Cholestasis and Hepatic Metastases: A Factor Contributing to Extreme Elevations of Carcinoembryonic Antigen [1,2,3]

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ABSTRACT—Records of 19 autopsied patients with metastatic carcinoma were studied to elucidate the contribution to the elevation of antemortem plasma carcinoembryonic antigen (CEA) levels (range, 5.9-136,000 ng/ml) of 1) liver pathology and dysfunction, 2) tumor morphology and CEA content, and 3) tumor spread and location. Liver function tests and plasma CEA recorded within 8 weeks of death, autopsy records of tumor spread, liver weight (as an index of liver tumor mass), and histologic sections were reviewed. Tissue CEA was demonstrated in 15 patients by an immunoperoxidase method. Cholestasis was seen in histologic sections of tissue from 8 of 10 patients, and elevated bilirubin was seen in 7 of 10 patients with hepatic metastases and CEA levels greater than 1,000 ng/ml. In contrast, histologically observed cholestasis and elevated bilirubin were seen in only 1 of 8 patients with CEA less than 500 ng/ml. A significant correlation was found between the plasma CEA level and histologically observed cholestasis (*P*<0.01). Serum bilirubin also correlated significantly (*P*<0.01), but alkaline phosphatase did not. Liver weight (tumor mass) showed a positive correlation with cholestasis (*P*<0.01) but not with circulating CEA. Markedly elevated plasma CEA levels (>1,000 ng/ml) seen preterminally may partially reflect impaired excretion of CEA by the hepatobiliary system rather than, or in addition to, preterminal increase in CEA-producing tumor.—JNCI 64: 1291-1294, 1980.

This study was prompted by the observation of an extraordinarily wide range of preterminal plasma CEA levels in patients who died with disseminated carcinoma; this wide range appeared to occur even in patients who had tumors of similar origin and extent of spread. Certain patients had extremely high CEA values that required explanation. In the present study we compare autopsy findings and preterminal liver function tests with plasma CEA values and report that, of the parameters measured, histologically observed cholestasis and elevated bilirubin levels correlated best with the degree of CEA elevation.

MATERIALS AND METHODS

Subjects and autopsy records.—To elucidate the factors accounting for this variation in plasma CEA elevation, we studied records of 19 autopsies of patients with metastatic carcinoma: 14 colon cancers, 1 breast cancer, 1 stomach cancer, 1 lung cancer, 1 pancreatic cancer, and 1 cholangiocarcinoma. All available autopsy records of patients with metastatic cancer at Peter Bent Brigham Hospital, Boston City Hospital, and University Hospital who had recorded CEA assays done by this laboratory within 8 weeks of death and who had routine liver function tests performed synchronously were studied retrospectively. Each patient's records were reviewed to document tumor primary site and extent of visceral spread. Liver weight was used as an index of tumor mass in that organ.

Histologic studies.—Sections from all of the cases were reviewed to determine tumor type and differentiation. All of the tumors were adenocarcinomas and were described as well differentiated (forming a predominantly glandular pattern), poorly differentiated (showing a predominant picture of anaplasia and no attempt at gland formation), and moderately differentiated (showing an intermediate degree of anaplasia and glandular differentiation). A detailed study of liver sections was performed (*see* below).

Immunohistochemistry studies.—For 15 cases an IMP PAP technique was used (*1*), and a monospecific anti-CEA antibody was used to detect CEA within sections of liver metastases. Paraffin sections 5μm thick were incubated in sequence with rabbit anti-CEA (New England Immunology Associates, Cambridge, Mass.), swine antirabbit IgG (Dako; distributed by Accurate Chemical and Scientific Corp., Hicksville, N.Y.) at 1/25 dilution, and PAP (Dako) at 1/100 dilution. The incubation time for the primary antibody was 24 hours at 0-4° C, followed by room temperature for 2 hours; both the bridge antibody and the PAP were kept for 30 minutes at room temperature. The stained sections were evaluated for the presence of CEA-positive staining. Tumor sections showing widespread positive staining were designated strongly positive; sections showing predominantly negative staining with focal positive areas were designated weakly positive. The

ABBREVIATIONS USED: CEA=carcinoembryonic antigen; IMP=immunoperoxidase staining; LDH=lactate dehydrogenase; PAP=peroxidase antiperoxidase; SGOT=serum glutamic-oxaloacetic transaminase.

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location of the tumor cell positivity, whether predominantly cytoplasmic, surface, or both patterns, was determined in each case. The CEA antibody was rendered monospecific by sequential absorption by groups A, B, and O cells and by lyophilized extracts of human lung (40 mg/ml), buffy coat (10 mg/ml), spleen (10 mg/ml), liver (10 mg/ml), and colon (10 mg/ml). Specificity and absence of cross-reactivity with purified colon carcinoma antigen-III (nonspecific cross-reacting antigen; gift of Roche Laboratories, Nutley, N.J.) were indicated by double immunodiffusion. Controls for the staining procedure consisted of replacement of the primary antibody with normal rabbit serum and with anti-CEA antiserum absorbed with purified CEA. A section of well-differentiated colon adenocarcinoma routinely was stained as a positive control.

Statistical analyses.—Because of the wide range of values (tables 2, 3), all of the data including liver weight, degree of cholestasis, and liver function tests were log transformed prior to statistical analysis. Correlations between plasma CEA levels and liver weight, degree of cholestasis, SGOT, LDH, alkaline phosphatase, and total bilirubin were performed by linear regression analysis.

RESULTS AND DISCUSSION

Table 1 lists the patients in order of decreasing plasma CEA values ranging from 136,000 to 6.0 ng/ml. Tumors from various primary sites were represented as were tumors with varying degrees of differentiation. Visceral metastases were present in all patients. All but 1 (#2) had liver metastases; 2 (#14, 19) had visceral metastases confined to the liver. The volume of liver metastases was estimated by organ weight. In 16 patients the pattern of liver metastases was that of irregular nodules throughout both lobes. Two patients (#1, 9) had multiple nodules and a large tumor mass at the hilus of the liver impinging on the common hepatic duct and producing obstruction and proximal dilatation of the biliary tree. Patient #2 was exceptional because of a high plasma CEA value (48,000 ng/ml) despite the absence of liver metastases; this patient had a large, poorly differentiated signet-ring-cell pancreatic carcinoma (700 g) that stained strongly for CEA and presumably produced large quantities of the antigen.

TABLE 1.—*Plasma CEA, tumor primary site, differentiation, and sites of metastases*

Patient No.	CEA, ng/ml	Primary site	Differentiation	Sites of metastases[a]	Liver wt
1	136,000	Colon	Well	Li, Pl, Lu	6,000
2	48,000	Pancreas	Poor	Ad, Ki	1,200
3	28,000	Stomach	Moderate	Li, Lu	2,700
4	13,000	Colon	Moderate	Li, Lu	3,170
5	8,600	Colon	Moderate	Li, Lu	6,480
6	6,500	Colon	Well	Li, Lu	8,800
7	4,500	Colon	Well	Li, Lu	4,150
8	1,900	Colon	Moderate	Li, Lu	3,312
9	1,500	Bile duct[b]	Moderate	Lu	3,700
10	1,200	Lung	Moderate	Li, Sp, Bo	2,000
11	1,000	Breast	Poor	Li, Co, Bo	1,100
12	340	Colon	Moderate	Li, Ad, Sp	1,240
13	320	Colon	Moderate	Li, Ce	2,260
14	250	Colon	Moderate to well	Li	9,050
15	240	Colon	Moderate	Li, Lu, Br, Ki, Ad, Je	5,130
16	210	Colon	Poor	Li, Lu, Ki, Ad, Pa	1,750
17	67	Colon	Well	Li, Lu, Pl, St, Pe, Sp	1,750
18	39	Colon	Poor	Li, Lu (1), Ad, Ki, Bo, Pa	1,580
19	6	Colon	Well	Li	1,200

[a] Ad=adrenal glands, Bo=bone, Br=brain, Ce=cecum, Co=colon, Je=jejunum, Ki=kidney, Li=liver, Lu=lung, Pa=pancreas, Pe=peritoneum, Pl=pleura, Sp=spleen, St=stomach.
[b] Cholangiocarcinoma.

Preterminal CEA and liver function tests (table 2).—Elevated serum bilirubin was associated with the highest values of plasma CEA. Of 10 patients with liver metastases and plasma CEA of 1,000 ng/ml or greater, 7 had elevated serum bilirubin in contrast to only 1 of 8 patients with plasma CEA less than 350 ng/ml. No correlation was found between either serum LDH or SGOT and plasma CEA. Moderate elevation of SGOT was found in 7 of the 10 patients with plasma CEA values greater than 1,000 ng/ml and may be attributable, in some instances, to the effects of significant cholestasis. Alkaline phosphatase was elevated in 17 of 19 patients. The correlation with the degree of CEA elevation was not statistically significant ($r=0.423$). Isoenzyme studies to determine the nature of the elevated alkaline phosphatase were not available.

Histologic evidence of cholestasis.—Cholestasis was looked for in sections of liver lobules uninvolved by tumor and was graded on a scale of 0 to 4+ (table 3). Grade 1: scattered intracellular bile pigment mainly in the centrilobular zones; grade 2: intracellular bile pigment and bile plugs extending into midzones; grade 3: numerous bile plugs throughout the lobules; and grade 4: bile plugs in ductules and bile ducts. The grading was performed independently by two observers in 16 of 19 cases. In the remaining 3 cases grading was determined by joint review. A correlation between grade of cholestasis and plasma CEA elevation was evident ($P<0.01$). Of 10 patients with liver metastases and plasma CEA of 1,000 ng/ml or greater, 8 showed histologic evidence of cholestasis in contrast to only 1 of 7 patients with plasma CEA less than 350 ng/ml. The highest plasma CEA value was found in association with a metastatic, well-differentiated carcinoma of the colon, which had grade 4+ cholestasis in the liver and complete obstruction of the common bile duct by tumor. The degree of cholestasis histologically observed also showed a significant correlation ($r=0.617$, $P<0.001$) with preterminal serum bilirubin levels. The degree of bile duct proliferation or inflammatory cell infiltration of the portal tracts, however, did not appear to correlate with the plasma CEA level.

Liver weight (table 1).—Liver weight showed a weaker degree of correlation with the plasma CEA ($r=0.446$) than did either the serum bilirubin ($r=0.592$)

TABLE 2.—*Comparison of plasma CEA value and liver function tests*[a]

Patient No.	CEA, ng/ml	LDH, mU/ml	Alkaline phosphatase, mU/ml	Bilirubin, mg/100 ml	SGOT, mU/ml
1	136,000	665	710	12.0	168
2	48,000	141	143	0.4	26
3	28,000	790	800	6.8	78
4	13,000	360	147	5.9	108
5	8,600	2,160	228	1.8	165
6	6,500	635	400	1.6	155
7	4,500	580	159	0.8	581
8	1,900	473	218	0.5	23
9	1,500	215	490	1.4	53
10	1,200	1,815	845	0.7	168
11	1,000	159	715	1.9	176
12	340	187	107	0.3	30
13	320	798	360	0.7	98
14	250	1,210	975	1.5	43
15	240	350	207	0.6	46
16	210	105	360	0.8	15
17	67	150	65	0.4	25
18	39	325	190	0.5	45
19	6	64	47	0.9	21

[a] Normal upper values: CEA, 2.5 ng/ml; LDH, 200 mU/ml; aklaline phosphatase, 85 mU/ml; bilirubin, 1.0 mg/100 ml; SGOT, 50 mU/ml.

or the degree of histologically observed cholestasis ($r=0.617$). A case in point, patient #14, had the greatest tumor mass (9,050 g) and a plasma CEA value of 250 ng/ml, whereas the highest plasma CEA value, 136,000 ng/ml, occurred in patient #1 with a liver weight of 6,000 g.

Localization of CEA by IMP.—CEA was demonstrated in all 15 tumors studied. In well-differentiated carcinomas the CEA was characteristically on the surface glycocalyx. Moderately and poorly differentiated tumors occasionally revealed intracytoplasmic staining. Inspissated secretions within glandular tumors showed positive staining, and a notable feature in many tumors was positive-staining "lakes," which corresponded to areas of necrosis (fig. 1). No attempt was made to correlate the extent of CEA lakes with the plasma CEA levels because we thought that necrosis seen in selected sections may not represent the extent of necrosis in the entire tumor. Hepatocytes, bile canaliculi, and bile ductules were negative for CEA in all 15 cases.

TABLE 3.—*Plasma CEA vs. tumor primary site, degree of cholestasis, and IMP staining of CEA*

Patient No.	Plasma CEA, ng/ml	Primary site of tumor	Cholestasis[a]	IMP staining[b]
1	136,000	Colon	4+	s
2	48,000	Pancreas	0	s
3	28,000	Stomach	3+	s
5	8,600	Colon	3+	w
6	6,500	Colon	1+	s
9	1,500	Bile duct[c]	3+	w
10	1,200	Lung	0	s
11	1,000	Breast	2–3+	w
14	250	Colon	1–2+	s
15	240	Colon	0	s
16	210	Colon	0	w
18	39	Colon	0	s

[a] Grades of cholestasis (0 to 4+) are defined in "Results and Discussion."
[b] s=strongly positive; w=weakly positive.
[c] Cholangiocarcinoma.

CEA content of tumors.—No correlation was found between the CEA content of the 15 tumors studied, as estimated by the IMP and the plasma CEA value (table 3). However, patients having both strongly positive staining for CEA and severe cholestasis (#1, 3) had extreme elevations of plasma CEA. Patients #14 and 15 with lower plasma CEA values despite strongly positive CEA staining had less or no cholestasis. A high plasma CEA value of 1,500 ng/ml was present in patients #5 and 9, who had little immunocytochemically demonstrable CEA but had grade 3+ cholestasis; #9 also had common bile duct obstruction at autopsy.

COMMENT

Many factors may apparently determine the degree of elevation of plasma CEA in the patient with metastatic carcinoma. Mayer et al. (*2*) and Shani et al. (*3*) have shown that wide and unexplained variability in CEA levels may occur in patients with widespread cancer. In addition to CEA production by the tumor, the tumor mass, and extent of blood vessel and lymphatic invasion (*4*), other factors such as tumor necrosis (*5, 6*) or increased vascularity may contribute. Previous studies have reported elevations of CEA in benign liver disease (*7-9*) and in benign biliary obstruction (*10*). In contrast to the extraordinarily high CEA levels reported in this series, CEA levels greater than 10 ng/ml are uncommon in patients with benign liver diseases, including fulminant hepatitis and obstruction of the extrahepatic biliary system. Levels greater than 100 are usually associated with liver metastases. Thomas and Hems (*11*) found that immunologically assayable ^{125}I-labeled CEA perfused in rats was detectable in the bile within 2 hours. Holyoke et al. (*12*) and Molnar et al. (*13*) also demonstrated the presence of CEA-like activity in human bile. Go et al. (*14*) reported a doubling of CEA concentration in duodenal juice following the emptying of cholecystokinin-stimulated gallbladder. Thus the frequent concomitance of cholestasis, intrahepatic and/or extrahepatic, with extraordinarily high CEA levels, such as those reported here, suggests at least an inability of the liver to excrete the very large amounts of CEA produced in the late stage of cancer. This finding may help to explain the lack of direct quantitative correlation between tumor mass and circulating CEA levels commonly observed.

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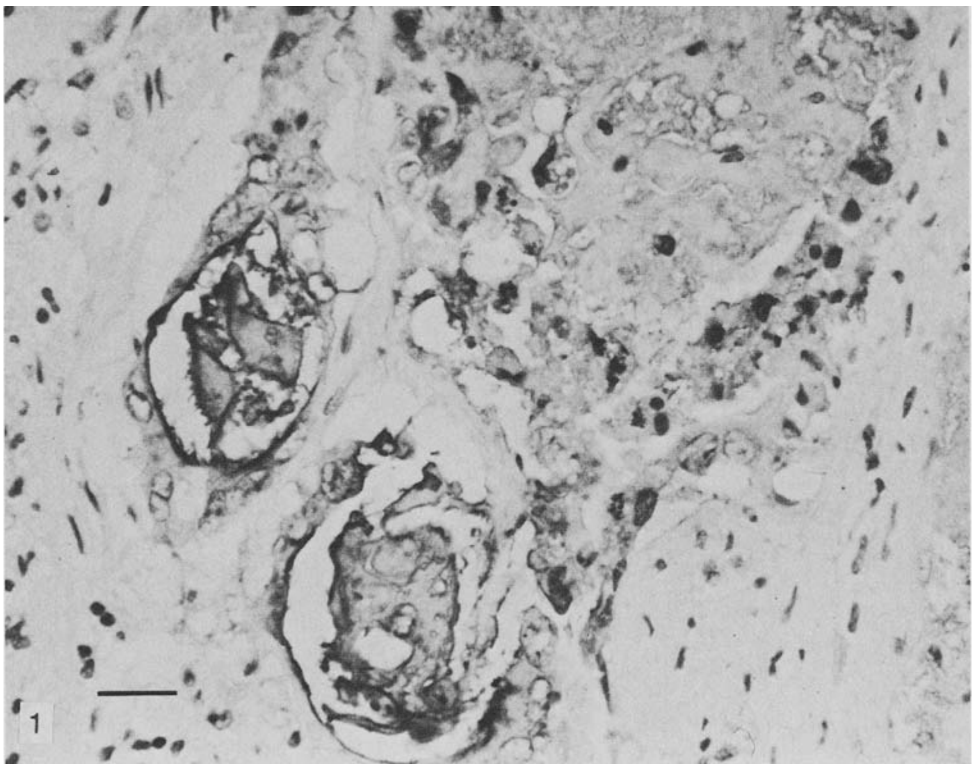

FIGURE 1.—Metastatic adenocarcinoma of the colon showing strongly positive staining of the glycocalyces and weaker staining of the adjacent necrotic tumor. IMP PAP technique; anti-CEA, 1/500 dilution. Hematoxylin counterstain. *Bar* = 50 μm.