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Radioimmunoassay of the Normal Serum Glycoprotein (CX 1) in Monitoring Ovarian Malignancy*

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Abstract—*A glycoprotein designated CX 1, of molecular weight 80,000, has been extracted from adenocarcinomas of the ovary and its measurement by radioimmunoassay established. CX 1 is present in the normal ovary and in normal serum. It is present in various non-ovarian carcinomas but in much greater amount in ovarian adenocarcinoma and in ascitic fluid associated with this cancer. Increased concentrations are found in the serum of patients with various cancers. In about 60% of patients with Stage III and IV ovarian adenocarcinoma, serum values are elevated and they correlate with the course of the disease, providing information which probably has clinical value.*

INTRODUCTION

ADENOCARCINOMA of the ovary is the commonest fatal gynaecological malignancy. In England and Wales deaths increased from 2,702 in 1958 to 3,784 in 1978; the peak incidence lies between 55 and 65 years of age [1]. Although surgery, radiotherapy and chemotherapy each contribute usefully to management, treatment of advanced ovarian cancer remains unsatisfactory. Good biochemical markers make a valuable contribution to clinical management and curability of choriocarcinoma and malignant teratoma, so similar markers for ovarian epithelial tumours have been sought but so far none have provided the sensitivity or reliability required to provide a basis for earlier diagnosis [2]. In some patients raised levels of carcinoembryonic antigen (CEA), placental alkaline phosphatase or human chorionic gonadotrophin (HCG) have been found, but even in advanced disease they have not occurred with sufficient frequency to command regular use. In this paper we report preliminary clinical experience with measurements of a normal serum glycoprotein, designated CX 1, which seems to be of value in monitoring the course of about 60% of patients with Stage III or IV ovarian adenocarcinoma. It may also be of value in distinguishing ascites due to ovarian adenocarcinoma from other causes. Preliminary description of this antigen [3] used the eponym OTAG, but this implied more specificity for ovarian tumours than is justified by later evidence.

MATERIALS AND METHODS

Isolation and properties of antigen CX 1

In summary, CX 1 was prepared from an homogenate of serous papillary cystadenocarcinoma in phosphate-buffered saline, pH 7.8. This was sonicated and centrifuged, and the supernant was dialysed against distilled H_2O and then freeze-dried. An antiserum raised in rabbits against this preparation showed lines of continuity in gel diffusion studies with homogenates from 6 similar tumours. The antiserum was absorbed against normal ovarian extracts and used to monitor further purification through ammonium sulphate precipitation, Con-A Sepharose affinity chromatography, immunosubtraction with anti-human serum bound to Sepharose and G-200 Sephadex gel-filtration. This preparation was in turn used to produce further antisera for the purification of CX 1 by affinity chromatography from ascitic fluids.

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The homogeneity of CX 1 used for radioimmunoassay was examined by crossed immunoelectrophoresis[4] against an unabsorbed antiserum R 53 (Fig. 1). SDS polyacrylamide electrophoresis[5] with 30 μg of sample gave 2 major bands close together at M. W. of approximately 80,000 with some faint bands indicating impurities. Molecular weight by G-200 Sephadex gel filtration was also close to 80,000. The isoelectric point of CX 1 purified from ascites was in the 4.9–5.8 range, whereas that purified from tumour homogenates had a pI near 7.0. CX 1 is destroyed by perchloric acid and by heating at 80°C for 5 min and it has β-electrophoretic mobility. Its amino acid and carbohydrate composition are shown in Fig. 2.

Using gel diffusion, rocket electrophoresis or radioimmunoassay, CX 1 has been distinguished from CEA [6], β-oncofetal antigen [7], SP_1 [8] and the commercially available preparations of human placental alkaline phosphatase, lactoferrin, transferrin and secretory piece. It does not bind [^{3}H]-dihydrostestosterone and the antiserum is not absorbed out by blood group O human erythrocytes. It has been distinguished from the OCAA antigen of Bhattacharya [9], embryonic pre-albumin (EPA) of Tatarinov [10] and FRGP of Hamazaki [11] by Dr. Axelson in a WHO Collaborative Study on Ovarian Tumour Associated Antigens. CX 1 has been negative for enzymic activity attributable to glycosidases, glycosyltransferases, γ-glutamyl transpeptidase and amylase.

Amino acid composition of CX1

	Residues/100 residues
Asp	10.68
Thr	7.12
Ser	10.69
Glu	12.43
Pro	3.62
Gly	10.75
Ala	7.16
½ Cys	1.50
Val	2.38
Met	0.53
Ile	2.19
Leu	10.15
Tyr	2.91
Phe	2.91
His	2.75
Lys	4.55
Arg	7.64

Carbohydrate composition of CX1

	Residues/100 amino acid residues
Glucosamine	6.74
Galactosamine	1.03
Mannose	3.25
Galactose	1.74
Sialic acid	TRACE

Fig. 2.

Radioimmunoassay

CX 1 was iodinated with [^{125}I] by the method of Greenwood *et al.* [12] and the labelled material further purified by G-200 gel filtration. A double antibody method was used with R 53 antiserum at a dilution of 1/60,000 and sheep anti-rabbit serum at a dilution of 1/280. Standards for assay use were prepared from an ascites fluid and CX 1-depleted normal serum was added to standards to provide equivalent non-specific protein concentrations with the samples. The assay procedure was automated [13] and the sensitivity of the assay for the results reported here was 3–5 μg/l. The standard line covered the range 750 μg/l CX 1 down to 2.9 μg/l by doubling dilutions. Each assay included pooled sera as quality controls at concentrations of 25, 100 and 250 μg/l. The coefficient of variations for these low, medium and high controls was 13.6, 8.6 and 12.7% respectively, between assays, and 13.8, 5.6 and 10% within assays.

Samples were obtained from hospital laboratory staff and from patients with cancer and other diseases attending for treatment or follow-up and having blood taken for other purposes. Sera were stored at −20°C. The cancer patients included patients in clinical remission as well as those with early or late clinical relapse.

CX 1, CEA and HCG were measured by double antibody radioimmunoassay on multiple serum samples from 18 patients with ovarian adenocarcinoma. For this purpose CX 1 values > 70 μg/l, CEA values > 10 μg/l and HCG values > 5 μg/l were scored as positive, and the patients studied were divided into 6 patients in Group A who showed no clinical or radiological evidence of disease during the period of study and 12 patients in group B who either relapsed during the period of study or who had evident disease throughout.

RESULTS

Sera from most normal subjects of both sexes, aged 19–70, contained CX 1 in concentrations of

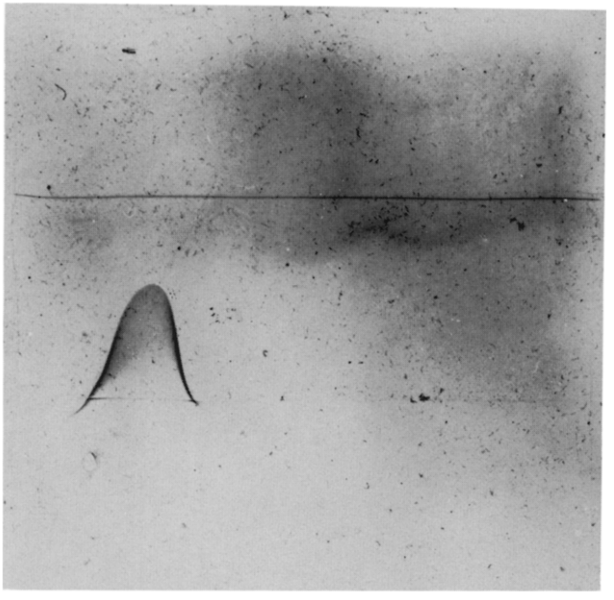

Fig. 1. *Crossed electrophoresis of purified* CX 1 (3 μg) *against* R53 *unabsorbed antiserum. Buffer was Tris–Barbital,* pH 8.6, I = 0.02. *First direction: in* 1% *agarose for* 1 hr *at* 10 V/cm; *second direction: the middle third of the plate was replaced with* 1% *agarose containing* 1% R53 *antiserum, and electrophoresis carried out at right angles for a further* 5 hr *at* 10 V/cm. *Staining was with Coomassie Brilliant Blue.*

30–70 μg/l; only 4% of 129 samples assayed exceeding 70 μg/l. Samples from women in early pregnancy assayed in the same range. A value of 70 μg/l was therefore adopted as a working upper limit of the normal range.

CX 1 was found in single samples of several biological fluids including foetal serum, seminal plasma and colostrum (Table 1). Sera from many patients with renal failure or vascular disease showed values > 70 μg/l (Table 2). CX 1 was also found in tissue culture media in which various malignant cell lines had been grown to confluence; the values ranged from 150 to 600 μg/l and were not higher in ovarian lines than in colonic, bronchial and choriocarcinoma cell lines. However, the concentration of CX 1 (calculated per mg protein) was much higher in homogenates from some ovarian adenocarcinomas than in homogenates from other tumours. Values for non-ovarian tumours were similar to those of normal ovary and in the range 5–20 times lower than in ovarian adenocarcinoma.

The concentration of CX 1 was > 70 μg/l in sera from some patients with ovarian or with non-ovarian cancer. The proportion of samples with increased values in patients with various cancers in remission or clinical relapse is shown in Table 3. Elevated values ranged from 70 to 2500 μg/l.

Concentrations of CX 1 in ascites fluids from patients with ovarian cancer, from non-malignant ovarian cysts, in serous effusions associated with other malignancies, and in non-malignant effusions and breast cyst fluids are shown in Fig. 3. It is evident that much higher concentrations of CX 1 (mg/l) are present in ascites fluid from some ovarian cancer patients, in contrast to the other malignant and to non-malignant effusions so far tested. One mesothelioma-associated effusion gave a value of 0.2 mg/l.

Serial measurements of CX 1 on 3 patients with ovarian cancer are shown in Figs. 4–6. Of 21 patients with Stage III and IV ovarian cancer, 13 (62%) showed elevated values before treatment; their values fell to, or almost to, the normal range in response to surgery and/or chemotherapy, and were raised again before, or by the time of, clinical recurrence.

On the other hand, in 8 patients (38%) serum values of CX 1 were hardly elevated at any time during the progression of their disease. Nonetheless, effusions arising in 2 patients in this group showed elevated values (500–700 μg/l). CX 1, CEA and HCG values on multiple samples from 18 patients with ovarian adenocarcinoma are summarised in Table 4.

DISCUSSION

We have found raised serum CX 1 values in 42% of randomly selected samples from patients with advanced, histologically proven

Table 1. CX 1 *concentrations in miscellaneous human biological fluids*

Specimen	Number examined	Concentration (μg/l)
Seminal plasma	1	887
Milk	1	203
Colostrum	1	768
Saliva	1	12
Urine	1	Not detectable
Amniotic fluid	2	135; 98
Foetal serum	2	527; 397

Table 2. CX 1 *Elevation in miscellaneous non-malignant pathological sera*

Diagnosis	Number of positives/total
Chronic renal failure	15/21
Thyrotoxicosis	1/1
Vascular disease	4/4
Others	5/31

Positive > 70 μg/l.

Table 3. CX 1 *Elevations in sera of patients with various cancers*

Diagnosis	Number of samples examined	% Positive
Ca-colon	10/55	18
Ca-lung	7/27	26
Ca-prostate	7/22	32
Ca-breast	20/57	35
Ca-ovary	291/694	42
Hepatoma	2/2	100
Leukaemia	13/82	16
Teratoma	10/55	18

CX 1 > 70 μg/l.

Table 4. *Comparison of number of samples positive for* CX 1, CEA *and* HCG *in* 18 *ovarian cancer patients followed serially*

	CX 1 +ve	CEA +ve	HCG +ve
Group A (remission)	1/66	5/58	1/67
Group B (relapsing)	95/133	4/80	10/141

ovarian cancer (Table 3). It is important to note that this group of results sometimes contains more than a single sample from one patient and that most of the patients were under treatment by surgery, chemotherapy or radiotherapy. Measurement of CX 1 at regular intervals of 1–2 months with full knowledge of clinical data has shown that CX 1 correlated well with the progress or regression of the disease in 13/21 patients. The marker does not generally give evidence of relapse much in advance of clinical or radiological evidence, although this is sometimes the case. It is possible that with more frequent sampling a short lead-in time would be seen more frequently.

One application of CX 1 appears to be in providing a sensitive quantitative indicator of response to therapy over a limited range of tumour burden. Responses to surgery, radiotherapy or chemotherapy appear to be reflected in falling serum values and progression of the disease in rising values. One of the main benefits of a good biochemical marker such as human chorionic gonadotrophin for choricarcinoma, for instance, lies in its change in concentration in serum reflecting tumour response or progression more promptly than radiological changes and thus allowing more effective use of available treatment. It is evident that measurement of CX 1 could not be used in

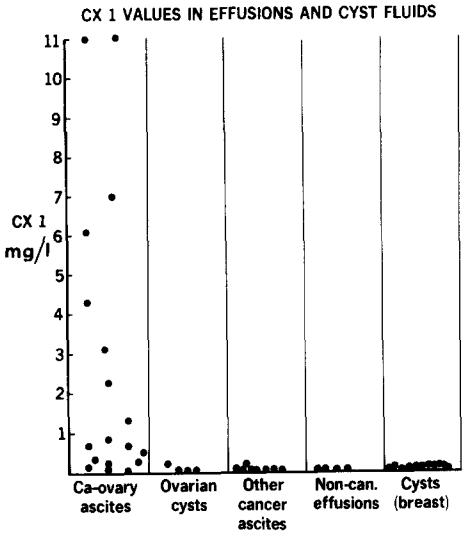

Fig. 3.

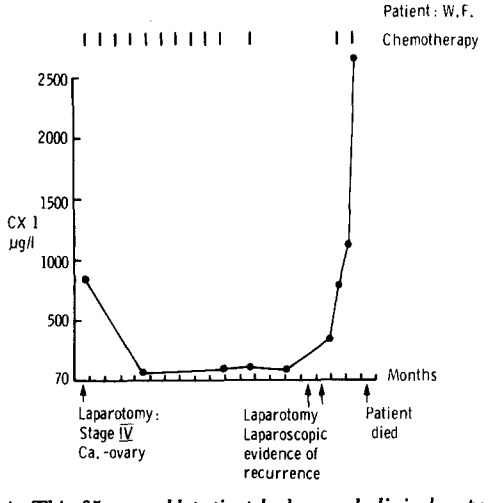

Fig. 4. This 65-year-old patient had a good clinical response to cis-Platinum and adriamycin. Following laparotomy for pelvic recurrence after one year, her disease progressed rapidly and renal failure may have contributed to the very high CX 1 values observed terminally. Rising values of CX 1 did not antedate clinical relapse although marginally elevated values had persisted. HCG values in this patient were marginally raised throughout her illness and increased terminally to 44 IU/l.

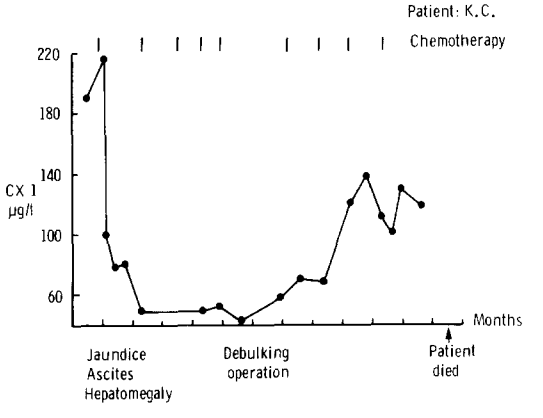

Fig. 5. This 54-year-old patient had Stage IV anaplastic carcinoma at presentation and had a good clinical response to cis-Platinum. At the debulking operation tumour was found microscopically in the excised omentum. CX 1 values increased 1–2 months before clinical relapse with hepatic metastases. This patient had an HCG value of 81 IU/l initially which fell with treatment and did not increase subsequently; her initially normal α-fetoprotein increased terminally to 49 µg/l.

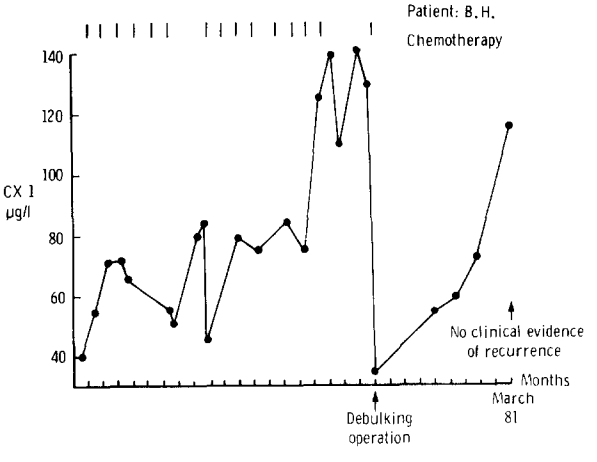

Fig. 6. A 56-year-old patient who had Stage III disease on presentation and had undergone chemotherapy for one year before CX 1 studies were started. Multidrug chemotherapy failed to prevent disease progression and surgical debulking was undertaken followed by a marked fall in CX 1 values. Following this she remained clinically and radiologically disease free. Rising CX 1 values anteceded clinical recurrence by 2–3 months.

early serological diagnosis since it is present in normal sera and the contribution from ovarian cancer is only evident in relatively advanced disease.

The regular presence of elevated values of CX 1 in ascites fluids caused by ovarian cancer may be valuable in distinguishing ovarian adenocarcinoma from other causes of ascites, but more samples need to be studied to determine its reliability in this context.

It is evident that more studies of CX 1 related to histological type, grade and stage of disease are required in ovarian adenocarcinoma. Tissue localisation studies are in progress. The range of variation in samples from normal individuals also needs to be determined. Although CX 1 falls far short of an ideal tumour marker it may have some clinical utility until such time as better markers become available. The use of CX 1 as a marker in other tumours remains to be determined, but it is noted that the incidence of positive samples from breast and prostatic cancer patients approaches that found in ovarian cancer.

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