

Carcinoembryonic antigen in serum in diseases of the liver and pancreas

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SYNOPSIS Carcinoembryonic antigen (CEA) was measured in whole serum and in serum extracted with perchloric acid by microradioimmunoassay in patients with benign and malignant diseases of the liver and pancreas. The level of detectability was 5 ng per ml. This level or greater was present in the serum of 50% of patients with chronic diffuse liver disease, 64% with pancreatitis, 94% with cancer of the digestive system, and 3% of controls. The incidence of levels of CEA of 5 ng/ml or more differed for various categories of chronic liver disease: from 22% in active chronic hepatitis, 46% in primary biliary cirrhosis, 63% in hepatoma, 78% in cryptogenic cirrhosis, and 88% in alcoholic cirrhosis; levels of CEA correlated with degrees of impairment of liver function as judged by bromsulphalein retention and serum levels of alkaline phosphatase and transaminase. In pancreatitis, 64% of cases had levels of CEA ranging from 5 to 20 ng/ml and in cancer of the pancreas 94% had levels above 5 ng/ml and 50% above 20 ng/ml.

Carcinoembryonic antigen (CEA) in serum was originally held to be specific for cancer of the digestive system (Gold and Freedman, 1965a, 1965b), but CEA-like activity has been reported in serum in various non-neoplastic diseases, particularly of the liver and pancreas (Lo Gerfo, Krupey, and Hansen, 1971; Moore, Kupchik, Marcon, and Zamcheck, 1971; Moore, Dhar, Zamcheck, Keeley, Gottlieb, and Kupchik, 1972; Krupey, Wilson, Freedman, and Gold, 1972; Warner, Khoo, MacSween, Bankhurst, and Mackay, 1973). Information is available on the relationship of the extent and spread of cancer, and its histological type, to the appearance of detectable CEA in serum. Thus Laurence, Stevens, Bettelheim, Darcy, Leese, Turberville, Alexander, Johns, and Munro Neville (1972) have reported a progressive increase in frequency of positive assays with spread of cancer of the colon and rectum, stomach, pancreas, bronchus, and breast; for example, the incidence of positive results in colo-rectal cancer increased from 13 of 29 patients with Dukes' stage A, through 22 of 29 patients with Dukes' stage B, to 20 of 20 patients with post-Dukes' stage C. Zamcheck, Moore, Dhar, and Kupchik (1972) reported similarly, citing an equivalent rise in the incidence of positive tests for

CEA in patients with colonic cancer from 19%, through 53% to 100%; equivalent findings were reported in a joint investigation (1972) on CEA in colonic cancer. Also, the amount of CEA detected was shown to be quantitatively greater when cancer had disseminated (Zamcheck *et al*, 1972; Laurence *et al*, 1972; Warner *et al*, 1973). However there did not appear to be any association between morphological differentiation or histological type of the tumour and the detection of CEA in serum according to Laurence *et al* (1972), and both squamous cell carcinoma and adenocarcinoma of the female reproductive tract were equally capable of resulting in raised CEA levels (Khoo and Mackay, 1973).

The present study, using microradioimmunoassay of CEA in whole serum, examines the association of CEA-like activity in serum of patients with chronic disease of the liver and pancreas and relates levels of CEA to diagnostic subtypes and functional status of the liver. For brevity, 'CEA' will be used instead of 'CEA-like activity', recognizing that CEA may represent various components with different physico-chemical properties.

Materials and Methods

OUTLINE OF STUDY

Levels of CEA in patients with diseases of the liver

and pancreas were compared with those in healthy persons, miscellaneous patients in hospital ('hospital controls'), and patients with cancer of the digestive system. The specificity of the assay on whole serum was assessed by re-assay of positive sera after extraction with perchloric acid. Levels of CEA were correlated with functional indices of liver disease including bromsulphalein retention (BSP), and levels of serum glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (AP), and bilirubin. Chi square analysis was used to test differences for statistical significance.

PATIENTS

The study comprised 505 patients, grouped as follows. (a) Liver disease (131), including 115 with diffuse parenchymal disease and 16 with hepatoma complicating cirrhosis, diagnosed histologically; the former 115 were subgrouped, on the basis of results of biochemical tests, autoimmune serological studies, and liver biopsy (Mackay, 1971), into alcoholic cirrhosis (35), active chronic hepatitis (58), cryptogenic cirrhosis (9), and primary biliary cirrhosis (13). (b) Pancreatic disease (30), including 16 with pancreatic cancer diagnosed at operation and 14 with acute pancreatitis or chronic relapsing pancreatitis diagnosed by clinical findings and serum amylase tests. (c) Healthy persons (130), including 30 adults aged 18-23 years in known good health, and 100 blood donors whose health status was not directly assessed by interview or examination. (d) 'Hospital controls' (100), including 25 with uterovaginal prolapse and 75 with cardiac, vascular, and psychiatric disorders, aged from 40 to 85 years. (e) Cancer of digestive system (138) with histological confirmation in all.

RADIOIMMUNOASSAY OF CEA

Carcinoembryonic antigen was measured by the competitive inhibition radioimmunoassay described by MacSween, Warner, Bankhurst, and Mackay (1972) and MacSween, Warner, and Mackay (1973), using purified CEA and goat antiserum to CEA provided by Dr P. Gold, Montreal. Samples (2-20 μg) of CEA were labelled with ^{125}I . Several weeks after iodination, the labelled antigen became less precipitable, and the non-precipitable component was removed by gel filtration on Sephadex G-200. Twenty-five μl of patient's serum was mixed with 25 μl of either a 1×10^{-5} or 5×10^{-6} dilution of goat antiserum to CEA; the tubes were held for two hours at 37°C and then overnight at 4°C ; 50 μl of ^{125}I CEA (concentration 1 ng/ml) was then added and the tubes were again held for two hours at 37°C and overnight at 4°C . Antigen-antibody complexes were precipitated with 100 μl of rabbit antiserum to

goat gamma globulin in a dilution of 1×10^{-1} or 2×10^{-2} . Each assay had three control systems, as follows: (1) pooled normal human serum and 3% bovine serum albumin in 0.01 M tris buffer replaced in duplicate tubes the goat antiserum to CEA; (2) normal human serum replaced in duplicate tubes the test serum; (3) a standard inhibition curve was constructed by adding known amounts per ml of CEA diluted in normal human serum. Under the conditions of our assay, the lowest amount of CEA detectable was 3 ng per ml; for the expression of results, the lower limit for positivity was taken as 5 ng per ml.

The following modification of the standard procedure of extraction of CEA from serum with perchloric acid was developed for small volumes of serum so as to minimize dilutional effects. Of 4M perchloric acid, 0.1 ml was slowly added to 0.3 ml of serum in a tube agitated in a Vortex mixer and the supernatant, after being held for one hour at 4°C and centrifuged at 4000g for 10 minutes, was dialysed against repeated changes of distilled water for 48 hours; the retentate was tested by radioimmunoassay for carcinoembryonic antigen.

BIOCHEMICAL INDICES OF LIVER DISEASE

Standard biochemical tests of liver function were performed by automated techniques in the Department of Biochemistry of the Royal Melbourne Hospital. Upper limits of normal ascertained from panels of healthy controls were for BSP retention, 10% of a 5 mg per kg dose injected intravenously 45 minutes beforehand, for serum GOT, 40 International Units (IU) per l, for serum AP, 13 King Armstrong (KA) units per 100 ml, and for serum bilirubin, 1.0 mg per 100 ml. Only results of biochemical tests performed on sera taken within seven days of obtaining sera for CEA estimation were considered.

Results

OVERALL INCIDENCE OF CEA IN SERUM (TABLE I)

Of the 230 sera from healthy persons and hospital controls, seven (3%) contained CEA and in six the level was low, 5-10 ng/ml; four were from blood donors and three from hospital controls diagnosed as cardiac failure, cerebral haemorrhage, and hysteria, respectively. Of the 129 sera from patients with non-malignant diseases of the liver (115) and pancreas (14), CEA was detected in 66 (51%) but levels were mostly low, between 5 and 20 ng/ml. For 138 sera from patients with cancer of the digestive system, CEA was detected in 127 (92%), and in some 45% the level exceeded 20 ng/ml.

Category and No. of Subjects	No. of Cases with CEA in Serum (ng/ml)			Percentage of Cases with Levels of CEA > 5 ng/ml
	5-10	11-20	>20	
Young healthy subjects (30)	0	0	0	0
Blood donors (100)	4	0	0	4
Hospital patients (100)	2	1	0	3
Diffuse liver disease (115)	20	23	14	50
Pancreatitis (14)	3	6	0	64
Cancer of digestive system (138) ¹	31	34	62	92

Table I *Carcinoembryonic antigen in serum in healthy persons, hospital patients, liver disease, pancreatic disease, and digestive system cancer*

¹Including colon (91), stomach (18), oesophagus (4), pancreas (20), bile duct and gall bladder (5)

CARCINOEMBRYONIC ANTIGEN IN SPECIFIC DISEASES OF THE LIVER AND PANCREAS

The incidence of positive results for CEA in serum differed for the four subtypes of chronic liver disease, being lowest in active chronic hepatitis (22%), intermediate in primary biliary cirrhosis (46%), and highest in cryptogenic (78%) and alcoholic cirrhosis (88%); moreover only in cryptogenic cirrhosis and alcoholic cirrhosis was there a substantial incidence (22-23%) of values over 20 ng/ml. The differences in incidence of positive results between the groups with alcoholic and cryptogenic cirrhosis, and those with active chronic hepatitis and primary biliary cirrhosis, were highly significant ($p < 0.005$). For the 16 patients with cirrhosis and hepatoma the incidence approached that in alcoholic and cryptogenic cirrhosis, 63% having detectable CEA and 13% having levels exceeding 20 ng/ml.

Of the 14 patients with acute or chronic pancreatitis, 64% had detectable levels but in no case

did the level exceed 20 ng/ml, and of the 16 patients with cancer of the pancreas, 94% had detectable levels and 50% had levels greater than 20 ng/ml.

EFFECT OF PERCHLORIC ACID EXTRACTION OF CEA FROM SERUM

For 51 sera which gave a positive result (5 ng/ml) in the assay on whole serum, CEA was extracted with perchloric acid; most sera remained positive but levels were lower (table II). Loss of CEA activity occurred particularly with sera from patients with primary biliary cirrhosis and pancreatitis; CEA became undetectable in seven of 13 such sera after extraction. Overall there was about a 25% loss of CEA activity in sera after extraction and loss of activity tended to be complete with pre-extraction levels between 5 and 10 ng/ml. Activity of CEA in whole serum was presumably identical with that in extracted serum, the lower levels after extraction being attributable to dilution.

Category of Disease	CEA in Whole Serum			No. of Sera tested	CEA in Serum before ¹ and after ² Extraction with Perchloric Acid				
	No. of Patients	No. of Cases with Serum Levels (ng/ml)			No. of Cases with Serum Levels (ng/ml)	>5	>10	>20	
		>5	>10						>20
Active chronic hepatitis	58	13 (22%)	7 (12%)	4 (7%)	13	13 ¹	7 ¹	4 ¹	
Cryptogenic cirrhosis	9	7 (78%)	5 (55%)	2 (22%)	7	7 ¹	5 ¹	2 ¹	
Alcoholic cirrhosis	35	31 (88%)	21 (60%)	8 (23%)	18	18 ¹	14 ¹	7 ¹	
Primary biliary cirrhosis	13	6 (46%)	4 (31%)	0 (0%)	5	5 ¹	3 ¹	0 ¹	
Hepatoma	16	10 (63%)	6 (38%)	2 (13%)		2 ²	2 ²	0 ²	
Acute or chronic pancreatitis	14	9 (64%)	6 (43%)	0 (0%)	8	8 ¹	7 ¹	4 ¹	
Carcinoma of pancreas	16	15 (94%)	10 (62%)	8 (50%)		4 ²	3 ²	1 ²	

Table II *Carcinoembryonic antigen in serum in diseases of the liver and pancreas*

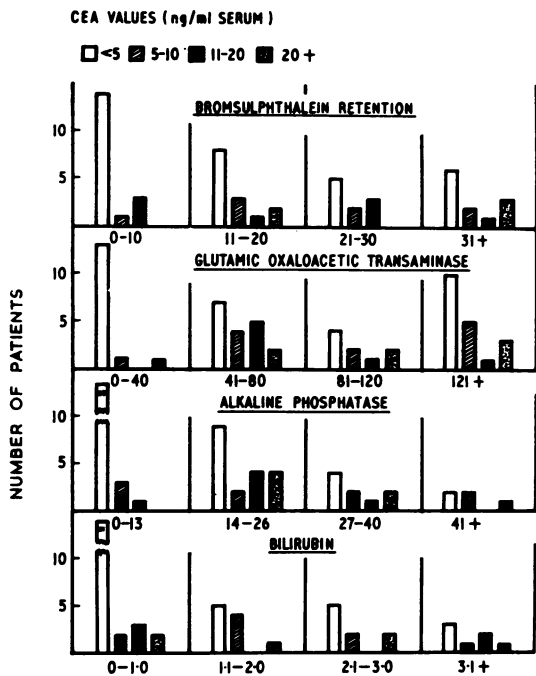


Fig Relation of levels of CEA in serum and functional impairment in diffuse liver disease.

CARCINOEMBRYONIC ANTIGEN AND LIVER FUNCTION

Tests of BSP retention and serum levels of GOT, AP, and bilirubin were classified either as 'normal' or 'abnormal' if the result was beyond the upper limit of normal. For this analysis, patients were grouped according to whether the level of CEA in whole serum was below or above 10 ng/ml. In the four tests considered, the incidence of levels of CEA above 10 ng/ml was significantly greater in the presence of abnormality of liver function (table III),

as assessed by BSP retention ($p < 0.005$), serum AP ($p < 0.001$), and serum GOT ($p < 0.02$). There was a trend for higher levels of CEA to be associated with more advanced abnormalities of most of the functional indices (fig 1).

Discussion

Radioimmunoassay for CEA in serum is being assessed as a diagnostic procedure for cancer of the gastrointestinal tract, pancreas, lung, breast, and to a lesser degree of the female reproductive system and other organs (Thomson, Krupey, Freedman, and Gold, 1969; Moore *et al*, 1971; Lo Gerfo *et al*, 1971; Reynoso, Chu, Holyoke, Cohen, Nemoto, Wang, Chuang, Guinan, and Murphy, 1972; Zamcheck *et al*, 1972; Warner *et al*, 1973).

The different immunoassays for CEA in common use include the 'Thomson-Gold' assay (Thomson *et al*, 1969) in which extracted serum is tested and CEA-antiCEA complexes are precipitated with ammonium sulphate, the 'Z-gel' assay of Lo Gerfo *et al* (1971) in which extracted serum is tested and complexes are precipitated by zirconyl gel, and the 'double antibody' assay in which whole serum is tested and complexes are precipitated by antibody to goat serum. For both the Thomson-Gold and the Z-gel assay, a level of 2.5 ng/ml has been defined as a positive result (Thomson *et al*, 1969; Lo Gerfo *et al*, 1971), and there was an overall correlation of 83 to 86% between them (Sorokin, Kupchik, Zamcheck, and Dhar, 1972). Laurence *et al* (1972) equated a result of 12.5 ng/ml of CEA by their 'double antibody' method with 2.5 ng/ml detected by the 'Z-gel' assay. Our present double antibody microradioimmunoassay detected 3 ng/ml, but we used a 'cut-off' level of 5 ng/ml. The cut-off level could be further raised to decrease 'background', but sensitivity is lost (Joint National Cancer Institute of Canada/American Cancer Society Investigation, 1972).

Liver Functional Index ¹		Cases with CEA > 10 ng per ml			Significance of Difference
		Active Chronic Hepatitis	Cirrhosis ²	Primary Biliary Cirrhosis	
BSP retention (10%)	Normal	0/32	0/3	3/3	3/38
	Abnormal	5/20	8/12	1/4	14/36
Serum GOT (40 IU/l)	Normal	0/10	1/5	—	1/15
	Abnormal	5/23	11/17	2/5	18/45
Serum AP (13 KAU/100 ml)	Normal	0/18	1/5	0/1	1/24
	Abnormal	6/12	8/15	3/6	17/33
Serum bilirubin (1 mg/100 ml)	Normal	2/19	3/8	0/1	5/28
	Abnormal	3/11	3/11	3/4	9/26

Table III Functional indices of the liver and incidence of positive results for CEA > 10 ng per ml

¹Upper limit of normal in brackets; ²Cirrhosis = alcoholic or cryptogenic cirrhosis; n.s. = not significant

Clinical interpretation of a positive test for CEA must allow for its presence in benign diseases of the liver and pancreas. Weakly positive tests for CEA could be indicative of either cancer or diffuse liver disease, but strongly positive results, particularly with normal or only mildly altered biochemical tests of liver function, would indicate cancer either in an extrahepatic site or metastatic in the liver. Carcinoma of the pancreas is not readily differentiated from pancreatitis on the basis of CEA levels, but higher levels are more indicative of cancer. The value of CEA assay in the detection of hepatoma is slight because of the high frequency of positive results in cirrhosis.

A high incidence (some 50%) of CEA in patients with benign diseases of the liver and pancreas was reported by Lo Gerfo *et al* (1971) and Moore *et al* (1971), although levels were lower than those in patients with cancer of the digestive system. Levels of CEA exceeding 2.5 ng/ml were detected in 40 of 88 patients with alcoholic liver disease but in none of 14 patients with non-alcoholic liver disease, and positive results correlated with alcoholic hyalin, hydropic degeneration, and fat in the liver (Moore *et al*, 1972). We found levels of CEA above 5 ng/ml in various forms of diffuse parenchymal liver disease, but the incidence and serum levels differed considerably according to the pathogenetic subtype, being greatest in alcoholic and cryptogenic cirrhosis and least in active chronic hepatitis. The magnitude of the CEA level correlated with the severity of the liver disease, as indicated by conventional tests of liver function, and the higher incidence of positive results in alcoholic and cryptogenic cirrhosis could be attributed to a greater impairment of function in these diseases.

The frequent detection of CEA in benign disease of the liver and pancreas poses the question of the specificity of the assay. Is CEA in effect being detected? Comparison of our results before and after extraction of CEA from serum with perchloric acid in patients with benign liver and pancreatic diseases indicates that positive tests for CEA remained even after extraction in 42 of 51 analyses. There was a moderate reduction in CEA level with sera extracted with perchloric acid, as compared with whole serum levels. The degree of reduction correlated with the dilution necessary for extraction, and the loss of CEA activity was therefore attributed to this. Other explanations would be interference with CEA activity by acid treatment, and loss of CEA activity via the dialysis membrane. Finally, in certain conditions, some of the CEA activity measured in our present assay might represent cross-reactive molecular configurations different from those measured in other assays; for example, in cases of

'active' ulcerative colitis, extraction and dialysis eliminated CEA-like activity detected in untreated serum (Khoo, Hunt, and Mackay, 1973).

The reason for elevated levels of CEA or CEA-line substances in benign liver diseases remains uncertain, as metabolism of CEA in the liver is yet to be known, and the following possibilities exist.

Production of CEA could accompany cellular regeneration in the liver; in cancer, CEA has been demonstrated on the glycocalyx of the tumour cell surface (Gold, Gold, and Freedman, 1968).

Inflammation in the liver could give rise to glycoproteins with CEA-like activity.

Impaired liver function could result in failure of excretion of small amounts of CEA possibly produced under normal conditions.

We note that only small amounts of CEA are extractable from normal and cirrhotic livers, much less than amounts demonstrable in colonic cancer (Kupchik and Zamcheck, 1972; Khoo, Warner, Lie, and Mackay, 1973); however, there is said to be identical immunological reactivity between extracts of liver, extracts from hepatic metastases of colonic cancer, and sera of cirrhotic patients (Kupchik and Zamcheck, 1972). Although our present results suggest that functional impairment of the liver is an important cause of raised levels of CEA in cirrhosis, other causes must be invoked to account for the finding in pancreatitis, and effects of inflammatory activity and cellular damage and regeneration remain to be assessed. Also the physicochemical nature of these CEA-reactive substances in non-neoplastic diseases of the liver and pancreas remains to be characterized.

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