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Onco-developmental Protein Concentrations in the Sera of Patients with Ovarian Cancer Prior to Treatment

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Abstract—*Eight onco-developmental proteins have been measured in the serum of women prior to their operation and treatment for ovarian cancer. Alpha-foetoprotein, CEA, hCG and PAPP-A were not often elevated whereas CPAP, SP_1, CanAg 50 and CA 125 were. Many of our findings differed from those of previous workers, possibly due to differences in clinical material and analytical technique. We conclude that large profiles of tumour markers are no longer of use in the investigation of ovarian cancer. At the present time we think that CA 125 could prove the most useful marker in the investigation of ovarian cancer.*

INTRODUCTION

IT HAS long been hoped that the discovery of specific tumour markers in the blood would enable the timely diagnosis of cancer to be achieved. In general, experience has shown tumour marker analyses to be of little use in cancer diagnosis, but their analysis may be valuable in following the course of treatment and detecting recurrence of the disease. Although the successful treatment of ovarian cancer is dependent upon early diagnosis and the skill with which the initial surgical treatment is undertaken, tumour marker analysis may provide a reliable method of monitoring subsequent treatment. To optimize this approach it is necessary to have baseline estimations of potential markers. To this end we have examined prior to treatment the following eight onco-developmental proteins, some recently discovered others long known, in the serum of women with ovarian cancer.

Alpha-foetoprotein (αFP), human chorionic gonadotrophin (hCG) and carcino-embryonic antigen (CEA) have been extensively used in the investigation of a variety of malignancies including ovarian cancer [1]. The association of carcino-placental alkaline phosphatase (CPAP) with cancer was originally described by Fishman *et al.* [2] in a case of bronchial cancer and more recently by Haije *et al.* [3] and Eerdekens *et al.* [4] in gynaecological malignancies. Pregnancy specific β_1 glycoprotein (SP_1) has been investigated in patients with ovarian cancer by Crowther *et al.* [5] but since then has been little used in that context. Pregnancy associated plasma protein A (PAPP-A) has not been used previously to investigate ovarian cancer and was included in this study because of an existing departmental interest in this protein. CA 125 has been proposed as a potentially useful marker for monitoring ovarian cancer [6], whereas CanAg 50 [7], although not specific for ovarian cancer, may be useful in the laboratory investigation of this disease if it is found to be elevated in a large proportion of patients.

MATERIALS AND METHODS

Clinical material

Blood was taken before or during operation from patients suspected of having ovarian cancer. The majority of blood samples were sent directly to the laboratory although a few were kept at 4°C for up to 24 h before being taken to the laboratory. On receipt, the blood samples were centrifuged and the serum stored at −20°C. Only patients diagnosed by surgical and histological criteria to have ovarian cancer were included in the study. No patients already undergoing treatment for ovarian cancer were investigated. In all, 33 patients were investi-

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gated although not all markers were assayed in all patients due to lack of serum sample.

Analytical methods

(a) *Alpha-foetoprotein* (*αFP*) was measured by an in-house radioimmunoassay utilizing ^{125}I labelled α-foetoprotein obtained from the Department Obstetrics, Ninewells Hospital, Dundee DD1 9SY, U.K. and anti-α-foetoprotein obtained from the Scottish Antibody Production Unit, Law Hospital, Carluke, Lanarkshire, ML8 5ER, U.K. Precipitation was carried out using polyethylene glycol. The assay system was standardized against International Reference Preparation 72/227.

(b) *Human chorionic gonadotrophin* (*hCG*) was measured by RIA using a kit produced by NMS Pharmaceutics Inc. Newport Beach CA. The antiserum used was raised against the β-subunit and the assay standardized against the Second International Reference Preparation.

(c) *Carcino-embryonic antigen* (*CEA*) was measured by in-house radioimmunoassay utilizing iodination grade CEA provided by Prof. K. Bagshawe (Charing Cross Hospital, London, U.K.) and anti-CEA provided by Dako Immunoglobulins, Copenhagen, Denmark. The assay was standardized against the First British Standard 73/601 [8].

(d) *Carcino-placental alkaline phosphatase* (*CPAP*) was measured by an immuno-enzymatic assay and standardized against a secondary serum standard prepared by heat-treating pooled pregnancy serum which was then assayed by standard enzymatic methods [9].

(e) *Pregnancy specific* β_1 *glycoprotein* (SP_1). This was assayed by an in-house ELISA [10, 11] modified to enhance the sensitivity. The modifications consisted of increased incubation times at each stage of the procedure and the use of undiluted test sera. The assay was standardized against a standard preparation provided by Hoechst Pharmaceuticals, Hounslow, TW4 6JH, U.K.

(f) *Pregnancy associated plasma protein A* (*PAPP-A*). This was measured by an in-house ELISA [12] and standardized against the World Health organization preparation IRP 78/610.

(g) *CA* 125 was measured by an immunoradiometric assay using test kits supplied by CIS (U.K.) Ltd, High Wycombe, Bucks, HP12 3RD, U.K. Results were calculated as U/ml with reference to standard preparations provided by the manufacturer.

(h) *CanAg* 50 was measured by RIA inhibition using a kit supplied by RIA (U.K.) Ltd, Washington, Tyne and Wear, NE37 3HS, U.K. Results were calculated according to the manufacturer's instructions.

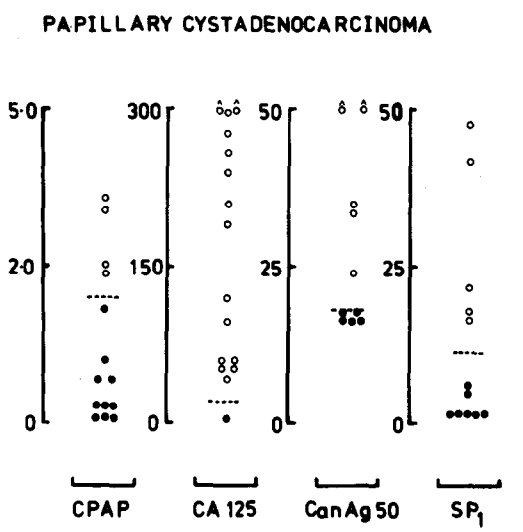

Fig. 1. The distribution of normal (•) and abnormal (○) results for CPAP, CA 125, CanAg 50 and SP_1 in patients with papillary cystadenocarcinoma. The upper limits of the reference ranges (shown as a broken line) and the units of measurement are designated in Table 1. Results which are high and off-scale are indicated thus: ô.

RESULTS

Thirty-three patients with histologically proven ovarian cancer were investigated. There were 16 patients with papillary cystadenocarcinoma, seven with mucinous cystadenocarcinoma, nine with solid adenocarcinoma and one with a granulosa cell carcinoma. Twenty-three patients (70%) had stage III or IV disease at diagnosis. The number of subjects examined for each tumour marker and the numbers of elevated results obtained is summarized in Table 1. Figures 1, 2 and 3 illustrate for the three main histological categories the results obtained for the four most commonly elevated tumour markers (CPAP, SP_1, CA 125 and CanAg 50). Sixteen subjects had CPAP, SP_1, CA 125 and CanAg 50 measured on the same samples and of these, 14 (88%) had raised Ca 125 antigen concentrations. Of the two remaining subjects one, that with the granulosa cell carcinoma, had slightly raised SP_1 and αFP concentrations. The other patient was a stage 1a papillary cystadenocarcinoma. She also had an elevated αFP level.

DISCUSSION

For many years the laboratory investigation of ovarian cancer has been hampered by a lack of suitable serum tumour markers. Many markers may be elevated, but few are found to be consistently raised in all patients with active disease. Of the eight putative tumour markers investigated in this study only CPAP, SP_1, CA 125 and CanAg 50 seemed worthy of further consideration. CA 125 has been promoted as a useful marker for monitoring ovarian cancer. CanAg 50, on the other hand, is said to be a general marker for epithelial cancers. CPAP has been recognised as a marker for a variety

Table 1.

Tumour marker	Number of subjects	Number with raised marker concentration
αFP	24	2 (8%)
hCG	24	0
CEA	24	0
CPAP	29	8 (28%)
SP_1	23	7 (30%)
PAPP-A	23	1 (4%)
CA 125	33	25 (76%)
CanAg 50	21	11 (52%)

Reference ranges:				
	αFP:	0–15 U/ml	SP_1:	0–13 μg/l
	hCG:	0–5 U/l	PAPP-A:	0–36 U/l
	CEA:	0–8 U/l	Ca 125:	0–20 U/l
	CPAP:	0–2 U/l	CanAg 50	0–17 U/ml

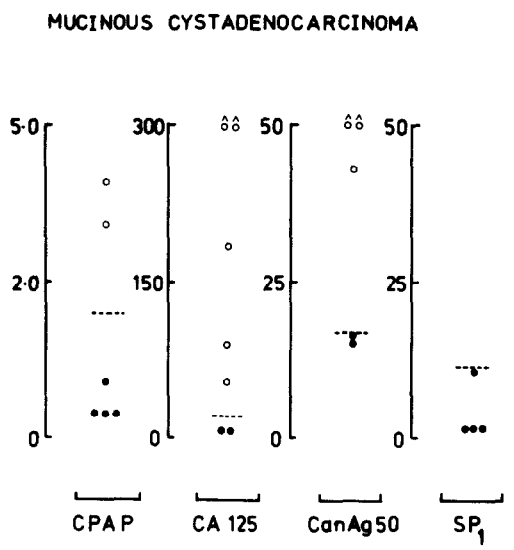

Fig. 2. *The distribution of results in patients with mucinous cystadenocarcinoma. Details as in Fig.* 1.

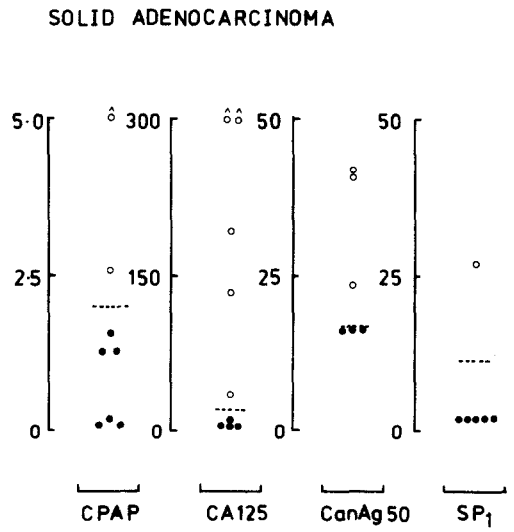

Fig. 3. *The distribution of results in patients with a solid adenocarcinoma. Detail as in Fig.* 1.

of malignancies, whereas SP_1 has been but little researched as a tumour marker.

When the three major histological categories are considered separately the percentage of raised CA 125 results ranges from 56% (solid adenocarcinoma) to 94% (papillary cystadenocarcinoma). In contrast SP_1 remained within normal limits in the four cases of mucinous cystadenocarcinoma, was raised in 6% (one patient) with a solid adenocarcinoma and 42% (five patients) with papillary cystadenocarcinoma. CPAP was raised in 25–33% of subjects and CanAg 50 in 50–60%, according to diagnosis.

In our hands αFP, CEA and hCG have been remarkably insensitive. This contrasts with the work of Cauchi *et al.* [1] who investigated the onco-foetal antigens, αFP, CEA and hCG in cancers of the ovary and cervix. They examined the consequences of alteration in diagnostic threshold or cut-off levels where one or more tests were positive and found that 53% of patients were positive when the cut-off was set at the lowest level. However, this level of sensitivity was bought at a cost of a false positive rate of 42–54%. Sarjadi *et al.* [13] compared a wide variety of enzymes, polyamines, onco-foetal proteins and other substances in patients with cancer of the ovary or uterine cervix. The most sensitive ovarian marker in patients with ovarian cancer was β2 microglobulin which was raised in 46% of patients. When this marker was considered with CEA, haemoglobin F and lactate dehydrogenase, 79% of patients had one or more markers raised. Eerdekens *et al.* [4] compared CPAP (which they call HPLAP) with CA 125 in patients with benign and malignant diseases. They reported that only 50% of their patients with ovarian cancer had raised CA 125 levels and 45% raised CPAP. These contrasting results may be partly explained by differences in clinical material. It is not clear from the details given when blood was taken in relation to the initial diagnosis and treatment; nor are the various tumour categories given.

When we were able to measure CPAP, SP_1, CA 125 and CanAg 50 in the same patients 88% had raised CA 125 concentrations, and 94% raised CA 125 *or* SP_1 concentrations. CPAP and CanAg 50 were never raised when CA 125 was normal. Thus a profile of the above four antigens does not appear to provide worthwhile information over and above Ca 125 and SP_1. Furthermore a much larger series will be needed to confirm the slight increase in information provided by simultaneous measurement of CA 125 and SP_1.

The sensitivity of CPAP in our hands was disappointing compared with the findings of Eerdekens *et al.* [4]. This may be due to our use of a polyclonal [9] rather than a monoclonal antibody. Our results clearly indicate that, overall, CA 125 is the most sensitive tumour marker, but Eerdekens *et al.* [4] drew attention to its lack of specificity. Both CPAP and CA 125 were raised in 20% of non-ovarian cancer patients, and in a variety of benign chronic diseases 23% of patients had a raised CA 125, whereas CPAP was elevated in only 2% of cases.

Canney *et al.* [14] also draw attention to the lack of specificity of CA 125, but report a positive association between progression or regression. A lack of diagnostic specificity may not be important unless the marker is to be used in population screening. Where a marker is to be used to follow the course of a diagnosed disease, sensitivity is more important.

Few other studies have compared so many potentially relevant markers for epithelial ovarian cancer. In conclusion, therefore, we are of the opinion that it is no longer worthwhile pursuing the use of large profiles of tumour markers in the investigation of ovarian cancer. Refinements in methodologies and careful consideration of the limits in specificity should enable the biochemical monitoring of the majority of ovarian cancer patients to be successfully undertaken. In particular, our studies indicate that CA 125 could prove to be the most useful of these markers, particularly in papillary cystadenocarcinoma.

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