

PS2 mRNA expression adds prognostic information to node status for 6-year survival in breast cancer

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Summary Expression of pS2, an oestrogen-regulated gene, has been associated with a good short-term prognosis and response to endocrine therapy. The aim of this study was to determine whether expression of mRNA for the pS2 gene in breast cancer could contribute useful information on disease behaviour and survival at medium-term follow-up. Northern blotting was used to detect pS2 messenger ribonucleic acid (mRNA) in the primary tumour tissue from each of 90 patients with breast cancer. Axillary node status was established by sampling or clearance, oestrogen receptor concentration by enzyme immunosorbant assay and follow-up was continued for at least 6 years or until death. At 83 months mean follow-up, 29 of 90 (32%) patients had recurrent disease and, of these, 18 (20%) had died from breast cancer. pS2 mRNA expression, present in 26 of 90 (29%) cancers, was associated with freedom from disease recurrence ($P = 0.026$) and was significantly associated with survival at a minimum of 6 years follow-up ($P < 0.001$). Pathological node status and tumour size were also significantly associated with disease recurrence ($P < 0.001$ and $P = 0.002$ respectively) and inversely with survival ($P < 0.001$ and $P < 0.001$ respectively). After multiple Cox regression analysis, pS2 expression was still a significant predictor of recurrence (but not survival) after adjusting for node status and tumour size; oestrogen receptor was an independent predictor of survival. The combination of node status and pS2 expression discriminated patients with particularly good prognosis (node negative, pS2 positive: no mortality at 6 years) or poor prognosis (node positive, pS2 negative; 41% mortality at 6 years). Evaluation of pS2 expression in breast cancer at diagnosis may provide additional useful prognostic information to conventional staging.

Keywords: breast cancer; pS2, prognosis

The detection of pS2 expression in response to an oestrogenic stimulus in breast cancer cells (Masiakowski et al, 1982) – an effect that can be antagonized by tamoxifen – led to speculation that pS2 could be an oestrogen-regulated gene of clinical importance. The pS2 gene (also known as BCE1, pNR-2, Md2) encodes a 600-base mRNA (Masiakowski et al, 1982), which is translated to a cysteine-rich protein with structural similarities to insulin-like growth factors I and II (Rio et al, 1987; Stack et al, 1988). The clinical interest in pS2 is the result of an association with oestrogen receptor expression and with other conventional markers of good prognosis (Rio et al, 1987; Stack et al, 1988; Schwartz et al, 1991; Predine et al, 1992; Thompson et al, 1993; Detre et al, 1994; Foekens et al, 1994; Speiser et al, 1994; Gibert et al, 1996; Tutschek et al, 1996). pS2 expression is associated with good prognosis in female – but not in male – patients at short-term follow-up (Foekens et al, 1990; Schwartz et al, 1991; Predine et al, 1992; Gion et al, 1993; Kardas et al, 1993; Thompson et al, 1993; Foekens et al, 1994; Speiser et al, 1994). Conversely, pS2-negative patients have a significantly shorter relapse-free survival (Gion et al, 1993; Foekens et al, 1994; Speiser et al, 1994; Schmidt et al, 1996) and overall survival (Gion et al, 1993; Speiser et al, 1994). pS2 also predicts a subsequent response to hormone manipulation as first-line therapy and on disease relapse (Henry et al, 1988,

1990, 1991; Schwartz et al 1991; Soubeyran et al, 1996). Thus, the detection of pS2 expression in breast cancers may define a subset of cancers with functional oestrogen receptors and hence patients with a good prognosis who are more likely to respond to endocrine manipulation.

The aim of this study was to determine whether pS2 gene mRNA expression could contribute useful information on disease behaviour and survival after a minimum of 6 years (medium term) follow-up.

PATIENTS AND METHODS

Ninety female patients with primary, previously untreated, histologically proven, invasive breast cancer (mean age 56 years, range 30–78 years) underwent surgery for breast cancer. The 2-year follow-up on a subgroup of these women forms part of a previous report (Thompson et al, 1993). Patients with impalpable disease or small tumours (when insufficient material was available for study) were excluded from the study. Fifty-six women were post-menopausal.

The tumour size on the resected specimen was measured as less than 2 cm (pT1) in nine patients, 2–4.9 cm (pT2) in 51 patients or greater than 5 cm (pT3) in 30 patients. Pathological axillary node status (based on 2–25 nodes per patient) was determined by axillary node sampling or clearance, with 46 of 90 patients node positive and 44 node negative at the time of diagnosis. The histology of the tumours was invasive ductal cancer of no special type (80 cancers), lobular breast cancer (seven) or special type (three). The

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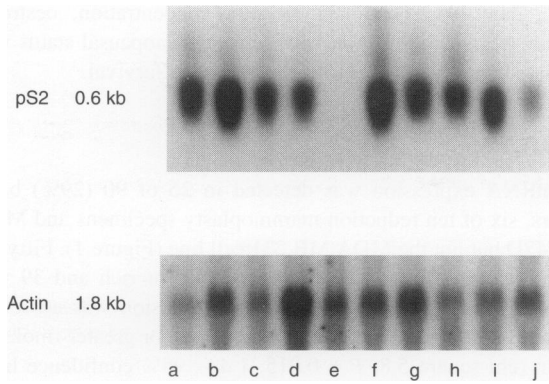


Figure 1 pS2 mRNA expression in breast cancers, breast cancer cell lines and reduction mammoplasty tissue. Northern blot autoradiographs probed sequentially with pS2 (0.6-kb mRNA, upper panel) and actin (1.8-kb mRNA, internal control for loading, lower panel). a, Breast cancer, node positive, no disease recurrence; b, breast cancer, node positive, no disease recurrence; c, breast cancer, node negative, no disease recurrence; d, reduction mammoplasty tissue; e, breast cancer cell line MDA MB 231 (oestrogen receptor 0 fmol mg⁻¹ protein); f, breast cancer cell line MCF 7 (oestrogen receptor 120 fmol mg⁻¹ protein); g, breast cancer cell line T 47 D (oestrogen receptor 40 fmol mg⁻¹ protein); h, breast cancer, node positive, disease recurrence; i, breast cancer, node positive, disease recurrence; j, breast cancer, node negative, disease recurrence

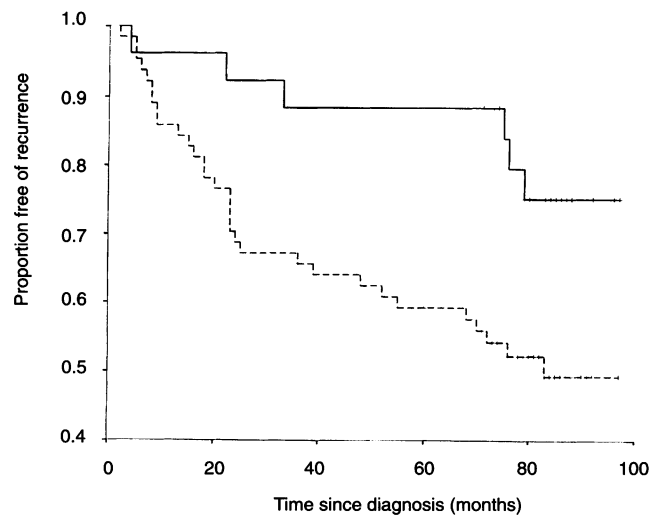


Figure 3 pS2 mRNA expression related to disease recurrence. Breast cancer recurrence against time since diagnosis (in months) for patients with pS2 mRNA expression in the primary cancer (—) and patients with no pS2 mRNA in the cancer (---), demonstrating a significant association between pS2 mRNA expression ($P < 0.026$) and freedom from disease recurrence

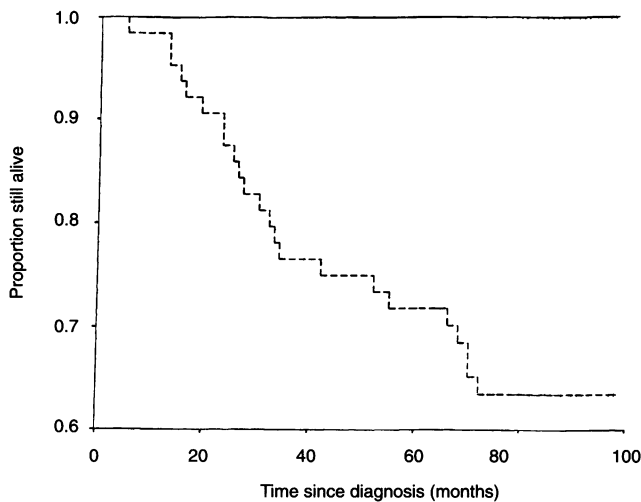


Figure 2 pS2 mRNA expression and survival in breast cancer. Patient survival against time since diagnosis (in months) for patients with pS2 mRNA expression in the primary cancer (—) and patients with no pS2 mRNA in the cancer (---), demonstrating a significant association between pS2 mRNA expression ($P < 0.001$) and survival

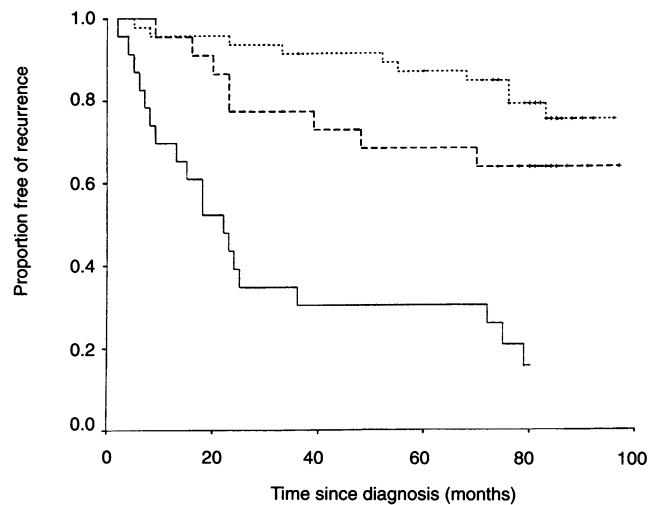


Figure 4 Axillary node metastasis and prognosis in breast cancer. Patient survival against time since diagnosis (in months) for patients subdivided into axillary node negative (· · ·), one to three axillary nodes containing tumour (---) or four or more axillary nodes involved with tumour (—)

oestrogen receptor content of the tumours was measured using an enzyme immunosorbant assay (ER-EIA, Abbott Laboratories, North Chicago, IL, USA) and expressed in fmol mg⁻¹ protein. Tumour oestrogen receptor concentrations of 20 fmol mg⁻¹ protein or more was considered oestrogen receptor moderate or rich (Anderson et al, 1989). Tumour grade and progesterone receptor status was not assessed, in keeping with our clinical and laboratory practice at that time.

In addition to surgical treatment, adjuvant therapy was administered to 79 of the 90 women: 66 received 20 mg of tamoxifen for a

minimum of 5 years, 11 CMF and two underwent surgical oophorectomy. Clinical and radiological follow-up (including annual mammography) were continued for at least 6 years or until death; follow-up ranged from 5 to 98 months with a mean follow-up of 83 months. Total RNA was extracted from snap-frozen tumour tissue using the lithium chloride-urea method, and pS2 expression was determined using the Northern blot technique, with a cDNA probe (Masaikowski et al, 1982) used to detect pS2 messenger ribonucleic acid (mRNA) and actin (Minty et al, 1981) as an internal control for RNA loading (Thompson et al, 1993).

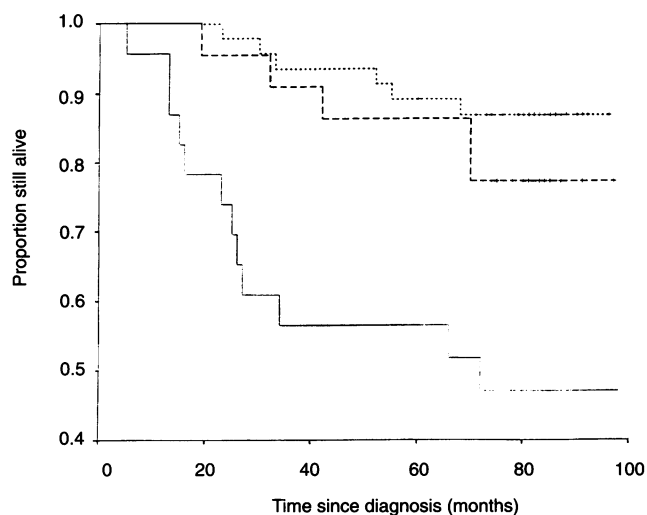


Figure 5 Axillary node metastases and disease-free interval. Disease recurrence plotted against time since diagnosis (in months) for patients subdivided into axillary node negative (•••), one to three axillary nodes containing tumour (---) or four or more axillary nodes involved with tumour (—)

Autoradiographs of the probed Northern blots were examined for tumour tissue from the 90 patients with primary breast cancer, ten patients who had undergone reduction mammoplasty and three breast cancer cell lines: MCF-7, T47D and MDA MB 231.

The chi-square test was used to compare pS2 mRNA expression and oestrogen receptor protein in the tumours and to compare recurrence and death rates at 6 years. Cox proportional hazards regression and multiple Cox regression were used to test whether pS2 mRNA expression, node status, tumour size on pathology

measurement, log oestrogen receptor concentration, oestrogen receptor status (low vs moderate/rich) or menopausal status were associated with recurrence of disease or with survival.

RESULTS

pS2 mRNA expression was detected in 26 of 90 (29%) breast cancers, six of ten reduction mammoplasty specimens and MCF7 and T47D but not the MDA MB 231 cell line (Figure 1). Fifty-one tumours were oestrogen receptor moderate or rich and 39 were oestrogen receptor poor. pS2 mRNA expression was associated with oestrogen receptor concentrations of 20 or greater fmol mg⁻¹ protein (chi-square 5.8, $P = 0.015$, 1 d.f., 95% confidence limits for oestrogen receptor 1.24–12.3). There was no association between pS2 expression and node status, tumour size or menopausal status. At a mean follow-up of 84 months, 29 of 90 (32%) patients had developed recurrent disease and 18 of 90 (20%) of these had died from breast cancer. All 26 patients with pS2 mRNA expression in the tumour at diagnosis were alive. On univariate analysis, pS2 mRNA expression was significantly associated with survival ($P < 0.001$) (Figure 2) and with disease-free interval ($P = 0.026$) (Figure 3). Taking the clinically useful cut-off of 20 fmol mg⁻¹ protein to discriminate between oestrogen receptor-poor (< 20) and oestrogen receptor-moderate/rich (≥ 20) tumours, or using oestrogen receptor as a continuous variable, or as the log of oestrogen receptor, oestrogen receptor expression was not associated with survival or disease-free interval by univariate analysis.

Axillary node metastasis, detected in 6 of 90 (51%) patients at diagnosis, was significantly associated both with poor prognosis ($P < 0.001$) (Figure 4) and with shorter disease-free interval ($P < 0.001$) (Figure 5). Tumour size, measured by the pathologist on the resected specimen, was also significantly associated with recurrence ($P < 0.001$) and survival ($P = 0.003$).

Table 1 Multiple Cox regression analysis of clinical and pathological parameters related to disease recurrence and survival in breast cancer

Factor	P-value			
	Recurrence		Survival	
	Univariate	Multivariate	Univariate	Multivariate
Node metastasis	< 0.001	< 0.001	0.001	< 0.001
Tumour size	< 0.001	0.002	0.003	< 0.001
pS2	0.026	0.020	< 0.001	0.92
Oestrogen receptor	0.90	0.52	0.10	0.001
Menopausal status	0.95	0.11	0.82	0.08

Table 2 Recurrence and survival by node status and pS2 mRNA expression.

	Node negative		Node positive		Total
	pS2 mRNA+	pS2 mRNA-	pS2 mRNA+	pS2 mRNA-	
Alive and well	13	27	10	11	61
Recurrent disease	1	0	2	8	11
Dead	0	5	0	13	18
Total	14	32	12	32	

pS2 mRNA+, pS2 mRNA positive; pS2 mRNA-, pS2mRNA negative.

By multiple Cox regression (Table 1), node status and tumour size both remained independently significant for both recurrence and survival; pS2 expression was still a significant predictor of recurrence after adjusting for both these variables, but was not a predictor for mortality. Oestrogen receptor status became independently predictive of mortality (but not disease recurrence) after adjusting for nodes and size. The combination of node status and pS2 expression (Table 2) defined patients with particularly good prognosis (node negative, pS2 positive; no mortality at 5 years) or poor prognosis (node positive, pS2 negative; 41% mortality at 5 years). When the cancer did not express pS2 mRNA, patients who were node positive were significantly more likely ($P < 0.001$) to develop disease recurrence than patients who were node negative.

DISCUSSION

This study examined pS2 mRNA expression in 90 breast cancers and related pS2 expression to clinical and pathological parameters and to disease behaviour at a minimum follow-up of 6 years.

pS2 expression was detected in 29% of the breast cancers studied, which is within the range (22–58%) for mRNA detection in breast cancer reported by others (Rio et al, 1987; Stack et al, 1988; Skilton et al, 1989; Henry et al, 1990; Zaretsky et al, 1990; Hahnel et al, 1991; Delvenne et al, 1992; Wysocki et al, 1994) and similar to the range of 27–68% of breast cancers that express pS2 protein (Foekens et al, 1990; Henry et al, 1991; Schwartz et al, 1991; Walker et al, 1995; Tutschek et al, 1996). The more recent development of reverse transcription polymerase chain reaction (RT-PCR), which may be a more sensitive method to detect pS2, allows both the detection and quantification of pS2 mRNA expression (Carr et al, 1995). It is unclear whether improved sensitivity would effect the prognostic value of pS2 mRNA.

pS2 expression was significantly related to oestrogen receptor protein expression as expected for an oestrogen-regulated protein (Rio et al, 1987; Stack et al, 1988; Skilton et al, 1989; Henry et al, 1991; Schwartz et al, 1991; Predine et al, 1992; Thompson et al, 1993; Detre et al, 1994; Foekens et al, 1994; Speiser et al, 1994; Stonelake et al, 1994; Walker et al, 1995; Gibert et al, 1996; Tutschek et al, 1996) and we have confirmed the observations of others that pS2 expression (whether measured as mRNA or protein) is not significantly associated with tumour size or node status (Foekens et al, 1990; Schwartz et al, 1991; Delvenne et al, 1992; Gion et al, 1993).

We have demonstrated that, at medium-term (6 year minimum, mean 83 months) follow-up, pS2 expression remains associated with good prognosis (Foekens et al, 1990; Schwartz et al, 1991; Predine et al, 1992; Thompson et al, 1993; Gion et al, 1993; Foekens et al, 1994; Speiser et al, 1994; Schmidt et al, 1996) in contrast to one other study of pS2 mRNA with a shorter follow-up (Wysocki et al, 1994). Although our study, like that of Speiser et al (1994), failed to identify pS2 as an independent prognostic factor for survival (which had been suggested by Gion et al, 1993; Foekens et al, 1994), oestrogen receptor protein was associated with survival after adjusting for node status and tumour size. As expected, axillary node status (based on the pathological examination of surgically resected axillary lymph nodes) and tumour size were significantly associated with disease recurrence and survival both on univariate analysis and multivariate analysis.

The most important clinical implication of this study is the confirmation that pS2 expression has a discriminant effect for better prognosis in both axillary lymph node-positive (Foekens et

al, 1990; Kausitz et al, 1994) and node-negative patients (Stonelake et al, 1994). Furthermore, the data presented here suggest that a combination of axillary node status and pS2 expression measured at the time of diagnosis can define patients at high and low risk of death for medium-term follow-up.

As pS2 expression is a good indicator of endocrine responsiveness both in the primary cancer and on relapse (Henry et al, 1988; Ramm et al, 1988; Skilton et al, 1989; Schwartz et al, 1991; Westley and May, 1991; Coradini et al, 1996; Soubeyran et al, 1996), the detection of pS2 expression in the primary cancer, whether at the mRNA or the protein level, may be useful both as prognostic information and as a guide to therapeutic intervention. In this study, patients with tumours that were oestrogen receptor moderate/rich at diagnosis (therefore including the tumours that express pS2) were treated with tamoxifen. Thus, as changes in pS2 protein expression have been demonstrated during tamoxifen therapy (Wilsher et al, 1996), it is possible that the apparent survival advantage at 5 years for the patients with pS2 mRNA detected in the tumour may be attributable to the effect of tamoxifen therapy mediated via pS2.

The usefulness of pS2 has been taken one step further in clinical practice; pS2 expression can be used to guide the likely response to tamoxifen therapy in patients with recurrent breast cancer (Foekens et al, 1994). While pS2 expression by Northern blotting may not be suitable for routine pS2 analysis, pS2 measured by immunohistochemical staining (Wilson et al, 1994) or competitive reverse transcription polymerase chain reaction (Carr et al, 1995) on fine-needle aspirates of breast cancer has been used to select patients over the age of 70 years for tamoxifen therapy. pS2 expression in breast cancer tissue is useful in itself as a marker of probable hormonal response to endocrine manipulation. This study has demonstrated that pS2 mRNA expression also provides useful prognostic information in addition to that provided by axillary node status when oestrogen receptor expression may be of limited value. pS2 should now be considered for routine clinical measurement as a response indicator and prognostic marker alongside oestrogen receptor protein.

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