Usefulness of Carcinoembryonic Antigen Monitoring Despite Normal Preoperative Values in Node-Positive Colon Cancer Patients

Zhaoshi Zeng, M.D.,* Alfred M. Cohen, M.D,* Carlos Urmacher, M.D.†

*From the Colorectal Service, *Department of Surgery and the †Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York*

PURPOSE: The aim of our study was to determine to what extent serial carcinoembryonic antigen (CEA) monitoring is helpful in detecting colorectal cancer recurrence in patients if their preoperative serum CEA is normal. Additional major objectives of this study were to correlate CEA immunohistochemical features of the primary tumor with serum CEA levels at the time of tumor recurrence in node-positive colorectal cancer patients with low preoperative CEA values. METHODS: One hundred fourteen node-positive colorectal cancer patients with preoperative serum CEA levels of <5.0 ng/ml undergoing clinically curative operations were studied. Primary tumors were evaluated for tissue CEA using the same monoclonal antibody as used for serum CEA determinations utilizing the avidin-biotin-peroxidase immunohistochemical technique. RESULTS: The exact preoperative serum CEA value did not correlate with tumor grade, immunohistochemical CEA intensity or pattern. In the 32 patients who developed recurrent cancer, the serum CEA at recurrence was greater than 5 ng/ml in 44 percent. All such patients had CEA present in their primary tumor. There was no correlation with the exact preoperative serum CEA, the intensity of the primary tissue CEA, or the localization of such CEA and subsequent serum elevation at recurrence. CONCLUSION: Serum CEA is a useful marker in the detection of recurrent colorectal cancer despite normal preoperative values. [Key words: Carcinoembryonic antigen; Colon cancer]

Zeng Z, Cohen AM, Urmacher C. Usefulness of carcinoembryonic antigen monitoring despite normal preoperative values in node-positive colon cancer patients. Dis Colon Rectum 1993;36:1063–1068.

The oncofetal protein carcinoembryonic antigen (CEA) is used clinically in patients with colorectal cancer to compliment pathologic staging in prognosis,[1–3] as a postoperative serum marker for the detection of recurrence,[4–6] and as an antigen for radioimmunodetection[7–9] and radioimmunotherapy.[4, 6] The presence of elevated preoperative serum levels correlates with the stage of disease.[4,6] However, only about one-half of node-positive colorectal carcinoma patients have elevated preoperative levels.[4, 6] It is unclear as to the usefulness of postoperative serial CEA levels in the detection of recurrence in patients whose preoperative levels are low. The major objectives of this study were to correlate tumor histologic and CEA immunohistochemical features of the primary tumor with preoperative serum CEA levels, as well as with serum CEA levels at the time of tumor recurrence in node positive colorectal cancer patients with low preoperative CEA values.

Address reprint requests to Dr. Cohen: 1275 York Avenue, New York, New York 10024.

PATIENTS AND METHODS

A group of 114 colon cancer patients treated in the Colorectal Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center from 1986 through 1990 were studied. These patients all had cancer that had spread to regional lymph nodes, had undergone potentially curative operations, and had a preoperative serum CEA value of less than 5.0 ng/ml.

Grade

The degree of differentiation of all invasive colorectal cancers are routinely categorized by our Department of Pathology into one of three groups: well differentiated (Grade I), moderately differentiated (Grade II), and poorly differentiated/anaplastic (Grade III).

Monoclonal Anti-CEA Antibody

The monoclonal anti-CEA antibody (catalogue no. 1199145) was commercially obtained from Boehringer Mannhein Hybritech, Indianapolis, Indiana. This monoclonal antibody (MoAb) is the same MoAb used in our clinical chemistry laboratory to determine serum CEA.

Controls

The control used with each batch of sections was a well-differentiated colonic adenocarcinoma from a patient whose preoperative serum CEA level was 249.0 ng/ml. Sections of colorectal cancer without the specific MoAb were used as a negative control.

Immunohistochemistry

For the immunohistochemical staining of CEA, formalin-fixed paraffin-embedded tissue was used. Four- to 6-μm-thick serial sections were cut from the primary tumor and lymph nodes and mounted on glass slides.

The avidin-biotin-peroxidase complex technique was used. The slides were incubated at 60°C overnight. After deparaffinization with xylene and rehydration through graded alcohol, the sections were incubated in 3 percent hydrogen peroxide for five minutes to quench endogenous peroxidase activity. Tissue sections were processed with 0.05 percent saponin solution (Sigma, St. Louis, MO) for 30 minutes at room temperature. The sections were then rinsed with phosphate-buffered saline (PBS).

For reduction of nonspecific background staining, all slides are placed in 1 percent bovine serum albumin/PBS, incubated with 1:20 normal horse serum for 30 minutes, and then incubated with murine anti-CEA MoAb. Preliminary studies had determined the optimum dilution and duration of the MoAb to be 1:3,000 with incubation at 4°C overnight. The specificity and accuracy of the immunoreaction was checked by a negative control using sections incubated with nonimmune serum instead of anti-CEA antibody. Sections were again rinsed in PBS and were incubated with 5 percent bovine serum albumin/PBS BHAM6 horse anti-mouse immunoglobulin diluted (1:500) at room temperature for 30 minutes, respectively. Following the latter step, tissue sections were rinsed in PBS and immunostaining was developed by immersion in 0.06 percent 3,3′-diaminobenzidine tetrahydrochloride solution dissolved in 0.5 percent Triton-X/PBS for two minutes. Sections were counterstained with modified Horris-hematoxylin (Fisher) and 0.3 percent ammonia water and passed through graded alcohol and xylene to dehydrate. Finally, the slides were coverslipped in balsam, then observed by conventional light microscopy.

Scoring Methods for Immunohistochemical Staining Analysis

Presence of reddish brown 3,3′-diaminobenzidine tetrahydrochloride precipitate was regarded as positive immunoreactivity. Sections showing no staining when compared with normal rabbit serum control were designated as negative.

The following parameters were used to record the staining of CEA in each case:

1. The *pattern* of CEA localization in tissue was classified based on the location of CEA. Four patterns were identified: 1) luminal or apical type, CEA is localized predominantly along the luminal surface of the cancer cells; 2) cytoplasmic type, CEA is localized in the cytoplasm; 3) both, CEA is localized in both luminal and cytoplasm pattern; and 4) stromal, CEA stained in the surrounding stroma as well as in the lumen and cytoplasm of cancer cells.

2. *Intensity* of anti-CEA reaction staining of the tumor cells was graded from + to +++ (+, weak; ++, moderate; +++, strong), according to the degree of brown pigment.

3. *A percentage of positive cells* (<25 percent, 25–50 percent, 50–75 percent, >75 percent) was assigned to score the distribution of cellular reactivity.

Statistical Analysis

Statistical analysis was carried out using Fisher's exact probability test. Probability values of <0.05 were considered to be significant.

RESULTS

Relationship of Preoperative Serum CEA Level and Tumor Differentiation in Node-Positive Colorectal Patients

By definition, the preoperative serum CEA of all patients involved in this study was below 5.0 ng/ml. Table 1 tabulates these data. No relationship was found between tumor differentiation and the exact preoperative serum CEA level.

Primary Tumor Tissue CEA Immunohistochemical Staining

Of 114 cases of node-positive colorectal cancer patients, 109 cases (95.6 percent) were positive and 5 (4.4 percent) negative for tumor tissue CEA (Table 2). The pattern of cellular localization was

Table 1.
Preoperative Serum CEA and Tumor Differentiation

Differentiation	No. of Patients	Preoperative Serum CEA Level			
		0	0.1–1.0	1.1–3.0	3.1–5.0
Well	8 (7.0)*	1 (5.6)	2 (9.1)	3 (6.3)	2 (7.7)
Moderate	77 (67.6)	10 (55.5)	11 (50.0)	37 (77.1)	19 (73.1)
Poor	29 (25.4)	7 (38.9)	9 (40.9)	8 (16.6)	5 (19.2)

* Numbers in parentheses are percentage of value.

Table 2.
Correlation Between Preoperative CEA and Immunohistochemical Tumor CEA

	No. of Patients	Preoperative CEA (ng/ml)				*P**
		0	0.1–1.0	1.1–3.0	3.1–5.0	
Tumor CEA						
Negative	5 (4.4)†	3 (16.7)	0 (0.0)	1 (2.1)	1 (3.8)	
Positive	109 (95.6)	15 (83.3)	22 (100)	47 (97.9)	25 (96.2)	=0.65
Tumor CEA location						
Luminal	56 (51.3)	7 (46.7)	11 (50.0)	25 (53.2)	13 (52.0)	
Cytoplasmic	8 (7.4)	1 (6.6)	1 (4.5)	3 (6.4)	3 (12.0)	
Both	42 (38.5)	7 (46.7)	9 (41.0)	18 (38.3)	8 (32.0)	=0.984
Stroma	3 (2.8)	0 (0.0)	1 (4.5)	1 (2.1)	1 (4.0)	
Intensity of CEA						
Weak(+)	40 (36.7)	9 (60.0)	11 (50.0)	10 (21.3)	10 (40.0)	
Moderate(++)	50 (45.9)	5 (33.3)	8 (36.4)	26 (55.3)	11 (44.0)	=0.111
Strong(+++)	19 (17.4)	1 (6.7)	3 (13.6)	11 (23.4)	4 (16.0)	
Cells positive for CEA (%)						
<25	10 (9.2)	3 (20.0)	4 (18.2)	0 (0.0)	4 (16.0)	
25–50	10 (9.2)	1 (6.7)	3 (13.6)	2 (4.3)	3 (12.0)	=0.023
50–75	15 (13.8)	3 (20.0)	4 (18.2)	5 (10.6)	3 (12.0)	
>75	74 (67.8)	8 (53.3)	11 (50.0)	40 (85.1)	15 (60.0)	

* *P* values refer to Fisher's exact probability test.
† Number in parentheses are percentage of value.

luminal (51.3 percent), both luminal and cytoplasmic type (38.5 percent), cytoplasmic type (8.3 percent), and the stromal type (2.7 percent). Staining of cells was generally ubiquitous with two-thirds of cases having >75 percent cells positive for CEA. The intensity of the positive reaction for CEA was variable: weak stain, 40 cases (36.7 percent); moderate, 50 cases (45.9 percent); and strong, 19 cases (17.4 percent).

Correlation of CEA Tissue Staining and Preoperative CEA Level

Of the five patients with negative CEA tumor cell staining, serum CEA levels for three patients was 0 and for the other two cases 1.3 and 4.9 ng/ml. As seen in Table 2, no correlation could be established between tumor CEA pattern and serum CEA level. Sixty percent of the cases with 0 serum CEA exhibited only weak(+) CEA staining. As the serum CEA level rose, the intensity of tissue CEA increased. However, these findings were not significantly different.

Correlation of CEA Tissue Staining and Tumor Differentiation

Table 3 shows the grade of tumor differentiation in relation to tumor CEA. Four of five negative tumor CEA stainings were in poorly differentiated cases. Positive CEA staining was observed in 100 percent of patients with well-differentiated tumor, 98.7 percent with moderately differentiated tumor and 86.2 percent with poorly differentiated tumor. There was a statistically significant difference ($P < 0.05$).

In well-differentiated and moderately differentiated tumors, the luminal pattern was present in 50.0 and 59.2 percent, respectively. For poorly differentiated tumors, the luminal pattern was expressed in only 28.0 percent. In regard to the correlation between CEA intensity and tumor differentiation, stronger staining (++ and +++) was found in the well-differentiated tumors than in the poorly differentiated tumor. Among 25 poorly differentiated tumors with positive staining, 6 cases (24 percent) had less than 25 percent of cells

Table 3.
Correlation Between Tumor Differentiation and Tumor CEA

	No. of Patients	Tumor Differentiation			*P**
		Well	Moderate	Poor	
Tumor CEA					
Negative	5 (4.4)†	0 (0.0)	1 (1.3)	4 (13.8)	=0.023
Positive	109 (95.6)	8 (100.0)	76 (98.7)	25 (86.2)	
Tumor CEA location					
Luminal	56 (51.3)	4 (50.0)	45 (59.2)	7 (28.0)	
Cytoplasmic	8 (7.4)	1 (12.5)	5 (6.6)	2 (8.0)	
Both	42 (38.5)	3 (37.5)	25 (32.9)	14 (56.0)	=0.073
Stroma	3 (2.8)	0 (0.0)	1 (1.3)	2 (8.0)	
Intensity of CEA					
Weak(+)	40 (36.7)	2 (25.0)	28 (36.8)	10 (40.0)	
Moderate(++)	50 (45.9)	5 (62.5)	34 (44.7)	11 (44.0)	=0.948
Strong(+++)	19 (17.4)	1 (12.5)	14 (18.5)	4 (16.0)	
Cells positive for CEA (%)					
<25	11 (10.0)	0 (0.0)	5 (6.6)	6 (24.0)	
5–50	9 (8.3)	0 (0.0)	1 (9.2)	2 (8.0)	=0.245
50–75	15 (13.7)	2 (25.0)	10 (13.2)	3 (12.0)	
>75%	74 (67.9)	6 (75.0)	54 (71.0)	14 (56.0)	

* *P* values refer to Fisher's exact probability test.
† Number in parentheses are percentage of value.

expressing CEA. None of these differences reached statistical significance.

Serum CEA at Subsequent Recurrence

In follow-up, 32 of the 114 patients have developed evidence of recurrence or metastases All had CEA present on tissue staining. There was no correlation between recurrence and other parameters (tumor differentiation, tumor CEA location, and intensity).

At recurrence, the serum CEA was greater than 5.0 ng/ml in 14 (43.8 percent) (Table 4). Ninety percent of the recurrences with elevated CEA were in patients with metastatic disease, as opposed to local recurrences. Ten of 21 patients with hepatic metastases and four of seven patients with lung metastases had elevated serum CEA at recurrence. There was no correlation with the exact preoperative serum CEA, the intensity of the primary tissue CEA, or the location of such CEA and subsequent elevation at recurrence.

DISCUSSION

CEA is a product of columnar and goblet cells in the normal colon as well as colonic cancer cells. It is expressed in normal colonic mucosa bordering the glandular lumen, in luminal secretions and in the tumor cell cytoplasm.[10, 11]

Immunohistochemical staining using monoclonal antibodies has been shown to be a sensitive method for demonstration of tissue CEA.[12–14] In the present study of 114 node-positive colorectal cancer patients with low preoperative serum CEA levels, 109 cases (94 percent) are tumor tissue CEA positive. Of these, 63.3 percent stained moderately or strongly positive.

The lack of correlation between serum levels and tumor tissue CEA has been reported by other investigators.[15–17] Page *et al.*[17] observed that in 37 colorectal cancer patients serum CEA measurement had a sensitivity of only 41.9 percent as compared with 90.3 percent for the immunohistochemical staining. Cunningham *et al.*[18] reported that 84.8 percent (13/17) of colorectal cancer patients with the serum CEA level below 2.5 ng/ml showed CEA-positive tumor staining not different from 71.1 percent (27/38) patients with serum CEA value above 2.5ng/ml. Midiri *et al.*[15] studied 57 colorectal cancer patients in which 11 had positive tumor tissue CEA with normal serum CEA.

Gold and Freedman[10] noted the greater the tumor cell differentiation, the stronger the staining for tumor CEA. Similar findings have been described by others. Nakopoulou and Zinozi[14] confirmed that more cases were positive and more strongly stained in well-differentiated carcinoma than in poorly differentiated carcinoma. Although we observed that 75 percent of well differentiated tumor showed moderate or strong CEA staining as compared with 60 percent of poorly differentiated

Table 4.
Correlation Between Primary Tumor and Serum CEA Level at Recurrence

Primary Tumor	Number (%)	CEA Level at Recurrence		P*
		Normal Number (%)	Elevated (>5.0 ng/ml) Number (%)	
Recurrence/metastases	32	18 (56.3)	14 (43.8)	
Preoperative Serum CEA				
0	6 (18.8)	4 (22.2)	2 (14.3)	
0.1–1.0	8 (25.0)	7 (38.9)	1 (7.1)	=0.120
1.1–3.0	11 (34.4)	5 (27.8)	6 (42.9)	
3.1–5.0	7 (21.8)	2 (11.1)	5 (35.7)	
Differentiation				
Well	3 (9.4)	0 (0.0)	3 (21.4)	
Moderate	21 (65.6)	13 (72.2)	8 (57.1)	=0.179
Poor	8 (25.0)	5 (27.8)	3 (21.5)	
Tumor CEA location				
Luminal	13 (40.6)	7 (38.9)	5 (42.9)	
Cytoplasmic	2 (6.3)	1 (5.5)	1 (7.1)	=0.784
Both	16 (50.0)	10 (55.6)	6 (42.9)	
Stroma	1 (3.1)	0 (0.0)	1 (7.1)	
Intensity of CEA				
Weak(+)	9 (28.1)	7 (38.9)	2 (14.3)	
Moderate(++)	17 (53.1)	9 (50.0)	8 (57.1)	=0.253
Strong(+++)	6 (18.8)	2 (11.1)	4 (28.6)	
Cells positive for CEA (%)				
<25	2 (6.3)	1 (5.7)	1 (7.1)	
25–50	0 (0.0)	0 (0.0)	0 (0.0)	=1.000
50–75	2 (6.3)	1 (5.7)	1 (7.1)	
>75	28 (87.4)	16 (88.8)	12 (85.8)	

* P value refer to Fisher's exact probability test.

tumors, these differences were not statistically significant. However, four of our five patients who stained negatively for CEA had poorly differentiated cancer. This is in agreement with the studies of Goslin *et al.*,[19] Denk and colleagues,[20] and Zamcheck.[21]

Several authors[22,23] found that the pattern of tumor tissue CEA localization influenced preoperative serum CEA level. In a study of tissue CEA patterns, Hamada *et al.*[22] reported that in 23 cases with the serum CEA level below 10 ng/ml, 30.5 percent were the luminal type and 4.4 percent were stromal, but in 28 patients with CEA values above 10 ng/ml, 3.6 percent were the luminal type and 42.9 percent were stromal. Our study of low preoperative serum CEA patients demonstrated luminal distribution in 51 percent, compared with stromal only in 2.8 percent. Our data confirm this correlation.

We were particularly interested in the correlation with preoperative tissue CEA as a predictor of elevated serum CEA at recurrence. Unfortunately, there was no correlation with the exact preoperative serum CEA, the intensity of the primary tissue CEA, or the pattern of localization of such CEA and subsequent serum CEA elevation at recurrence. Most important, however, approximately one-third of patients with poorly differentiated as well as moderately differentiated primary tumor did have an elevated serum CEA at recurrence.

We conclude that serum CEA is a useful marker in the detection of recurrent colorectal cancer despite normal preoperative values.

ACKNOWLEDGMENTS

The authors thank Mr. Antonio Scorza, Mrs. Maryann D.Gangi, and Mrs. Dina Gildor for their technical assistance with tissue sections.

REFERENCES

1. Kohler JP, Simnonowitz D, Paloyan D. Pre-operative CEA level: a prognostic test in patients with colorectal carcinoma. Am Surg 1980;46:449–52.
2. Wolmark N, Fisher B, Wieaund HS. The prognostic value of modifications of Dukes' C class of colorectal cancer: an analysis of the NASBP clinical trials. Ann Surg 1986;203:115–22.

significance of carcinoembryonic antigen in colorectal carcinoma, serum levels before and after resection and before recurrence. Arch Surg 1991;126:314–6.

4. Martin EW Jr, Minton JP, Carey LC. CEA-directed second-look surgery in the asymptomatic patient after primary resection of colorectal cancer. Ann Surg 1985;202:310–17.
5. Goslin R, Steele G, MacIntyre J, *et al.* The use of pre-operative plasma CEA levels for the stratification of patients after curative resection of colorectal cancer. Ann Surg 1980;192:747–51.
6. Wanebo JH, Stearns MW, Schwartz MK. Use CEA as an indicator of early recurrence and as a guide to a selected second-look procedure in patients with colorectal cancer. Ann Surg 1978;188:481–92.
7. Goldenberg DM, Goldenberg H, Sharkey RM, *et al.* Imaging of colorectal carcinoma with radiolabeled antibodies. Semin Nucl Med 1989;19:262–81.
8. Zeng ZS, Divgi CR, McDermott KC, *et al.* Radioimmunodetection of colorectal cancer. Perspect Colon Rectal Surg 1991;4:35–67.
9. Lind P, Lechner P, Arian-Schad K, *et al.* Anti-carcinoembryonic antigen immunoscintigraphy (technetium-99 m-monoclonal antibody BW 431/26) and serum CEA levels in patients with suspected primary and recurrent colorectal carcinoma. J Nucl Med 1991;32:1319–25.
10. Gold P, Freeman SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med 1965;122:467–81.
11. Bordes M, Michiels R, Martin F. Detection by immunofluorescence of carcinoembryonic antigen in colonic carcinoma, other malignant or benign tumors and non-cancerous tissues. Digestion 1973;9:109–15.
12. Crowson M, Hockey MS, Newman J, *et al.* An immunocytochemical study of carcinoembryonic antigen (CEA) expression in colorectal tumors and their metastases using a monoclonal antibody (abstr). Br J Surg 1984;71:376.
13. Goldenberg DM, Sharkey RM, Primus FJ. Immunocytochemical detection of carcinoembryonic antigen in conventional histopathology specimens. Cancer 1978;42:1546–53.
14. Nakopoulou L, Zinozi M, Theodoropoulos G, *et al.* Carcinoembryonic antigen detection by immunocytochemical methods in carcinomas of the colon and stomach. Dis Colon Rectum 1983;26:269–74.
15. Midiri G, Amanti C, Benedetti M, *et al.* CEA tissue staining in colorectal cancer patients. Cancer 1985;55:2624–29.
16. Boyd CR, Bivens BA, Kashmire R, *et al.* Plasma CEA, tumor CEA, tumor histology. J Surg Oncol 1976;8:507–12.
17. Page M, Dalifard I, Bertrand G. Immunostaining of colorectal cancer with monoclonal anti-CEA antibodies compared to serum and tumor CEA content. Anticancer Res 1986;6:893–96.
18. Cunningham L, Stocking B, Halter SA, *et al.* Immunoperoxidase staining of carcinoembryonic antigen as a prognostic indicator in colorectal carcinoma. Dis Colon Rectum 1986;29:111–6.
19. Goslin R, O'brien MJ, Steele G, *et al.* Correlation of plasma CEA and CEA tissue staining in poorly differentiated colorectal cancer. Am J Med 1981;71:246–53.
20. Denk H, Tappeiner G, Eckerstorfer R, *et al.* Carcinoembryonic antigen in gastrointestinal and extragastrointestinal tumors and its relationship to tumor cell differentiation. Int J Cancer 1972;10:262–72.
21. Zamcheck N. The expanding field of colorectal cancer marker: CEA, the prototype. Cancer Bull 1981;33:141–51.
22. Hamada Y, Yamamura M, Hioki K, *et al.* Immunohistochemical study of carcinoembryonic antigen in patients with colorectal cancer, correlation with plasma carcinoembryonic antigen levels. Cancer 1985;55:136–41.
23. Nagura H, Tsutsumi Y, Shioda Y, *et al.* Immunohistochemistry of gastric carcinoma and associated disease: novel distribution of carcinoembryonic antigen and secretory component on the surface of gastric cancer cells. J Histochem Cytochem 1992;31(Suppl 1A):193–8.