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Short Communication

Pregnancy-specific β_1-glycoprotein (SP_1) in serum and tissue from patients with benign and malignant breast tumours

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The pregnancy specific β_1-glycoprotein (SP_1) is synthesized by the human placenta and secreted into the maternal circulation. However, SP_1 does not seem to be specific to pregnancy since it has been detected by radioimmunoassay in sera from 3 to 54% of healthy persons (Searle *et al.*, 1978; Würz, 1979; Tatarinov, 1980) and in sera from patients with a variety of malignant diseases, for instance 8–55% of breast cancer patients (Searle *et al.*, 1978; Würz, 1979; Tatarinov, 1980), depending on the detection limit of the assay. By means of a histological immunoperoxidase technique SP_1 has been demonstrated in 37–76% of malignant tumours of the breast (Horne *et al.*, 1976; Inaba *et al.*, 1980; Walker, 1981). Furthermore, the survival time was significantly longer for women with SP_1 negative tumours than those with SP_1 positive tumours (Horne *et al.*, 1976).

A prospective study (3 years) was undertaken to clarify the value of determining SP_1 in serum taken preoperatively and/or detecting SP_1 in tumour tissue as a prognostic indicator in the selection of patients with malignant breast tumours for chemotherapy.

The study comprised 113 women selected at random from patients admitted to the Department of Surgery during the course of about 6 months for investigation of and treatment for a suspected breast tumour. The histological classification was done as recommended by the WHO (Azzopardi *et al.*, 1982). Benign breast disease was found in 79 patients. The histological diagnoses were mammary dysplasia/fibrocystic disease (66 patients), fibroadenoma (5 patients), intraductal papilloma (5 patients), lipoma (2 patients), and phyllodes tumour (1 patient). Malignancy was histologically confirmed in 34 women, 7 of whom had previously had a contralateral malignant breast tumour, whereas breast cancer had previously not occurred in the remaining. Twenty four of the latter were given a total mastectomy with partial axillary dissection and, depending on the histological findings, were treated postoperatively with radiotherapy and systemic adjuvant treatment (Andersen *et al.*, 1981). The other 3 patients had only the tumour removed because of age or a histological diagnosis of non-invasive ductal carcinoma. The seven patients with previous contralateral breast tumours were given individual treatment. The patients with cancer were followed up for at least 3 years after operation.

The determination of SP_1 in serum was performed with a highly sensitive radioimmunoassay described elsewhere (Sørensen and Trentemøller, 1983). The assay consisted of standards, controls, unknown samples and controls for non-specific binding (NSB) of ^{125}I-SP_1 in standards and in all samples. As NSB of ^{125}I-SP_1 for standards and samples was different (usually 7–8% and 5–6%, respectively) the percentage of binding for standards and samples was calculated by subtracting the corresponding NSB from antibody bound radioactivity and the total amount of radioactivity added, respectively. A spline function programme (Reinsch, 1967) was used to calculate the SP_1 concentration in the samples.

Serial dilution of 4 serum samples with a concentration of $SP_1 > 2.0\,\mu g\,l^{-1}$ were parallel with the standard curve (Figure 1 (a–d)). Furthermore, if various amounts of SP_1, 25–400 pg, (pregnancy serum) were added to a non-pregnancy serum pool (from patients) a constant difference was found, corresponding to an SP_1 concentration of $1.4\,\mu g\,l^{-1}$. However, the dose-response for samples with low SP_1 values was less steep than the standard curve (Figure 1 (e, f)).

Interassay variation was estimated by repeated analysis of a normal serum pool and a pregnancy serum pool diluted 1:50 and 1:25 with assay buffer. The mean values were 1.3, 2.3 and $4.5\,\mu g\,l^{-1}$ and the coefficient of variation was 11.8–13.2% ($n = 10$–14).

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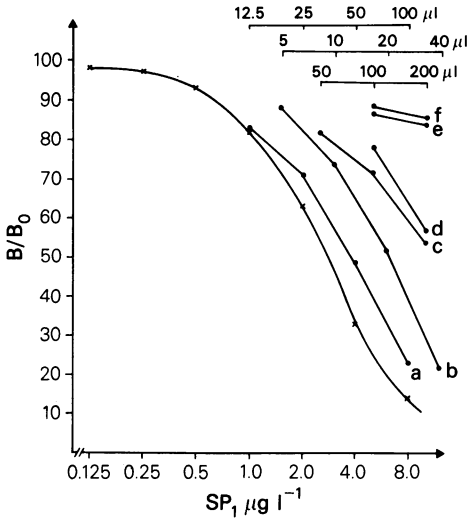

Figure 1 Parallelism of standard curve (X) and serial dilutions of serum samples (●) from patients with high (a–d) and low (e–f) concentrations of SP_1.

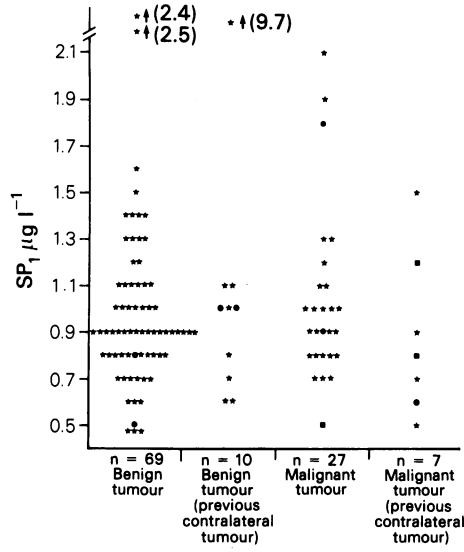

Figure 2 SP_1 level in sera and degree of staining for SP_1 in tumour tissue from patients with benign or malignant breast tumours. Tumour cells negative or uncertain weak reaction (★), tumour cells weak SP_1 positive (●), tumour cells strong SP_1 positive (■).

The detection limit of the assay was $0.5\,\mu g l^{-1}$, the smallest concentration of SP_1 which could be distinguished from a standard without SP_1 (the zero standard). A 95% confidence interval for the estimate of zero standard ($n=15$) differed from a 95% confidence interval for the estimate of $0.5\,\mu g l^{-1}$ standard ($n=15$).

The pathological material consisted of conventional formalin-fixed, wax-embedded histological sections of tumour tissue from the patients. By means of indirect immunoperoxidase technique (Heyderman, 1979), the breast tumours were investigated for the presence of SP_1 with rabbit anti-human SP_1 (Lot No. 018 C, Dakopatt, Denmark) at a dilution of 1:30. The degree of staining was assessed in the epithelium of ducts, within the lumen, in myoepithelial cells, in stroma and if present, in the cells of the tumour. If no reaction or a doubtful weak reaction developed the staining was regarded as negative. When staining was positive the most positive staining was registered as weak or strong.

Sections from SP_1 positive breast carcinomas to confirm antibody specificity were incubated with antiserum, which had been absorbed with purified SP_1 (Sørensen & Trentemøller, 1983). The staining completely disappeared. In addition no colour reaction was apparent in the sections when buffer replaced anti-SP_1 antiserum.

The tumours were classified by H & E stained sections.

Almost all the values of SP_1 in the sera of women with benign breast tumours were from <0.5 to $1.6\,\mu g l^{-1}$ (Figure 2). One woman had a slightly increased concentration of $2.5\,\mu g l^{-1}$ and another on hormonal substitution therapy with oestradiol and norgestrel had an inexplicable high value of $24\,\mu g l^{-1}$. In 10 patients with benign tumours and previous contralateral breast cancer the range was similar to that of women with malignant breast disorders except for one patient, who had an increased concentration of $9.7\,\mu g l^{-1}$. She died during the follow-up period from a recurrence of her breast cancer. In 34 patients with breast cancer only 3 patients had a slightly increased concentration of SP_1 ($1.8–2.1\,\mu g l^{-1}$), whereas 31 patients had an SP_1 concentration ranging from $<0.5\,\mu g l^{-1}$ to $1.5\,\mu g l^{-1}$, corresponding to the level in patients with benign breast diseases.

Immunohistochemical investigation with indirect immunoperoxidase technique for SP_1 in various benign breast diseases was negative except in 2 patients with intraductal papilloma where a few tumour cells showed weak SP_1 activity and in 2 patients with a simultaneous relapse of their first breast cancer where normal duct epithelium showed slight activity. SP_1 could be demonstrated in tumour cells in 6/34 (18%) malignant breast tumours. In all tumours the SP_1 reactivity was heterogeneous varying from negative to different degrees of positive staining. The SP_1 reactivity was in all tumours localized in the cytoplasm of the tumour cells (Figure 3). Only one of 6 patients with

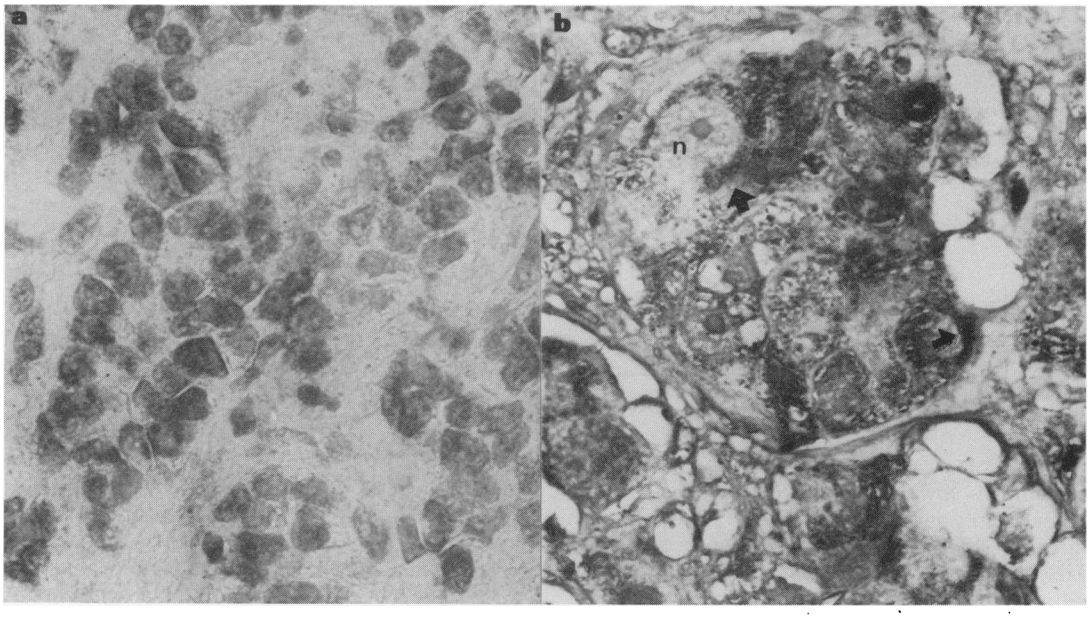

Figure 3 Mammary carcinoma incubated with anti-SP_1. **A**: tumour cells without SP_1 reaction. **B**: tumour cells with SP_1 localized in the cytoplasm of cells (arrows). Immunohistochemical staining $\times 590$ n: nucleus.

positive SP_1 staining in the tumour died from the breast cancer during the observation period of 3 years (Table I).

The detection limit chosen does not include non-specific interference (noise) (Hunter & Bennie, 1979). When sera from subjects who might be expected to have little or no SP_1 in the circulation were assayed, responses were close to the detection limit, but significant. Determinations of these samples in two dilutions seemed to display responses which were less steep than the corresponding part of the standard curve, Figure 1 (e, f). This might indicate non-specificity – although the values were derived from the upper more imprecise part of the curve – or presence of a minor SP_1 component, $SP_1(\gamma)$, (Sørensen & Trentemøller, 1983). The non-specific reactivity may be large enough to obscure specific determinations, particularly at low levels (Hunter & Bennie, 1979). However, a parallelism was found between the standard curve and dilutions of serum samples with an SP_1 concentration $>2\,\mu g\,l^{-1}$ (Figure 1).

A range for serum SP_1 in women with benign breast tumour was obtained which agreed with that in healthy subjects (Kaminska *et al.*, 1979; Würz, 1979; Rosen *et al.*, 1982). For patients with breast cancer the SP_1 concentration was of the same level as that in women with benign tumours. No values above $3\,\mu g\,l^{-1}$ were found which agreed with other studies (Bremmer *et al.*, 1981; Rosen *et al.*, 1982), but conflicted with studies previously reported to have from 22% to 29% of SP_1 determinations $>3\,\mu g\,l^{-1}$ (Searle *et al.*, 1978; Würz, 1979) and 8% to 11% $>10\,\mu g\,l^{-1}$ (Würz, 1979; Tatarinov, 1980). In a less sensitive assay with a detection limit of $10\,\mu g\,l^{-1}$, SP_1 was observed in only one of 42 patients with malignant breast disorders (Grudzinskas *et al.*, 1980). However, SP_1 has been measured in the majority of homogenates of breast tumour tissue, both malignant and benign (Bremmer *et al.*, 1981) although the concentrations measured were close to the detection limit.

By means of an indirect immunoperoxidase technique, SP_1 was absent in all benign tumours except 4. Two of these had intraductal papillomatosis. In another study no benign tumours out of 12 were found to be SP_1 positive (Horne *et al.*, 1976). In malignant breast tumours SP_1 was present in only 17% of the patients compared with 76, 53 and 37% in other studies (Horne *et al.*, 1976; Inaba *et al.*, 1980; Walker, 1981). The explanation may be differences in the methods, the antisera, or the representativeness of the histological sections since SP_1 positive cells are irregularly distributed in the tumour, or in the composition of the tumours. No correlation seems to exist between the intensity of SP_1 staining in the tumour and the serum SP_1 level. Strong SP_1 positive tumours had normal serum SP_1 concentration and *vice versa*. The significance of the degree of differentiation for the presence of SP_1 is

Table I Clinical and histological findings in breast cancer patients with serum $SP_1 > 1.6\,\mu g\,l^{-1}$ or positive SP_1 staining of the tumour

	Histological diagnosis (WHO)	*Histologically involved ratio of lymph nodes*	*Preoperative tumour size (cm)*	*Relapse*	*S-SP_1 $\mu g\,l^{-1}$*
S-$SP_1 > 1.6\,\mu g\,l^{-1}$	Invasive ductal carcinoma	—/—	2	No	1.8
	Invasive ductal carcinoma	11/16	—	No	1.9
	Mucinous carcinoma	0/7	2	No	2.1
Positive SP_1 staining	Invasive ductal carcinoma	4/18	$1\frac{1}{2}$	No	<0.5
	Intraductal carcinoma	—	2	Yes, died	0.6
	Papillary carcinoma	0/5	$2\frac{1}{2}$	No, died	0.8
	Invasive ductal carcinoma	—	—	Yes	0.9
	Invasive ductal carcinoma	0/7	$2\frac{1}{2}$	No	1.2
	Invasive ductal carcinoma	—/—	2	No	1.8

uncertain. A low occurrence of SP_1 was found histochemically in poorly differentiated carcinomas (Walker, 1981), whereas homogenates of poorly differentiated carcinomas had a higher concentration of SP_1 than those of well differentiated tumours (Bremmer *et al.*, 1981).

The presence of SP_1 in malignant tumours might indicate a shorter survival (Horne *et al.*, 1976), but the low incidence of SP_1 positive tumours and a follow-up period of only 3 years in this study meant that the number of patients was too small to permit satisfactory statistical analysis. Furthermore, various postoperative chemotherapeutic regimes may influence the survival.

In conclusion, quantification of SP_1 in sera or an investigation for the presence of SP_1 in tumour tissue seem to be of little clinical value in the management of patients with breast cancer. On the other hand, SP_1 has been demonstrated in some breast cancers and it remains to be elucidated whether this detection indicates local production or an uptake of SP_1 from the circulation. Finally, a study is required to determine whether the serum SP_1 levels obtained are truly being assayed or arise from a matrix effect in the radioimmunoassay.

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References

ANDERSEN, K.W., MOURIDSEN, H.T., CASTBERG, Th. & 8 others. (1981). Organisation of the Danish adjuvant trials in breast cancer. *Dan. Med. Bull.*, **28**, 102.

AZZOPARDI, J.G., CHEPICK, O.F., HARTMANN, W.H. & 10 others. (1982). The World Health Organization histological typing of breast tumours – second edition. *Am. J. Clin. Pathol.*, **78**, 806.

BREMMER, R.D., NISBET, A.D., HERRIOT, R. & 4 others. (1981). Detection of placental protein five (PP5) and pregnancy-specific glycoprotein (SP_1) in benign and malignant breast disease. *Oncodev. Biol. Med.*, **2**, 55.

GRUDZINSKAS, J.G., COOMBES, R.C., RATCLIFFE, J.G. & 4 others. (1980). Circulating levels of pregnancy specific β_1 glycoprotein in patients with testicular, bronchogenic and breast carcinomas. *Cancer*, **45**, 102.

HEYDERMAN, E. (1979). Immunoperoxidase technique in histopathology: applications, methods and controls. *J. Clin. Pathol.*, **32**, 971.

HORNE, C.H.W., REID, I.N. & MILNE, G.D. (1976). Prognostic significance of inappropriate production of pregnancy proteins by breast cancers. *Lancet*, **ii**, 279.

HUNTER, W.M. & BENNIE, J.G. (1979). Reduction of non-specific serum responses in human pituitary gonadotrophin radioimmunoassays. *J. Endocrinol.*, **80**, 59.

KAMINSKA, J., CALVERT, I. & ROSEN, S.W. (1979). Radioimmunoassay of "pregnancy-specific" β_1-glycoprotein (SP_1). *Clin. Chem.*, **25**, 577.

INABA, N., RENK, T., WURSTER, K., RAPP, W. & BOHN, H. (1980). Ectopic synthesis of pregnancy specific β_1-glycoprotein (SP_1) and placental specific tissue proteins (PP_5, PP_{10}, PP_{11}, PP_{12}) in nontrophoblastic malignant tumours. Possible markers in oncology. *Klin Wochenschr.*, **58**, 789.

ROSEN, S.W., GAIL, M.H. & TORMEY, D.C. (1982). Use of circulating pregnancy specific β_1-glycoprotein as a marker in carcinoma of the breast in women. *J. Natl Cancer Inst.*, **69,** 1067.

REINSCH, C.H. (1967). Smoothing by spline functions. *Num. Math.*, **10,** 177.

SEARLE, F., LEAKE, B.A., BAGSHAWE, K.D. & DENT, J. (1978). Serum SP_1-pregnancy-specific-β-glycoprotein in choriocarcinoma and other neoplastic disease. *Lancet*, **i,** 579.

SØRENSEN, S. & TRENTEMØLLER, S. (1983). Purification of the major component of the pregnancy specific β_1-glycoprotein (SP_1) by affinity chromatography, and application to a highly sensitive radioimmunoassay. *Oncodev. Biol. Med.*, **4,** 351.

TATARINOV, Y.S. (1980). The diagnostic value of circulating trophoblast-specific β_1-glycoprotein (TSG) in cancer patients. *Br. J. Cancer*, **41,** 821.

WALKER, R.A. (1981). Differentiation of human breast carcinomas: An immunohistological study of appropriate and inappropriate protein production. *J. Pathol.*, **135,** 87.

WÜRZ, H. (1979). Serum concentrations of SP_1 (pregnancy-specific-β_1-glycoprotein) in healthy, non-pregnant individuals, and in patients with nontrophoblastic malignant neoplasms. *Arch. Gynecol.*, **227,** 1.