Short Communication

Pregnancy-specific $\beta_1$-glycoprotein (SP$_1$) in serum and tissue from patients with benign and malignant breast tumours

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The pregnancy specific $\beta_1$-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$^{-1}$ were parallel with the standard curve (Figure 1 (a–d)). Furthermore, if various amounts of SP$_1$, 25–400 pg, (pregnancy seru.m) 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$^{-1}$. 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$^{-1}$ and the coefficient of variation was 11.8–13.2% ($n = 10–14$).
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 antisera, 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
positive SP₁ staining in the tumour died from the breast cancer during the observation period of 3 years (Table 1).

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 SP₁ 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 SP₁ component, SP₁(γ), (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 SP₁ concentration >2 μg l⁻¹ (Figure 1).

A range for serum SP₁ 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 SP₁ concentration was of the same level as that in women with benign tumours. No values above 3 μg 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 SP₁ determinations >3 μg l⁻¹ (Searle et al., 1978; Würz, 1979) and 8% to 11% >10 μg l⁻¹ (Würz, 1979; Tatarinov, 1980). In a less sensitive assay with a detection limit of 10 μg l⁻¹, SP₁ was observed in only one of 42 patients with malignant breast disorders (Grudzinskas et al., 1980). However, SP₁ 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, SP₁ 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 SP₁ positive (Horne et al., 1976). In malignant breast tumours SP₁ 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 SP₁ positive cells are irregularly distributed in the tumour, or in the composition of the tumours. No correlation seems to exist between the intensity of SP₁ staining in the tumour and the serum SP₁ level. Strong SP₁ positive tumours had normal serum SP₁ concentration and vice versa. The significance of the degree of differentiation for the presence of SP₁ is

Figure 3 Mammary carcinoma incubated with anti-SP₁. A: tumour cells without SP₁ reaction. B: tumour cells with SP₁ localized in the cytoplasm of cells (arrows). Immunohistochemical staining × 590 n: nucleus.
Table I Clinical and histological findings in breast cancer patients with serum SP$_1$ > 1.6 μg l$^{-1}$ or positive SP$_1$ staining of the tumour

| SP$_1$ diagnosis (WHO) | Histologically involved ratio of lymph nodes | Preoperative tumour size (cm) | Relapse | S-SP$_1$, μg l$^{-1}$ |
|-----------------------|---------------------------------------------|------------------------------|---------|---------------------|
| S-SP$_1$ > 1.6 μg l$^{-1}$ | Invasive ductal carcinoma | 2 | No | 1.8 |
| | Invasive ductal carcinoma | 11/16 | No | 1.9 |
| | Mucinous carcinoma | 0/7 | No | 2.1 |
| Positive SP$_1$ staining | Invasive ductal carcinoma | 4/18 | 1.5 | <0.5 |
| | Intraductal carcinoma | --- | 2 | Yes, died | 0.6 |
| | Papillary carcinoma | 0/5 | 2 | No, died | 0.8 |
| | Invasive ductal carcinoma | --- | --- | Yes | 0.9 |
| | Invasive ductal carcinoma | 0/7 | 2 | No | 1.2 |
| | Invasive ductal carcinoma | --- | 2 | No | 1.8 |

A low occurrence of SP$_1$ was found histochromically 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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