Serum carcinoembryonic antigen in clinical disorders

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SUMMARY Carcinoembryonic antigen (CEA) levels have been measured in the serum of 490 patients and 93 normal controls using the double antibody radioimmunoassay technique. Levels were elevated in 71 of patients with carcinomata of the gastrointestinal tract and in 42% with other types of malignancy. In patients with non-neoplastic disease of the gastrointestinal tract and liver, elevated levels were found in 14 and 66% respectively. In general the CEA level tends to be higher in cancer patients with haematogenous dissemination. Following complete surgical removal of a tumour, levels fall to normal within 14 days in the majority of patients. Of 33 patients studied during follow up, elevated levels were found in 12, 10 of whom had evidence of recurrence.

The significance of these findings and the possible application of CEA assay in clinical practice are discussed.

In recent years many attempts have been made to detect macro-molecular substances specific for human tumours, which if liberated into the body fluids could form the basis of an assay useful in the early diagnosis and management of malignant disease. One of the best such examples is the assay of chorionic gonadotrophin in patients with chorio-carcinomata (Bagshawe, 1969).

Well documented evidence has existed for some time of specific antigens in experimentally induced tumours of animals (Old, Boyse, Clarke, and Carswell, 1962). The same is not true for human tumours. Although there have been many reports of apparently human tumour-specific antigens, in the majority of instances this specificity is now open to doubt. In 1965 Gold and Freedman described an antigen which was identical in 12 different colonic carcinoma specimens examined (Gold and Freedman, 1965a). They later detected the same antigen in extracts of other gastrointestinal carcinomata together with extracts of foetal intestine, liver, and pancreas during the first two trimesters of pregnancy (Gold and Freedman, 1965b). Further, they were unable to demonstrate this antigen either in normal tissues or in non-entodermally derived malignant tumours, and therefore called it carcinoembryonic antigen (CEA). The existence of this antigen in other than carcinomatous or foetal tissue has since been extensively studied and found in normal human

colon (Martin and Martin, 1970) and pooled normal serum (Chu, Reynoso, and Hansen, 1972). This change in view of the specificity of CEA is reflected in the reports of radioimmunoassay results in a wide range of clinical groups. An initial claim of 97% positivity in patients with carcinoma of the colon (Thomson, Krupey, Freedman, and Gold, 1969) has not been reproduced (Lo Gerfo, Krupey, and Hansen, 1971; Moore, Kupchik, Marcon, and Zamcheck, 1971; Joint Canadian and American, Study, 1972; Laurence, Stevens, Bettelheim, Darcy, Leese, Turberville, Alexander, Johns, and Neville, 1972) and substantial overlap is now agreed between malignant and non-malignant groups. Clinical conditions on which attention has been focused include those posing diagnostic difficulty with carcinoma, hence the importance of overlap between results in inflammatory bowel disease and carcinoma of the colon and also between peptic ulcer and gastric cancer.

Radioimmunoassay has been carried out in three major ways. The first, the Farr assay, used by Gold and his collaborators (Thomson *et al*, 1969), utilizes precipitation of the antigen-antibody complex by ammonium sulphate. Hansen replaced ammonium sulphate with zirconyl phosphate gel (Z-gel) (Lo Gerfo *et al*, 1971) and this forms the basis of the test developed for commercial purposes by Hoffman La Roche Inc. Finally, a double antibody test was developed by Todd using horse antigoat IgG to

precipitate the CEA-anti-CEA complex (Egan, Lautenschleger, Coligan, and Todd, 1972). This is the only method which does not involve preliminary perchloric acid extraction of the specimens.

In this study, the second using the double-antibody technique, we have been able to include sizeable groups of patients suffering from ulcerative colitis, Crohn's disease, and cirrhosis.

Patients and Methods

The majority of subjects included in this study were either patients in the wards or attending outpatient clinics at Birmingham General Hospital. The normal controls, whose ages range from 19 to 63 years, were either members of staff of the Department of Experimental Pathology, University of Birmingham, or were participating in a normal population biochemical profile screening programme.

In all patients a definite diagnosis had been made by standard clinical, radiological, and laboratory methods, and where appropriate, eg, for all malignant tumours, by histological examination. All gastric, colo-rectal, and breast neoplasms were graded with respect to the clinical, operative and pathological findings, the TNM grading being used for breast and gastric lesions (Kennedy, 1970) and Dukes' classification for colonic tumours (Dukes, 1940). In patients with inflammatory bowel disease the diagnosis was established by radiological or operative findings supported in almost all cases by histological examination. The severity of the disease process was graded with respect to clinical and

biochemical indices and the extent of the disease by radiological or surgical findings.

Samples of blood (10 ml) were drawn by venepuncture into a sterile container and the serum was separated within two hours. Each sample was coded and stored at -20° until use and assays were performed without knowledge of the diagnosis in every case.

The assay method used is the double antibody technique (Egan *et al*, 1972) as modified by Laurence *et al* (1972). Serum samples (0.2 ml) were assayed in duplicate. The assay is capable of measuring CEA in amounts from 0.5 to 70 ng/0.2 ml with an agreement in duplicate tubes that is normally within 10%. Repeated observations on the same serum sample established a standard error for the assay of ± 0.6 ng/ml for a mean value of 13.2 ng/ml. Purified CEA, specific CEA antiserum, and horse antioat IgG, for use in the assay, were kindly supplied by Dr C. W. Todd, City of Hope National Medical Center, Duarte, California. The CEA has been shown to be immunologically indistinguishable from that of Gold and was prepared by perchloric acid extraction of hepatic metastases followed by sequential gel filtration on Sepharose 4B and Sephadex G200 (Coligan, Lautenschleger, Egan, and Todd, 1972). The CEA antiserum was prepared in goats by injecting microgram quantities of the purified CEA subcutaneously in complete Freund's adjuvant (Egan *et al*, 1972). The CEA for the assay was labelled with ^{125}I weekly by the chloramine T method (Egan *et al*, 1972) and standard curves were produced for each preparation.

Fig 1 The overall distribution of serum CEA values in 583 patients and normal controls.

Results

The overall distribution of serum CEA levels in 490 patients and 93 normal subjects plotted logarithmically is shown in figure 1.

The sera of 93 normal controls have been examined and in 91 of these the level was 12.5 ng/ml or less. In two subjects with levels of 15 and 17 ng/ml there was no clinical evidence of malignant disease. The range of 0-12.5 ng/ml is consistent with results obtained by Laurence *et al* (1972) and has therefore been accepted as the upper limit of normality for the purpose of this study.

GASTROINTESTINAL DISORDERS

Results obtained from the sera of 273 patients suffering from a variety of gastrointestinal diseases are summarized in table I. In a total of 96 estimations from patients with gastrointestinal malignant disease elevated levels were found in 71%, and in 35% levels were greater than 55 ng/ml. In 32 of these patients levels ranged from 100 to over 500 ng/ml. Of 53 patients with colonic carcinomata, the range did not differ significantly from the larger group, 68% having elevated levels, 34% being in excess of 55 ng/ml. The positivity rate in carcinoma of the pancreas, gallbladder, and bile ducts appears marginally higher (85%) but numbers are small and the disease had reached an advanced state in most of these cases. The one patient with a pancreatic carcinoma who showed a normal CEA is of interest, in that at the time the specimen was taken an indurated

head of the pancreas had been noted at an operation carried out for benign pathology elsewhere in the abdomen. He died eight months later, postmortem examination revealing generalized dissemination from a primary pancreatic carcinoma. Unfortunately the serum CEA was not estimated on this second occasion.

All three patients with colonic polyposis had normal CEA levels. Of these one had a solitary adenoma of the sigmoid colon containing carcinoma *in situ*, and another was a patient with familial polyposis also with premalignant changes.

Inflammatory diseases of the gastrointestinal tract are important in the differential diagnosis of endodermally derived malignancy. Of 172 patients with these disorders, 86% showed CEA levels within the normal range. A major proportion of elevated levels in this group occurred in patients with ulcerative colitis (11% positive) and Crohn's disease (14% positive). In patients with ulcerative colitis there was no correlation between the level of CEA and the length of history, degree of activity, or extent of colonic involvement. Three patients had premalignant changes (Morson and Pang, 1967) on rectal biopsy or on examination of the total colectomy specimen, and in one of these the CEA level was raised. The one patient in the group who developed an overt carcinoma had a normal CEA level whereas no tumours were found in six patients with raised levels. There was also no relationship between CEA concentration and the extent or activity of disease in the group with Crohn's disease. Nonetheless all patients with elevated levels did have disease of not less than seven years' standing (range 7-30 years: mean 18.1).

The results in the group of patients with liver disease are summarized in table II. Raised levels were evident in a high proportion, 21 of 32 being positive. There was no significant difference between the CEA levels in various categories or cirrhosis and it is of interest that two patients with acute viral hepatitis also

	No.	<12.5	12.5-55	>55	Percentage Positive
Malignant Tumours					
Colon/rectum	53	17	18	18	68
Stomach	23	8	6	9	65
Pancreas	8	1	5	2	
Ampulla of Vater	1	1	0	0	
Gallbladder	3	0	1	2	
Bile duct	1	0	1	0	
Hepatoma	1	0	1	0	
Oesophagus	1	0	1	0	
Disseminated	5	1	1	3	
Total malignant tumours	96	28	34	34	71
Benign Tumours					
Colon	3	3	0	0	
Inflammatory Diseases					
Peptic ulcer	21	18	3	0	14
Cholecystitis	5	4	1	0	
Ulcerative colitis	61	54	7	0	11
Crohn's disease	58	50	8	0	14
Diverticular disease	9	7	2	0	
Adult coeliac disease	9	7	2	0	
Pancreatitis	4	3	1	0	
Other	5	4	1	0	

Table I Serum CEA in gastrointestinal disease (ng/ml)

	No.	<12.5	12.5-55	>55	Percentage Positive
Alcoholic cirrhosis	5	1	4	0	
Cryptogenic cirrhosis	9	3	6	0	
Primary biliary cirrhosis	3	2	1	0	
Secondary biliary cirrhosis	1	0	1	0	
Chronic aggressive hepatitis	7	3	4	0	
Alcoholic hepatitis	3	1	2	0	
Viral hepatitis	2	0	2	0	
Other	2	1	1	0	
Total	32	11	21	0	66

Table II Serum CEA in liver disease (ng/ml)

showed raised values. In one patient with cryptogenic cirrhosis the level was within the normal range on a preliminary measurement but six months later after the development of progressive jaundice the CEA level had risen to 27 ng/ml. He subsequently died and postmortem examination revealed the presence of a hepatocellular carcinoma.

NON-GASTROINTESTINAL DISEASE

The serum samples of 109 patients with malignant tumours arising in organs other than those of the gastrointestinal system have been examined, elevated levels being found in 42%. The single patient whose level was in excess of 500 mg/ml suffered from IgG myeloma.

The majority of patients with benign tumours and all those with benign breast and prostatic disease had normal levels. In other disease groups levels were above the normal range in a significant proportion with rheumatoid arthritis, chronic respiratory disease, and other inflammatory disorders (table III).

RELATIONSHIP TO TUMOUR STAGING

The CEA levels have been analysed with respect to the tumour stage in colonic, gastric, and breast carcinomata, and the results are displayed in

Fig 2 Comparison of serum CEA levels and tumour dissemination.

Group 1 Localized tumours without lymph node metastases.

Group 2 Tumours with local lymph node metastases.

Group 3 Spread to liver, lung, or bone.

	No.	<12.5	12.5-55	>55	Percentage Positive
Malignant Tumours					
Bronchus	19	10	7	2	47
Breast	42	27	10	5	36
Ovary	4	4	0	0	
Cervix/uterus	4	1	1	2	
Bladder	3	1	2	0	
Prostate	1	0	1	0	
Disseminated	10	6	4	0	
Lymphoreticular	4	3	1	0	
Leukaemia	12	8	3	1	
Other	10	3	7	0	
Total malignant tumours	109	63	36	10	42
Benign Tumours					
Bladder	3	2	1	0	
Ovary	3	2	1	0	
Thyroid	3	3	0	0	
Salivary	2	2	0	0	
Other Disorders					
Benign breast disease	11	11	0	0	
Benign prostatic disease	3	3	0	0	
Rheumatoid arthritis	11	7	4	0	
Chronic respiratory disease	6	3	3	0	
Other inflammatory disorders	21	14	7	0	
Postmyocardial infarction	12	11	1	0	
Uraemia	1	1	0	0	

Table III Serum CEA in non-gastrointestinal disorders (ng/ml)

figure 2. A minority of patients without lymph node involvement (group 1) have raised levels. Where spread has occurred to the local lymph nodes, but resection is still practicable (group 2), the proportion rises, and where generalized dissemination has occurred 92% are positive (group 3). In many of these the level was greater than 100 ng/ml.

THE RESPONSE TO THERAPY AND THE DETECTION OF RECURRENT TUMOUR

We have studied 19 patients at intervals up to 14 days after the removal of a colonic carcinoma, and results from 11 patients with localized tumours are shown in figure 3. In three, raised levels fell to normal by the end of the first week and although two others showed elevated concentrations for seven days, these became normal within two weeks. One patient with an initially normal level developed a raised level at the end of the first week, but she unfortunately died from a pulmonary embolism two days later. A second with an initial value of 100 ng/ml showed a fall in CEA level at the end of the first week, but seven days later it had risen again to 70 ng/ml. This rise was associated with the development of intra-abdominal sepsis and three months after the opera-

Fig 3 The effect of surgical excision of localized tumours on the serum CEA concentration.

X—patient died from a pulmonary embolism.

Y—patient developed intraabdominal sepsis.

tion there was no clinical evidence of tumour recurrence although a further CEA estimation has not been made.

Estimations of CEA have been made on 33 patients attending a follow-up clinic between three months and six years after resection of a colonic or rectal carcinoma. In 21 of these, levels were normal and one patient had evidence of tumour recurrence at the site of a previous anastomosis. Of 12 patients with elevated levels, 10 had evidence of tumour recurrence or have developed signs of recurrence within three months of the CEA estimation.

Discussion

The double antibody radioimmunoassay for the estimation of CEA in peripheral blood samples has been shown by Laurence *et al* (1972) to produce results comparable in a clinical context with those using the Farr technique (Moore *et al*, 1971; Joint Canadian and American Study, 1972) or the Z-gel assay (Lo Gerfo *et al*, 1971). It does not involve perchloric acid extraction, is quicker to perform, and requires less serum (0.4 ml). It also appears to be more readily adaptable to automation procedures, and thus it is imperative to define its value in the assessment of practical clinical problems.

The higher range of normal values obtained by this method is possibly related to the use of unextracted serum, and it is possible that other serum proteins which would be removed by perchlorate extraction might interfere with the assay thus producing a positive result. Comparison of serum

concentrations of a range of eight such proteins with CEA has been completed and this possibility appears unlikely (Paine, King, Leonard, Booth, and Dykes, 1973). Carcinoembryonic antigen has been demonstrated in normal human plasma (Chu *et al*, 1972) but it remains to be established whether high control values are due to CEA or to some other substance interfering with the assay. The clinical value of the assay, however, does not depend on the upper limit set to the normal range but rather on the effective separation of normal from abnormal. Further studies on this normal range are in progress and for this report we have chosen to use the range of 0 to 12.5 ng/ml suggested by Laurence *et al* (1972), which appears compatible with our own findings. By this standard, the present results are compatible with those obtained either by the double antibody or other assays, approximately 70% positivity being obtained in carcinoma of the colon/rectum, stomach, and pancreas, and 40% from those with other malignant tumours. A much smaller yield of positive results might be acceptable provided they include early lesions, and it is important to find that for tumours of colon, stomach, and breast which have not yet apparently involved lymph nodes, positive results were found in 37, 33, and 21% respectively. It further appears that in patients with carcinoma of the colon values greater than 100 ng/ml indicate widespread metastases, a finding which might also be of clinical value.

As for most laboratory tests, potential value depends both on the unambiguous separation of the pathological from the normal, and on a similarly clear separation from other disease groups, which frequently give diagnostic difficulty. By this latter criterion, CEA assay has, after the first exciting report from Thomson *et al* (1969), been disappointing. The diagnostic usefulness now depends on proportional rates of positivity, although recently Laurence *et al* (1972) have emphasized the importance of concentration. We were fortunate in having large groups of patients suffering from inflammatory bowel disease, and within this other 'control' group no patient had a CEA concentration of greater than 55 ng/ml. This level also applies to non-gastrointestinal non-malignant disease, and therefore appears to be the best additional level to set to give information of maximal clinical value. Below this level, CEA concentration could still be of diagnostic value, as all patients in the non-malignant disease groups had clear diagnostic labels and abnormal values would not necessarily lead to confusion. The difficulties in assessing a new clinical test in groups of fully diagnosed patients are, however, well known, and the more important question of its value in the undiagnosed patient is one which we are now

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studying. Claims have already been made that abnormal values in patients with apparently non-malignant diseases frequently turn out to indicate the presence of cancer (Lo Gerfo *et al*, 1971), but this question urgently requires further study.

One further limitation to the usefulness of this test is the relationship of concentration to the spread of the disease, and it is disappointing that early lesions are usually associated with normal values. Nevertheless, by combining data from this and the other published study utilizing the double antibody test (Laurence *et al*, 1972), it appears that approximately one fifth of patients with early and localized lesions have CEA concentrations greater than 40 ng/ml and in at least two-fifths of patients it is greater than 12.5 ng/ml. Whether this information is of diagnostic usefulness will only be answered by the prospective study of undiagnosed patients which is now in progress.

Another and perhaps more promising area in which CEA might be of value is in the progressive study of patients with pre-malignant or treated malignant conditions. The small amount of data we have of positive values in longstanding ulcerative colitis is not encouraging in terms of the predictive value in the development of cancer, but much more information is necessary before an authoritative view is possible. In cancer follow up, however, correlation with recurrence appears better, as previously reported (Laurence *et al*, 1972). Following initial surgical resection it has been shown that the fall in concentration is related to the completeness of removal (Thomson *et al*, 1969; Laurence *et al*, 1972; Dhar, Moore, Zamcheck, and Kupchik, 1972), a finding supported by our own data. Further, the presence of elevated concentrations in 10 of 12 patients with recurrence of colonic cancer underlines the possible applications in follow up. Surgical opinion in this condition often favours second-look surgery (Gilbertsen and Wangenstein, 1962), the timing of which could readily be influenced by this test. Whether such increases occur early enough to be of real value is a problem of great importance and is also under active study.

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