Prediction of Relapse and Survival in Breast Cancer Patients by pS2 Protein Status

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ABSTRACT

Application of systemic adjuvant therapy for primary breast cancer patients requires a more accurate identification of patients at high risk for recurrence. We have quantitatively assessed the cytosolic levels of estrogen-regulated pS2 protein in tumors of 205 breast cancer patients (median follow-up, 47 mo). There were no significant associations between the level of pS2 protein and tumor size, lymph node status, and differentiation grade. Using length of relapse-free survival (RFS) and overall survival (OS) as end points, 11 ng of pS2 protein/mg of cytosol protein were found as the best cut-off level to discriminate between positive (pS2+) and negative (pS2−). Patients with pS2− tumors showed significantly shorter RFS and OS (P < 0.0001) than patients with pS2+ tumors. Also after adjustment for tumor size, lymph node status, and estrogen receptor (ER) status, pS2− negativity was associated with earlier recurrence and death. Tumors positive for pS2 (55 of 205, 27%) were almost exclusively confined to the subclass of ER+ tumors (53 of 55, 96%). The death rate for patients with pS2+ tumors was one-tenth of the death rate for patients with pS2−/ER+ tumors. In the patients with ER+ tumors, the prognostic power of the pS2 status was especially present in patients whose tumors were also positive for the progesterone receptor (5-yr RFS and OS, 85% and 97% for ER+/PgR+/pS2+ tumors compared with 50% and 54% for the patients with ER+/PgR+/pS2− tumors). In patients with axillary lymph node involvement (N+), pS2 status could discriminate strongly between a good and bad prognosis group (5-yr RFS and OS, 65% and 88% for N+/pS2− compared with 32% and 34% for N+/pS2+). A similar phenomenon was observed in patients without axillary lymph node involvement (5-yr RFS and OS, 89% and 95% for N/ pS2− compared with 58% and 82% for N/pS2+).

We conclude that the pS2 status of human primary breast tumors is an important variable for the identification of patients at high risk for recurrence and death. Knowledge of the cytosolic pS2 status appeared of particular importance to identify patients at high risk in the ER+/PgR+ subclass of tumors, and in both the N0 and N+ subclasses of patients.

INTRODUCTION

Approximately 50% of the patients presenting with primary breast cancer can be cured by local surgery and radiotherapeutic treatment. The other half of the patients already have micrometastases at the time of diagnosis. Once detectable distant metastases have developed, none of the existing systemic therapies is curative but merely palliative. It is therefore essential to have methods to identify those patients which are likely to develop macrometastases, justifying adjuvant therapy to delay or possibly prevent a relapse. Several patient and tumor characteristics are currently used to classify breast tumors (1–10). However, many breast carcinomas having similar cell biological properties differ markedly in their recurrence behavior. A combination of various prognosticators may better identify patients at high risk of recurrence and survival. For the management of breast cancer, ER2 and PgR status of the primary breast tumor is not completely predictive for response to endocrine therapy and recurrence, since approximately half of the patients with ER+ tumors and a quarter of the patients with ER−/PgR+ tumors will not respond to endocrine therapy or have a worse prognosis. In analogy with the PgR (7–10), the specific transcription of the estrogen-related pS2 gene may reflect a more intact ER machinery. The pS2 gene was initially characterized as a gene whose expression is specifically controlled by estrogen in the MCF-7 human breast cancer cell line (11, 12). It was recently shown, with a complementary DNA probe for pS2 mRNA and with specific polyclonal antibodies against the pS2 protein, which is an 84 amino-acid-long secretory protein of unknown function (13), that pS2 was predominantly expressed in ER+ primary breast tumors (14). Moreover, the simultaneous determination of ER, PgR, and pS2 gene expression revealed a functional heterogeneity in ER+ tumors. It was found that, in ER+ tumors, both the PgR and pS2 genes were induced in 62%, the PgR gene without the pS2 gene in 26%, and the reverse in 5% of the cases. On the other hand, ER− tumors appeared homogeneous in this respect, with 96% lacking expression of either of these genes (14). pS2 mRNA was also expressed at various levels in some metastatic nodes of pS2 mRNA-positive primary breast tumors, but was never observed in metastatic nodes of pS2 mRNA-negative cancers or in benign breast tumors and in normal axillary nodes (14). Similar observations were done with an immunocytochemical technique for detection of pS2 protein in paraffin-embedded sections. It was shown that staining was cytoplasmic in specific ductal breast carcinomas and metastatic nodes, but was absent in normal duct epithelium and benign breast tumors (14). Moreover, no significant staining of pS2 protein was observed in a variety of normal human specimens (such as colon, pancreas, liver, lung, prostate, kidney, endometrium, ovary, and adrenals) (15). However, pS2 was specifically expressed and secreted by ER− mucosa cells of the normal stomach (antrum and body) of both female and male individuals (15).

For an evaluation of the role of pS2 in breast cancer growth, we have analyzed tumor specimens for the amounts of pS2 protein in tumors of 205 breast cancer patients (median follow-up, 47 mo). The characteristics with respect to T-N-M status are currently used to classify breast tumors (1–10). However, many breast carcinomas having similar cell biological properties differ markedly in their recurrence behavior. A combination of various prognosticators may better identify patients at high risk of recurrence and survival. For the management of breast cancer, ER2 and PgR status of the primary breast tumor is not completely predictive for response to endocrine therapy and recurrence, since approximately half of the patients with ER+ tumors and a quarter of the patients with ER−/PgR+ tumors will not respond to endocrine therapy or have a worse prognosis. In analogy with the PgR (7–10), the specific transcription of the estrogen-related pS2 gene may reflect a more intact ER machinery. The pS2 gene was initially characterized as a gene whose expression is specifically controlled by estrogen in the MCF-7 human breast cancer cell line (11, 12). It was recently shown, with a complementary DNA probe for pS2 mRNA and with specific polyclonal antibodies against the pS2 protein, which is an 84 amino-acid-long secretory protein of unknown function (13), that pS2 was predominantly expressed in ER+ primary breast tumors (14). Moreover, the simultaneous determination of ER, PgR, and pS2 gene expression revealed a functional heterogeneity in ER+ tumors. It was found that, in ER+ tumors, both the PgR and pS2 genes were induced in 62%, the PgR gene without the pS2 gene in 26%, and the reverse in 5% of the cases. On the other hand, ER− tumors appeared homogeneous in this respect, with 96% lacking expression of either of these genes (14). pS2 mRNA was also expressed at various levels in some metastatic nodes of pS2 mRNA-positive primary breast tumors, but was never observed in metastatic nodes of pS2 mRNA-negative cancers or in benign breast tumors and in normal axillary nodes (14). Similar observations were done with an immunocytochemical technique for detection of pS2 protein in paraffin-embedded sections. It was shown that staining was cytoplasmic in specific ductal breast carcinomas and metastatic nodes, but was absent in normal duct epithelium and benign breast tumors (14). Moreover, no significant staining of pS2 protein was observed in a variety of normal human specimens (such as colon, pancreas, liver, lung, prostate, kidney, endometrium, ovary, and adrenals) (15). However, pS2 was specifically expressed and secreted by ER− mucosa cells of the normal stomach (antrum and body) of both female and male individuals (15).

For an evaluation of the role of pS2 in breast cancer growth, we have analyzed tumor specimens for the amounts of pS2 protein. In addition we have correlated the findings with ER and PgR status of the tumor, with patient and other tumor characteristics (size and differentiation grade), and with tumor recurrence and patient survival.

PATIENTS AND METHODS

Patients

This study was performed on a group of 205 patients with operable breast cancer who underwent breast-conserving surgery or modified radical mastectomy with axillary lymph node dissection in the years 1978 through 1984.

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The abbreviations used are: ER, estrogen receptor; PgR, progesterone receptor; RFS, relapse-free survival; OS, overall survival; DCC, dextran-coated charcoal; EIA, enzyme immunoassays; IAI, irsotic regression analysis; ANOVA, analysis of variance; RHR, relative hazard rate; EGF-R, epidermal growth factor receptor; N0, nodes negative; N+, nodes positive.

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characteristics of the patients at the time of surgery and postoperative treatment are listed in Table 1. Note that, due to a few missing patient characteristics, the number of patients in the table does not always add up to 205. Perimenopausal patients have been defined as patients having irregular less frequent menstruations (less than once per 3 mo) and their last menstruation not longer than 1 yr ago. The somewhat high percentage of node-positive patients (61%) might be explained by the fact that very small tumors (commonly showing less lymph node metastasis) were underestimated in this series because all available tissue was mainly used for histological diagnosis and routine steroid receptor assays.

In those years patients with medially or centrally located (T1/T2) tumors, or T3/T4 tumors, were irradiated on parasternal lymph nodes. Ultimately, nearly all patients (19 excepted, including 6 M1 patients who already had distant metastasis at time of primary surgery) received some form of irradiation on the breast/thoracic wall (especially after breast-conserving surgery) and/or on one or more lymph node areas. Women under 56 yr of age and with positive lymph nodes generally received adjuvant chemotherapy (cyclophosphamide, methotrexate, 5-fluorouracil).

Methods

Tumors. Processing and pathological examination of tumors were performed as described previously (16). Steroid Hormone Receptor Assays. For routine biochemical steroid binding assays of ER and PgR, the DCC method and multiple-point Scatchard analysis of the binding data were used, exactly according to procedures as recommended by the EORTC (17). The buffer used for preparing cytosols consisted of 10 mM KH2PO4, 1.5 mM dipotassium dihydrogen phosphate, 10 mM sodium azide, 15% monothioglycerol, and 10% (v/v) glycerol (pH 7.4). In 9 cases, receptor values obtained by ER EIA and PgR EIA (Abbott Laboratories, Abbott Park, IL) because not enough cytosol was available to perform two complete multiple-point Scatchard analyses. It was shown before that data obtained with these EIAs are comparable with those obtained with the conventionally used DCC assays (18). The EIA values for the 9 cases missing on DCC were all clearly positive or negative, and their classification would not have changed with a different cutoff level. Before analysis of ER and PgR, cytosols were stored at -80°C for up to 3 mo. The prognostic power of ER and PgR values of the used archival material was not different when compared with those of the originally performed ER and PgR assays on cytosols prepared from other parts of the tumor biopsies (18). Protein assay was performed with the Bio-Rad method (Coomassie brilliant blue) with human serum albumin (Kabi Diagnostica) as a standard.

Assay of pS2 Protein (ELSA PS2”). For preparation of monoclonal antibodies, the pS2 protein (PS2”, CIS Bioindustries, Gif-sur-Yvette, France) was first isolated from culture medium of MCF-7 human breast cancer cells. Monoclonal antibodies were prepared by fusion of mouse myeloma cells X63 with spleen cells from BALB/c mice immunized with purified pS2 (19). Hybridoma culture supernatants were screened for their ability to bind 125I-labeled pS2. The selected hybridomas were produced on Pristane-primed BALB/c mice, and antibodies were purified by affinity chromatography on Protein A-Sepharose (Pharmacia, Uppsala, Sweden).

Next, two monoclonal antibodies (BC6 and BC4), directed against different epitopes on the pS2 molecule, allowed us to develop an immunoradiometric assay based on the ELSA” technology (ORIS Patent 7805040 of February 22, 1978). The first antibody was coated on the ELSA solid phase while the second one, radionabeled with 125Iodine, was used as a tracer. Twenty μl of sample (after storage of the cytosols for up to 18 mo at -80°C), or standard, and 300 μl of monoclonal anti-pS2 tracer were added into a fitted ELSA tube. Single step immunoassay was performed at 18-25°C for 1 h under agitation. Following three washes with distilled water, ELSA tubes were counted. Bound radioactivity was converted to pS2 protein concentration by reference to the standard curve obtained in each experiment.

Follow-up. All patients were routinely examined every 3 to 6 mo during the first 5 yr and once a year thereafter (median follow-up, 47 mo). Of the 205 patients included in this study, 71 have died (9 of whom without evidence of relapse). They all count as failures in the OS analysis. Eighty patients showed evidence of disease during follow-up. These patients together with the patients who died without evidence of recurrence (the cause of death of these patients is, in fact, very hard to verify) count as failures in the RFS analysis. Primary M1 patients (n = 9) were excluded in analyses for RFS and OS.

Statistics. For ER and PgR the choice of a cut point between values labeled as negative and values labeled as positive was based on the results of IRA (20). With IRA the hazard rate for failure (relapse or death) is estimated as a function of the receptor value under the assumption of a monotone-decreasing failure rate with increasing receptor levels. For this series of patients the established cutoff levels were 18 fmol/mg of protein for ER and 26 fmol/mg of protein for PgR, respectively (18). The IRA procedure was also applied to determine the best cut point for a dichotomies of the pS2 values in negative and positive.

The associations between pS2 protein and menopausal status, tumor size, nodal status, and differentiation grade of the tumor were studied by the F test (ANOVA). The associations between pS2 protein and steroid receptors (excluding 9 for which ER and PgR were estimated with EIAs) and age of the patients were described by Spearman rank correlation coefficients. In the analysis the (relapse free) survival was estimated by the method of Kaplan and Meier. The likelihood ratio test in the univariate Cox regression model was used to test for differences. The Cox model was also applied for multivariate analyses. In this way the strength and the direction of the association between pS2 protein and (relapse free) survival were estimated and tested, given the information of other well-known prognostic factors as tumor size, nodal status, and ER. The multivariate analysis was replicated with a different choice of the cutoff for ER and PgR, i.e., 10 fmol/mg of protein. This had no effect on the estimated strength of the association of pS2 and prognosis (results not presented).

A significance level of 5% was chosen as a criterion for entering variables in the multivariate model. The results of the multivariate analysis (as shown in Table 4 of "Results") are expressed in terms of RHRs derived from the estimated regression coefficients. The RHRs

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<table>
<thead>
<tr>
<th>Characteristics of patients, tumors, and treatment</th>
<th>Frequency</th>
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<tr>
<td><strong>Patients</strong></td>
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<tr>
<td>Total no.</td>
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<tr>
<td>Age, mean</td>
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<td>T2 (2–5 cm)</td>
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<tr>
<td>T3/4 (≥5 cm)</td>
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<tr>
<td>N+</td>
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<tr>
<td>M1</td>
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<td>Surgery of the axilla</td>
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<tr>
<td>Other lymph node areas without</td>
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<tr>
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<td>Chemotherapy (CMF)</td>
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<tr>
<td>Hormonal therapy</td>
<td>10</td>
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</table>

* Numbers in parentheses, range.

* CMF, cyclophosphamide, methotrexate, 5-fluorouracil.
of the binary variables $N$ ($N^0$ or $N^+$), ER, and pS2 express the average ratio of the failure rates in both categories of the variables.

RESULTS

Associations between pS2 and (Relapse-free) Survival. The levels of pS2 varied from 0 to 274 ng/mg of protein (median, 3.6; mean ± SD, 13.4 ± 30.6). To establish possible significance of pS2 levels as a predictor for RFS and OS, univariate Cox regression analysis was done with $\log(pS2)$ as factor and RFS and OS as end points. In both analyses pS2 showed a strong negative association with failure rate (test for trend; RFS, $P = 0.002$; OS, $P = 0.0004$). Graphically this was also shown by division of the range of pS2 into four groups of equal size; patients with levels of pS2 in the highest quartile had the lowest risk of failure or death. This justified the approach of isotonic regression analysis to search for a cutoff point for pS2 to enable us to classify the tumors as negative (pS2--) and positive (pS2+).

With both end points RFS and OS, the same cutoff point (11 ng of pS2/mg of cytosolic protein) was found. Five-yr actuarial RFS and OS analyses (Fig. 1) clearly show that pS2 status was a significant predictor of early recurrence and death. Among patients with pS2 ≤11 ng/mg of protein, no further association between pS2 and RFS or OS was observed.

Table 2 shows the fractions with 5-yr RFS and OS, and the corresponding $P$ values based on the likelihood ratio test in univariate Cox analysis for pS2, and for known prognostic factors as tumor size, lymph node status, differentiation grade, and ER and PgR. The 5-yr probability for recurrence and death is more than 2.5, respectively, 5 times as high in patients with pS2 tumors compared with pS2+ tumors. Moreover, the data in Table 2 confirm the prognostic power of the known prognostic factors in breast cancer.

Associations between pS2 and ER, PgR, and Age of the Patients. There was a clear significant association between pS2 and ER and PgR (Spearman rank correlation coefficients, $r_s = +0.36$ and $r_s = +0.42$, respectively). There was no overall correlation between pS2 levels and age of the patients ($r_s = -0.07$). However, the level of pS2 was lower ($P = 0.01$) in postmenopausal patients ($\log(pS2) ± SD = 0.9 ± 1.9$ ng/mg of protein) compared with premenopausal patients ($1.7 ± 1.8$ ng/mg of protein).

Table 3 shows the relationships between pS2 and ER and PgR. All but two pS2-- tumors (53 of 55, 96%) were also ER+. About one-third of all ER+ tumors, however, were pS2+. In addition, pS2 positivity was more frequent in ER+/PgR+ tumors (41%) than in ER+/PgR-- tumors (25%).

Associations between pS2 and Tumor Size, Lymph Node Status, and Differentiation Grade. Relationships between pS2 and tumor size or lymph node status were evaluated with the $F$ test (ANOVA) and with the grade of differentiation assessed with a test for trend. There were no statistically significant associations between the level of pS2 and tumor size ($P = 0.45$), lymph node status ($P = 0.15$), or differentiation grade ($P = 0.28$).

Multivariate Analysis of pS2 Status. The combined effect of several clinical and biochemical parameters in combination with other factors was assessed with Cox multivariate analysis for RFS and OS. In both analyses, tumor size, lymph node status, ER, and pS2 were included in the model (Table 4). Given one of the two steroid receptors (ER or PgR), the other did not contribute any more to a better fit, while including either one of these two factors was statistically significant. This is due to the strong association between ER and PgR. In the case of analysis of more patients, it may well be that ER, pS2, and also PgR will be significant parameters in the multivariate analysis. The RHR for ER+ patients compared with ER-- patients was 0.38 for RFS and 0.39 for OS. The RHR for pS2+ patients compared with pS2-- patients was 0.49 for RFS and 0.30 for
OS (Table 4). Note that pS2 is positive almost exclusively when ER is positive (Table 3). This implies that the RHR for the ER+/pS2* group compared with the ER+/pS2~ group is 0.18 (0.38 × 0.49) for RFS and 0.11 for OS (0.39 × 0.30). Thus, the death rate in pS2+ patients is almost one-tenth of the death rate in ER patients.

PgR, age, and menopause did not significantly add to the prognostic information and were therefore excluded from the multivariate models (Table 4). Differentiation grade also showed a statistically significant negative association with the RFS. It was not included in the above models because of too many missing values.

RFS and OS Stratified by ER, PgR, and pS2 Status. In view of the association between ER and PgR with pS2, and the occurrence of significant amounts of ER+/PgR* and ER+/PgR~ tumors which were pS2~ (Table 3), we have constructed 5-yr actuarial RFS and OS curves stratified by ER, PgR, and pS2 status. Since there were only 3 ER+/PgR* tumors and one ER~/PgR~/pS2* tumor, these patients were not included in the analyses. Analyses for 5-yr RFS (Fig. 2A) and OS (Fig. 2B) clearly showed that, in the group with ER+/PgR~ tumors, the subgroup of pS2~ patients (53 of 90, 59%) had a significantly higher risk of failure (50% versus 15% for pS2*; Fig. 2A) and death (46% versus 3% for pS2*; Fig. 2B). Patients with tumors having an ER+/PgR~/pS2* phenotype showed the worst prognosis, in analyses for both RFS (83% failures after 5 yr, Fig. 2A) and OS (59% deaths after 5 yr, Fig. 2B). In the group of patients with tumors having the ER+/PgR~ phenotype, the pS2 status was not discriminative with respect to RFS (Fig. 2A) or OS (Fig. 2B).

RFS and OS Stratified by Lymph Node Status and pS2 Status. There was an increased risk of recurrence and death (for both, R < 0.0001) for patients with involved axillary nodes (Table 2). Since there appeared to be no significant association between the level of pS2 and lymph node involvement, the impact of pS2 status on length of RFS (Fig. 3, A and C) and OS (Fig. 3, B and D) in the “good prognosis” group of the N0 patients and in the “bad prognosis” group of the N+ patients was studied. Fig. 3A shows that, in the group of N0 patients, the pS2− subgroup had a significantly higher risk for failure (42% relapses after 5 yr) than the pS2+ subgroup (11% relapses). A similar dramatic effect was observed for patients with involved axillary lymph nodes. In these patients pS2 status also showed a strong discrimination, i.e., 68% failure after 5 yr for the pS2− and 35% for the pS2+ subclass (Fig. 3C).

Also with respect to 5-yr OS, pS2 negativity was associated with an earlier death in both the N0 group (18% for pS2− and 5% for pS2*; Fig. 3B), and the N+ group of patients (66% for pS2− and 12% for pS2*; Fig. 3D). Patients with increased risk for earlier failure and death (pS2*) represent 66% (50 of 76) in the N0 group and 78% (92 of 118) in the N+ group (Fig. 3C).

DISCUSSION

In the current discussion regarding application of systemic adjuvant therapy in primary breast cancer, identification of high-risk and low-risk patients is a major issue (21). Several classical (tumor size, lymph node status, histopathology, steroid receptor status) and second-generation prognostic factors (proliferation rate, DNA ploidy, oncogenes, growth factor receptors, and some glycoproteins) are available for making therapeutic decisions (22, 23). Unfortunately, at present no single prognosticator is sufficiently powerful to be used alone for treatment decisions. Although combinations of prognostic factors can improve the prediction of a patient’s prognosis, no group of prognosticators completely fulfills the objective to fully distinguish high- and low-risk patients (21). In the present study we report the prognostic value of a new powerful prognosticator for breast cancer, i.e., the pS2 protein, which appears to be able to strongly discriminate between high- and low-risk groups within the patient population with ER+ tumors. The follow-up of the patients (median, approximately 4 yr) is, however, relatively short for breast cancer. One should therefore keep in mind that the strength of the association of pS2 with prognosis may become weaker or stronger with longer follow-up or after addition of more patients. In addition, for the patient population used, optimal cutoff levels for ER, PgR, and pS2 were determined by isotonic regression analysis. The best value for the cutoff level may change after inclusion of more patients or be different for another group of patients.

The expression of the pS2 gene in ER+ subclasses of human primary breast tumors was shown in an earlier series of 180 breast tumors (14). The present study, in which the pS2 protein was assessed quantitatively, confirms this strong association between ER and pS2. After establishment of the clinically significant cutoff level by isotonic regression analysis, pS2 appeared almost exclusively to be associated with ER-positive breast tumors; i.e., 35% of ER+ tumors were also positive for pS2, whereas the ER− tumors, only 4% had pS2 levels above the prognostically significant level of 11 ng/mg of protein (Table 3). It was furthermore interesting to note that, of the ER+/PgR+ tumors, 41% appeared pS2+, and of the ER+/PgR−
caused by the fact that EGF-R is predominantly associated with tumors, only 25%, implying a functional heterogeneity of the estrogen receptor. Using a clinically significant cutoff level to discriminate between pS2+ and pS2~, as in this series, the amount of defined pS2+ tumors was lower than when compared with the earlier series (27% versus 49%), in which pS2 status was assessed by pS2 mRNA analysis and pS2 immunocytochemistry (14). This is due to the fact that, in the present series of 205 tumors, detectable levels below 11 ng/mg of protein were considered negative, in spite of the presence of amounts of pS2 (median value, 3.6 ng/mg of protein) which were defined as positive by other techniques in the earlier series of 180 tumors (14).

The possible prognostic value of pS2 in the ER~ subclass could not be evaluated, since only 2 of 54 ER~ tumors were positive for pS2. With respect to length of RFS and OS, in the subclass of ER~ tumors, pS2 especially added to the predictive power of ER and PgR in the ER+/PgR~ subclass (Fig. 2). This suggests that pS2 plays a role in the less malignant behavior of breast tumor cells only if PgR is also present and, thus, in the presence of a complete biologically active ER system. Additional knowledge of the pS2 status of the tumor may identify patients in specific ER/PgR subclasses which will fail or respond to a selected therapy. In this respect, Sainsbury et al. showed that EGF-R status provides additional prognostic information, especially in patients with ER~ tumors (3, 24), caused by the fact that EGF-R is predominantly associated with ER~ tumors. In the present study, pS2 positivity is almost exclusively confined to ER~ tumors and discriminates clearly between high- and low-risk patients within this group. It is therefore tempting to speculate that the approximately half of the patients in the ER~ subclass who yet do not respond to hormonal therapy can be identified by a pS2~ phenotype. It is at present unknown whether pS2 status will provide additional information concerning the clinical responsiveness to therapy. However, a preliminary evaluation of our present data showed that patients with pS2+ tumors have a shorter survival from relapse than patients with pS2~ tumors (data not shown).

In this study we confirm that tumor size, lymph node status, differentiation grade, and steroid receptor status of the tumor are important predictors for the length of RFS and OS. The very strong association of pS2 with ER and with relapse and survival suggested a relationship between pS2 and tumor differentiation. In this series of tumors we were unable to ascertain a significant relationship between pS2 levels and differentiation grade of the tumor, nor with tumor size and lymph node status.

Overall, tumor status of pS2 was a highly significant predictor for early recurrence or death (Cox univariate analyses; $P < 0.001$). In addition to the prognostic power of the pS2 status in the ER+/PgR~ subclass, a strong discriminative effect of pS2 alone in both the N0 and the N+ patient population was observed. For the N0 patients this observation may be of extreme importance, since there is no agreement whether these patients have to receive any further systemic treatment after local therapy. Moreover, if breast cancer screening will detect a higher population of patients without node involvement, of which eventually 20 to 30% will experience a recurrence and are therefore not cured by surgery alone, the clinician will imperatively need tools to select the patients at high risk. The ER status reveals only an 8 to 10% difference between ER~ and ER~ groups in both RFS and OS in large series of node-negative cancer patients (22, 25). McGuire (22) and Fisher et al. (25) concluded that knowledge of ER values can certainly contribute to a treatment decision, but that additional information is required. In our study, we found in the N0 patient group a 31% difference in RFS (Fig. 3A) and a 13% difference in OS (Fig. 3B) between pS2+ and pS2~ patients. In view of the relatively large difference in RFS it might be expected that the 13% difference in OS will increase after longer follow-up. In this N0 group other (modern) prognosticators (nuclear grade, tumor size, ploidy, S-phase fraction, labeling index, and cathepsin D) showed a difference in RFS after 5 yr of follow-up between high- and low-risk patients varying from 13% to 20% (1, 2, 22, 25, 26). Sainsbury et al. (3) described in this N0 patient group a significant difference in RFS between patients with EGF-R-positive and EGF-R-negative tumors after 2.5 yr of follow-up. These data, are in conflict with our findings in a larger series of patients, indicating mainly a discriminatory effect of EGF-R status in N+ patients after 5 yr of follow-up (16). HER2/neu or c-erbB-2 oncogene amplification or expression did not even have clinical value in N0 patients (22, 27). In general there is no agreement on the statistical association between c-erbB-2 amplification and lymph node status or patient prognosis (28).
In addition to a strong discriminatory capacity, a particular advantage of the measurement of pS2 protein over other new prognosticators (e.g., EGF-R, ploidy, proliferation rate) lies in the fact that pS2 status can be obtained with an easy and rapid assay on the very same cytosols which are routinely prepared for the assessment of steroid receptor status. Also in the N+ patient group, pS2 levels were able to strongly discriminate between high- and low-risk patients, showing a 33% difference in RFS (Fig. 3C) and a 54% difference in OS (Fig. 3D) between pS2+ and pS2− patients.

Patients with an ER+/PgR+/pS2+ tumor experienced an 83% failure, and 59% were dead after 5 yr. With respect to consequences for treatment decisions it awaits further study if patients with pS2− tumors will benefit from aggressive adjuvant chemotherapy (with or without addition of hormonal therapy), and if patients with pS2+ tumors ought not to be treated, or only with hormonal agents.

In summary, pS2+ status of the tumor is a valuable tool for predicting an overall excellent prognosis. On the other hand, pS2− status is strongly associated with an early recurrence and death in subsets of patients. In particular, pS2− negativity was associated with a worse prognosis in the patients with ER+/PgR+ tumors, in patients with N0 disease, and in patients with N+ disease. We conclude that measurement of cytosolic pS2 protein status, together with ER and PgR, will help the clinician to refine the choice of treatment for each individual patient.

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Prediction of Relapse and Survival in Breast Cancer Patients by pS2 Protein Status

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