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Cancer-associated proteins in effusion fluids

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SUMMARY Two cancer-associated proteins, carcinoembryonic antigen (CEA) and pregnancy-associated α_2 glycoprotein (PAG), together with 13 normal serum proteins were measured in the serum and effusion fluid of patients with ascites and pleural effusions. The results indicate that CEA measurement in effusion fluid is more effective than serum measurement in distinguishing cancerous from congestive or inflammatory effusions. Comparisons with the results of cytological examination suggest that fluid CEA estimation may prove a useful clinical tool. Serum PAG levels were higher in patients with cancer, but fluid determination offers no advantage in separating the disease groups. Similarly, the estimation of individual normal serum proteins in effusion fluids is unlikely to be of diagnostic value.

Patients with ascitic or pleural effusion often present difficult diagnostic problems. Various investigative aids are used in clinical practice, including effusion total protein estimation and cytological examination of the fluid for malignant cells (Rovelstad *et al.*, 1958; McGuckin *et al.*, 1959). These techniques do not, however, always ensure a correct diagnosis, and in the absence of positive cytology it may be difficult to differentiate between neoplastic and inflammatory effusions without resorting to exploratory surgery.

Several cancer-associated proteins have been described, the serum concentrations of which are frequently increased in cancer patients (Neville and Cooper, 1976). Carcinoembryonic antigen (CEA), the protein most extensively studied, has been found to be of strictly limited value as a diagnostic test when measured in serum, even in patients with advanced disease (Laurence *et al.*, 1972; Booth *et al.*, 1973). Similarly, pregnancy-associated α_2 glycoprotein (PAG) may be raised in the serum of patients with cancer, but increases are also encountered in normal pregnancy and in association with oestrogen therapy (Horne *et al.*, 1973; Stimson, 1975).

Tissue culture studies show that colonic carcinoma cells *in vitro* secrete CEA into the surrounding culture fluid (Burtin *et al.*, 1970; Egan and Todd, 1972), a situation in many ways comparable to the carcinomatous effusion. This concept encouraged the investigation of the diagnostic value of CEA measurement in effusion fluids. In addition, the serum and fluid concentration of PAG and 13 normal serum proteins have also been studied.

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Patients and methods

Fifty-six patients were included in the study, 31 with ascites and 25 with pleural effusions. The diagnostic groups are indicated in Table 1. Matched serum and fluid specimens were obtained in 45 cases. Serum PAG and other protein determinations were made in 32 cases.

Diagnoses were established by clinical and laboratory evaluation. The diagnosis of cirrhosis was confirmed by liver biopsy and malignant disease by positive histology except in four individuals in whom clinical and radiological features were strongly suggestive although positive histology could not be obtained.

CEA estimations were performed using the modified double antibody radioimmunassay (Egan *et al.*, 1972), giving a sensitivity of approximately 4 ng/ml. PAG was measured by rocket immunoelectrophoresis (Laurell, 1966) using an antiserum prepared by Berne (1973) giving a sensitivity of approximately 1 ng/ml. The normal proteins were measured by two-dimensional immunoelectrophoresis modified to allow precise quantitation (Bradwell and Burnett, 1975). The proteins measured were pre albumin, albumin, orosomucoid, α1 antitrypsin, α1 β glycoprotein, group component protein, α1 antichymo-

Table 1 *Patients included in the study*

Disease group	*Ascites*	*Pleural effusion*
Cancer*	15	12
Congestive cardiac failure	2	3
Cirrhosis	14	—
Inflammatory disease	—	10
Total	31	25

*Individual tumours:
Ascites: colon 3, stomach 2, pancreas 1, liver 1, ovary 2, cervix 1, uncertain origin 5
Pleural effusions: Breast 5, bronchus 7

trypsin, ceruloplasmin, α_2 macroglobulin, haptoglobin, haemopexin, transferrin, and the C_3 component of complement. Cytological examination for malignant cells was undertaken by the hospital pathology laboratories.

Results

CARCINOEMBRYONIC ANTIGEN

Serum CEA levels discriminated poorly between cancer and non-cancerous disease in patients with either ascites or pleural effusions (Fig. 1). By contrast, ascitic fluid CEA concentration separated 60% of the cancer patients from the non-malignant group. Whenever the fluid CEA concentration was increased in association with cancer it was always several times higher than the respective serum concentration whereas the levels in the cirrhotic fluids were always lower than the corresponding serum level.

A similar, though less striking situation was observed in the group with pleural effusions (Fig. 1). However, raised fluid CEA levels were also recorded in three patients considered to have inflammatory effusions. One such patient presented with bronchopneumonia, complicated by a pleural effusion, although 12 months previously he had undergone treatment for prostatic carcinoma. Currently there is no clinical evidence of recurrent malignancy. A second had bronchopneumonia without any evidence of malignancy and was also a negro, a racial group sometimes associated with raised serum CEA concentrations in normal individuals (Laurence *et al.*, 1972). There was no explanation for the third patient having raised levels of CEA.

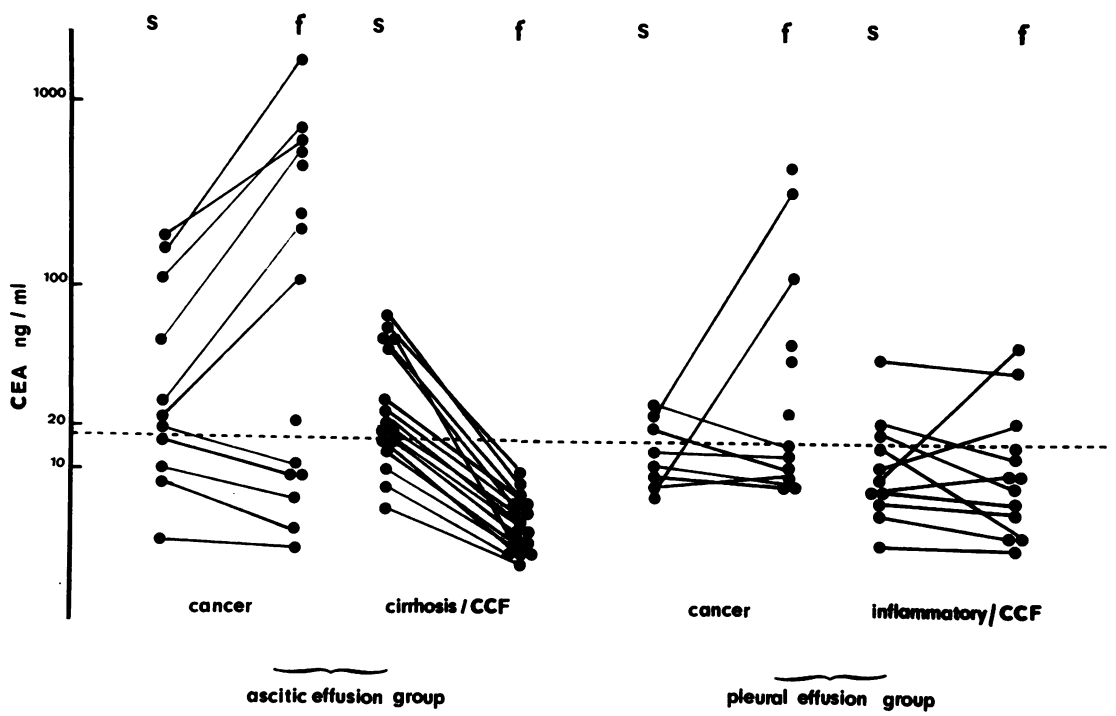

Fig. 1 *Serum (S) and effusion fluid (f) CEA concentrations in patients with ascites and pleural effusions. (Dotted line represents upper limit of normal for serum CEA.)*

COMPARISON OF FLUID CEA AND CYTOLOGY

The results of a comparison between fluid CEA concentration and cytological examination in malignant effusions are given in Table 2. In only five cancer patients were cytology and CEA levels both positive, but in seven more the CEA value alone was raised and cytology alone was positive in a further six. Thus, although cytology was positive in less than half of the cancerous effusions, by using both techniques the positive diagnostic rate rose to 75%. In addition, one patient with cirrhotic ascites was erroneously diagnosed as carcinomatous ascites on the basis of 'positive' cytology, although at necropsy no evidence of cancer was found.

Table 2 *Comparison between fluid CEA concentration and cytology in patients with cancer*

	CEA < 20ng/ml	*CEA > 20ng/ml*
Ascitic effusions		
Cytology positive	2	3
Cytology negative	4	3
Pleural effusions		
Cytology positive	4	2
Cytology negative	2	4

Cytology positive — 11/24 CEA positive — 12/24
CEA + cytology 18/24

PREGNANCY-ASSOCIATED α_2 GLYCOPROTEIN

A marked difference between serum PAG concentrations was observed for the carcinomatous and the cirrhotic ascites patients (Fig. 2). In the pleural effusion group, however, there was very little difference between the carcinomatous and inflammatory groups. In contrast to CEA, PAG levels in the effusion fluids of the cancer patients were in all cases much lower than the serum levels and were of no value in separating the disease groups.

NORMAL SERUM PROTEINS

In the patients studied, the concentration of individual proteins in the effusion fluids was always lower than in the serum. As expected, levels were higher in patients with cancerous and inflammatory effusions

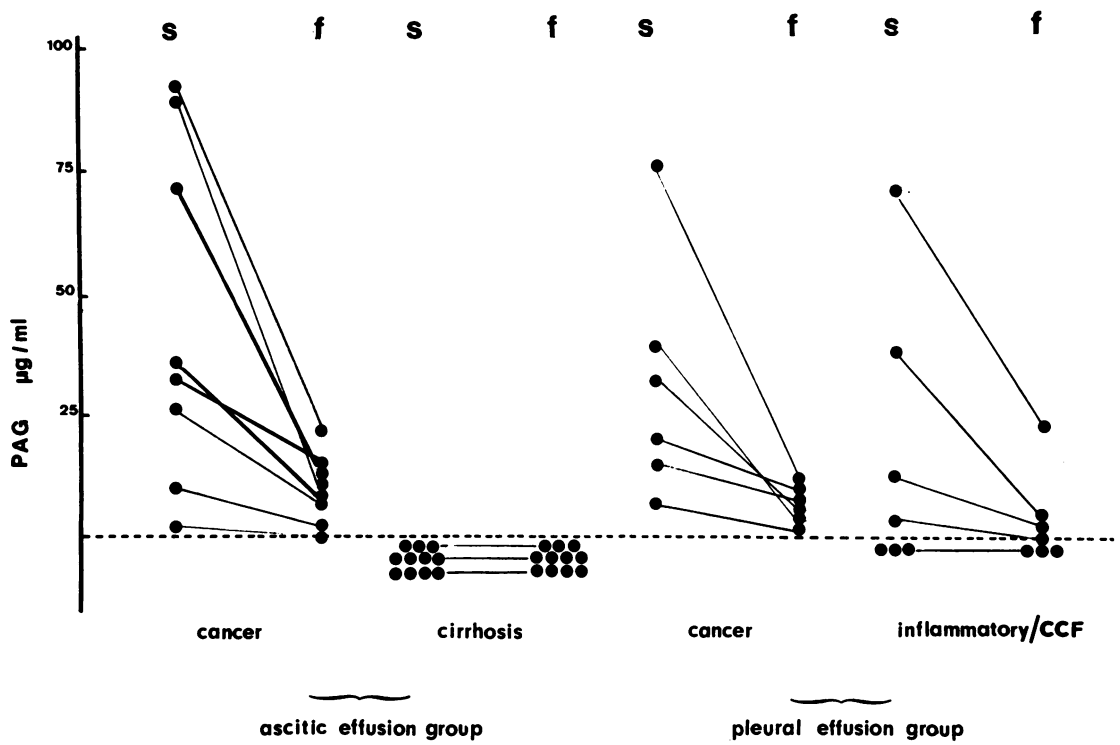

Fig. 2 *Serum (S) and effusion fluid (f) PAG concentrations in patients with ascites and pleural effusions. (Dotted line represents the limit of sensitivity for the assay.)*

although a large overlap existed. No individual protein appeared to offer better discrimination between the disease groups although these groups were too small for adequate statistical analysis.

Discussion

The demonstration that CEA levels in cancerous effusions are frequently several times higher than corresponding serum levels is striking and presumably reflects CEA secretion by the tumour cells. In contrast, the fluid levels of PAG were always lower than the serum levels, possibly reflecting PAG synthesis by peripheral blood leucocytes (Stimson and Blackstock, 1975). Similarly, there is no evidence from this study that tumours liberate normal serum proteins into effusion fluids as has previously been suggested (Rovelstad *et al.*, 1958).

The finding that fluid CEA levels in patients with non-cancerous conditions are almost always lower than the corresponding serum level is important and suggests that CEA estimation in effusion fluids may be a useful tool in clinical practice. In this limited series the comparison of CEA estimation and cytological examination for malignant cells indicates that both tests, when used in combination, should increase the diagnostic accuracy in patients with effusions.

The results of serum PAG estimation confirm the findings of Berne (1973) and Stimson (1975) that raised levels may be found in association with a variety of tumours. Although serum PAG estimation appears to differentiate cancer patients from those with cirrhosis, raised levels also occur in inflammatory disorders. Furthermore, the measurement of PAG and normal serum proteins in the effusion fluids does not appear to offer additional diagnostic information.

The extremely high concentration of CEA demonstrated in malignant effusions suggests that this fluid might prove a suitable biological fluid for the study of known tumour-associated proteins including hormones and surface antigens. Further studies along these lines might also help to evaluate and separate other tumour-related substances.

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