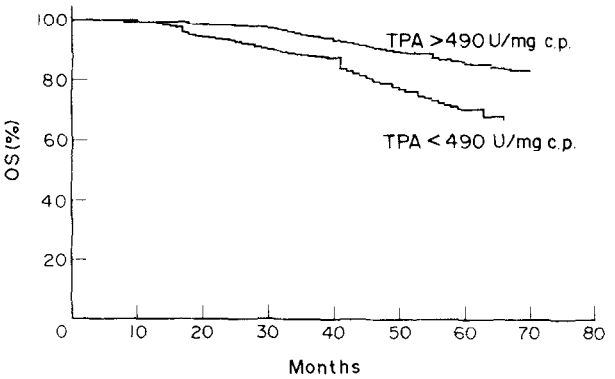

Fig. 3. Proportional hazard overall survival according to TPA (covariates in the model: tumour size, nodal status, cathepsin D, ER, PgR).

Table 1. Results of Cox's stepwise proportional hazard model: DFS analysis

Covariate	β	Relative risk*	P	Global χ^2
Cytosolic TPA	1.23	3.42 (1.86–6.30)	0.0001	15.5
Lymph-node status	1.13	3.11 (1.82–5.26)	0.0001	31.1
Cathepsin D	0.93	2.54 (1.40–4.57)	0.001	39.9
PgR	0.56	1.74 (1.03–2.97)	0.038	43.0

β is the estimated regression coefficient of the hazard function.
* The 95% confidence limits are given for the relative risk.

Tables 1 and 2 report the results for both DFS and OS. Axillary nodal status and cytosolic TPA showed an independent prognostic value both in OS and DFS analysis. Cathepsin D was prognostic only for DFS, whereas PgR prognostic value was highly significant for OS and weakly significant for DFS. TPA is a very powerful prognostic indicator for both DFS and OS, as it is shown from the global χ^2 value (15.5 for DFS, 11.4 for OS).

Given PgR, ER was no longer a significant prognostic indicator in the multivariate analysis, probably due to the close association between ER and PgR. Concerning the lack of prognostic role of tumour size in the multivariate analysis, it should be noted that in the present series the tumour size ranged between 1 and 3 cm in 87% of cases. According to the result of the study of Carter *et al.* on 24740 cases [15], prognosis should not be expected to be significantly related to variations in tumour size when variations occur in this range.

DISCUSSION

The choice of patients to treat with adjuvant therapies is an important goal in breast cancer management. The most powerful prognostic indicator is the axillary lymph-nodal status. However, 30% of node-negative cases will have a recurrence within 10 years. Adjuvant therapies, which significantly reduce the risk of recurrence in node-positive patients, have been therefore proposed also for node-negative patients [16]. On the other hand, adjuvant chemotherapy is toxic and the strategy of treating 70% of node-negative patients which will have no recurrence, in order to benefit the 10% of the remaining 30% of cases without axillary metastasis, could be questionable [17]. Therefore, it should be critical to identify criteria to select node-negative patients with higher risk of recurrence [18].

Table 2. Results of Cox's stepwise proportional hazard model: OS analysis

Covariate	β	Relative risk*	P	Global χ^2
Lymph-node status	1.63	5.11 (2.29–11.3)	0.0001	14.8
Cytosolic TPA	1.28	3.60 (1.46–8.85)	0.0004	26.2
PgR	1.19	3.30 (1.51–7.17)	0.002	34.9

β is the estimated regression coefficient of the hazard function.
* The 95% confidence limits are given for the relative risk.

Several parameters have been proposed as effective prognostic indicators such as tumour size [15], nuclear grade [19], ER, PgR [20], DNA ploidy [21], EGF receptor [22], oncogene expression [23], ^{3}H thymidine labelling index [24] and cathepsin D [25]. Concerning tumour markers, the prognostic role of epithelial antigens of the mucin family have been evaluated through immunohistochemical methods. The expression of epitopes recognised by the monoclonal antibodies NCRC-11 and HMGF-1, which reflect a differentiation event, was shown to indicate a favourable prognosis [26, 27]. So far, among the biochemical parameters, only ER/PgR status and cathepsin D are measured by fully standardised methods and are indeed available for routine clinical use. The other parameters are affected by methodological variability or interpretation pitfalls [18].

When studying tumour markers in cytosol of breast cancer we noted that high TPA levels were related to a better prognosis [6]. Two possible interpretations were hypothesised to explain this unexpected relationship (high tumour marker, better prognosis): (1) higher cytosolic TPA levels are found in more differentiated tumours, as it is suggested by the direct relationship between TPA cytosol levels and ER/PgR positivity [5]; (2) more aggressive tumours might secrete TPA very quickly showing therefore lower cytosolic TPA levels. The latter interpretation is in accordance with the reported findings that higher TPA serum levels indicate a less favourable prognosis in breast cancer [9, 28].

Apart from any possible interpretation, cytosolic TPA seems to be a powerful prognostic indicator, being in the present patient series the most important variable in both node-negative and node-positive cases. Moreover, it is independent from and more powerful than PgR and cathepsin D. Although the present study has been carried out retrospectively, the patient series evaluated seems not to suffer a selection bias as has been demonstrated by data on the relationship between prognosis and lymph-nodal status, receptor status and cathepsin D which are in agreement with those published by other groups. Considering that TPA assay is simple, relatively low-cost, accurate and reproducible, we conclude that cytosolic TPA should be extensively evaluated in prospective studies.

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Tumour Infiltrating Lymphocytes as an Independent Prognostic Factor in Transitional Cell Bladder Cancer

P.K. Lipponen, M.J. Eskelinen, K. Jauhiainen, E. Harju and R. Terho

The prognostic value of tumour infiltrating lymphocytes (TIL) was assessed in a cohort of 514 patients with a transitional cell bladder cancer (TCC) during a follow up period of over 9 years. The density of TIL were positively correlated to WHO grade ($P < 0.0001$), non-papillary growth architecture ($P < 0.0001$), morphometric nuclear factors ($P < 0.007$) and volume corrected mitotic index (M/V index) ($P < 0.0001$). Dense TIL predicted progression in Ta–T1 tumours ($P < 0.0006$) whereas in a multivariate analysis they had no independent predictive value. Dense TIL were related to short recurrence-free survival in Ta–T1 tumours in a univariate analysis ($P = 0.06$) as well as in a multivariate analysis ($P = 0.005$). Dense TIL predicted unfavourable prognosis in the entire cohort ($P = 0.0316$) and in papillary tumours ($P = 0.062$) whereas in nodular tumours TIL were a sign of good prognosis ($P = 0.0141$). Also in T3–T4 tumours TIL were related to less aggressive behaviour of TCC ($P = 0.0259$). In a multivariate analysis including clinical stage (T-category), WHO grade, papillary status, six morphometric nuclear factors and M/V index dense TIL were a highly significant indicator of a favourable prognosis ($P = 0.007$). Particularly TIL categorised rapidly proliferating TCC into prognostic groups ($P = 0.001$). The results show that TIL are a sign of efficient host defence mechanisms in TCC and TIL predict a favourable prognosis in invasive TCC.
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INTRODUCTION

TODAY, SEVERAL significant prognostic factors related to tumour size, tumour cells and to their special characteristics are known in transitional cell bladder cancer (TCC) [1–5]. Recent analyses, however, indicate that tumour host interactions have a significant role in predicting the disease outcome in several human neoplasms [6–13]. In rapidly proliferating breast tumours TIL (tumour infiltrating lymphocytes) are a sign of favourable prognosis [6, 10] and the presence of histiocytes around other tumours correlates to less aggressive behaviour of neoplasms [8, 9, 13]. The majority of TIL in human tumours consist of T-cell populations [14–16] which suggest that cytotoxic antitumour