The prognostic value of preoperative serum levels of CEA, CA19-9 and CA72-4 in patients with colorectal cancer

Chao Xu Zheng¹, Wen Hua Zhan¹, Ji Zong Zhao², Dong Zheng³, Dong Ping Wang¹, Yu Long He¹ and Zhang Qing Zheng¹

INTRODUCTION

Carcinoembryonic antigen (CEA), originally described by Gold and Freedman[¹] in 1965, is now an acknowledged member of immunoglobulin superfamily[²], with a role as an intracellular adhesion molecule[³]. Carbohydrate antigen 19-9 (CA19-9), obtained with a monoclonal antibody produced by immunizing a mouse with a colonic cancer cell line in 1979[⁴], is a ligand for E-selectin that plays an important role in the adhesion of cancer cells to endothelial cells[⁵-⁶]. More recently, tumor-associated glycoprotein 72 (TAG-72), has been identified with the monoclonal antibody B72.3[⁷]. This antigen has been found in a variety of human adenocarcinomas (colorectal, gastric, ovarian, breast, and lung), but rarely expressed in benign and normal adult tissue[⁸-⁹]. The CA72-4 antigen is an antigenic determinant of TAG-72 which is recognized by B72.3 and CC-49 monoclonal antibodies[¹⁰]. CEA, CA19-9 and CA72-4 represent the currently most useful tumor markers for gastrointestinal malignancies. Elevated serum levels of CEA, CA19-9 and CA72-4 have been found in many patients with colorectal, gastric, biliary tract, and pancreatic carcinoma[¹¹-¹⁷]. Today, these tumor markers are primarily used in early diagnosis and monitoring of therapeutics[¹⁸,¹⁹], and determining the prognosis of patients who have undergone tumor resection. Since April 1996, these three tumor markers have been used as routine in our hospital for detecting colorectal cancer. The aim of this study was to assess their correlations with the tumor pathology and their prognostic value.

MATERIALS AND METHODS

Patients

A total of 107 patients with histologically diagnosed colorectal carcinoma were included in the study group. All had been treated surgically between April 1996 and March 1997. There were 74 men and 33 women. The mean age of the patients was 56.1 years (range: 26-81 years). The pathological findings (types and size of tumor, morphology, depth of invasion and number of positive lymph nodes) of the patients were reviewed. The patients were staged in accordance with Dukes classification. Ninety seven patients (90.7%) were followed up for at least 36 months or till death. Ten patients lost to follow up were excluded from the final assessment.

The control group included 155 healthy adults who received routine physical examination between December 1995 and July 1996 in our hospital, among whom, 72 were men and 83 were women. The mean age was 47.5 years (range: 20-89 years).

Serum assays for CEA, CA19-9 and CA72-4

Peripheral blood samples were obtained from the patients preoperatively and those from healthy subjects in the control group after fasted overnight. Sera were promptly separated and stored at -80°C for use. Serum CEA was measured with a one-step, solid-phase enzyme immunoassay commercial kit (CEA EIA Kit, General Biologicals Inc., Taiwan). Serum CA19-9 was determined with a two-step, solid-phase enzyme immunoassay commercial kit (CA19-9 EIA Kit, Maxim Biotech Inc., USA). A solid-phase two-site radioimmunometric assay kit, CA72-4 RIA kit (Centocor Inc., USA), was used for determination of serum levels of CA72-4. The upper limit and cut off values of serum CEA, CA19-9 and CA72-4 in the control group 99% confidence interval were 5.0 µg/L, 31.0 KU/L and

1Department of General Surgery, 1Laboratory of Surgery, 2Department of Medicine, First Affiliated Hospital, Sun Yat-Sen University of Medical Sciences, Guangzhou 510080, Guangdong Province, China

Dr. Chao Xu Zheng, now working as a surgeon and lecturer in the Department of General Surgery, First Affiliated Hospital, Sun Yat-Sen University of Medical Sciences, who is a Ph.D. student, having 7 papers published.

This study was supported by the research grant from Administration of Key Disciplines for “Project 211” of Sun Yat-Sen University of Medical Sciences, Grant No.98097.

Correspondence to: Dr. Chao Xu Zheng, Department of General Surgery, First Affiliated Hospital, Sun Yat-Sen University of Medical Sciences, 58 Zhongshan 2nd Road, Guangzhou 510080, Guangdong Province, China

Tel. 0086-20-7755766 Ext.8720, Fax. 0086-20-87750632
Email. zcxzd@163.net

Received 2000-11-13 Accepted 2000-11-30
markers.

The results also suggested the more advanced the Dukes stage, the higher the positive rates of all these tumor markers and the pathological findings. The relation between recurrence rates and serum levels of these tumor markers was also evaluated by Chi-square test. The Kaplan-Meier method was used to calculate the cumulative survival rates and plot survival curves, and Log Rank test was used for the evaluation of statistical differences between the curves. P values of less than 0.05 were considered to be statistically significant.

RESULTS

The mean serum levels of CEA, CA19-9 and CA72-4 in 107 patients with colorectal cancer were 5.9 ± 10.1 µg/L (range: 0.1 - 70.0 µg/L), 61.5 ± 302.1KU/L (range: 0.1 - 5105.0 KU/L) and 8.4 ± 19.5 KU/L (range: 1.1 - 188.2 KU/L), respectively. The positive rates of serum CEA, CA19-9 and CA72-4 in these patients were 29.9%, 25.2% and 32.7%, respectively.

Relationship between serum levels of tumor markers and pathological findings

The correlation between positive rates of serum tumor markers and the pathological variables is shown in Table 1. Positive rate of serum CA72-4 was significantly higher in poorly-differentiated colorectal cancer than in well and moderately-differentiated types (P = 0.031). There was no statistical significance between the positive serum tumor markers and the size and morphology of the colorectal cancer, but were well correlated with the depth of invasion. Both serum CA19-9 and CA72-4 were found in 30, 25 and 31% of cases, respectively. The overall 3-year survival rate was 53.3% in serum CEA-positive patients and 73.6% in CA19-9-negative patients (P = 0.042, Figure 1); 48.0% in CA19-9-positive patients and 73.6% in CA19-9-negative patients (P = 0.011, Figure 2); 48.4% in CA72-4-positive patients, and 75.8% in CA72-4-negative patients (P = 0.003, Figure 3).

Relationship between serum levels of tumor markers and pathological findings

The correlation between positive rates of serum tumor markers and the pathological variables is shown in Table 1. Positive rate of serum CA72-4 was significantly higher in poorly-differentiated colorectal cancer than in well and moderately-differentiated types (P = 0.031). There was no statistical significance between the positive serum tumor markers and the size and morphology of the colorectal cancer, but were well correlated with the depth of invasion. Both serum CA19-9 and CA72-4 were found in 30, 25 and 31% of cases, respectively. The overall 3-year survival rate was 53.3% in serum CEA-positive patients and 73.6% in CA19-9-negative patients (P = 0.042, Figure 1); 48.0% in CA19-9-positive patients and 73.6% in CA19-9-negative patients (P = 0.011, Figure 2); 48.4% in CA72-4-positive patients, and 75.8% in CA72-4-negative patients (P = 0.003, Figure 3).

Preoperative serum levels of CEA, CA19-9 and CA72-4 as prognostic indexes

Of the 97 followed up patients, 86 were classified as Dukes A, B and C stage according to the pathological findings. All underwent curative resection. The overall recurrence rate was 32.6%. In the serum CA19-9 or CA72-4 positive patients, the recurrence rates were significantly higher than that in patients with negative result (Table 2), while serum level of CEA did not correlate well with the recurrence rate (P = 0.102).

Among these 97 patients, positive serum CEA, CA19-9 and CA72-4 were found in 30, 25 and 31 cases, respectively. The overall 3-year survival rate was 67.0%. Survival curves of the patients according to positive and negative serum CEA, CA19-9 and CA72-4 are shown in Figures 1-3, respectively. Significant differences in survival curve were found between the positive and negative ones. The 3-year survival rate was 53.3% in serum CEA-positive patients and 73.1% in CEA-negative patients (P = 0.042, Figure 1); 48.0% in CA19-9-positive patients and 73.6% in CA19-9-negative patients (P = 0.011, Figure 2); 48.4% in CA72-4-positive patients, and 75.8% in CA72-4-negative patients (P = 0.003, Figure 3).

Table 1  Positive rates of CEA, CA19-9 and CA72-4 in accordance with pathologic findings

| Pathological factors | No. of cases | CEA positive (%) | P value | CA19-9 positive (%) | P value | CA72-4 positive (%) | P value |
|----------------------|--------------|------------------|---------|---------------------|---------|---------------------|---------|
| Histological type    |              |                  |         |                     |         |                     |         |
| Well, moderately     | 73           | 30.1             | 0.001   | 24.7                | 0.001   | 26.0                | 0.001   |
| Poorly               | 34           | 29.4             | 0.011   | >0.05               | 0.001   | >0.05               | 0.001   |
| Tumor size (cm)      |              |                  |         |                     |         |                     |         |
| ≤5                   | 57           | 31.6             | 0.001   | 22.8                | 0.001   | 28.1                | 0.001   |
| >5                   | 50           | 28.0             | 0.001   | >0.05               | 0.001   | >0.05               | 0.001   |
| Gross morphology     |              |                  |         |                     |         |                     |         |
| Polypoid, fungating  | 45           | 28.9             | 0.001   | 26.7                | 0.001   | 33.3                | 0.001   |
| Ulcerative           | 41           | 29.3             | 0.001   | 24.4                | 0.001   | 31.7                | 0.001   |
| Diffusely infiltrative | 21       | 33.3             | 0.001   | >0.05               | 0.001   | >0.05               | 0.001   |
| Depth of invasion*   |              |                  |         |                     |         |                     |         |
| Within sm           | 22           | 13.6             | 0.001   | 9.1                 | 0.001   | 13.6                | 0.001   |
| dm, s               | 54           | 25.9             | 0.001   | 24.1                | 0.001   | 29.6                | 0.001   |
| p, s                | 31           | 48.4             | 0.001   | 38.7                | 0.001   | 51.6                | 0.001   |
| No. of positive lymph nodes |      |                  |         |                     |         |                     |         |
| 0                   | 61           | 29.5             | 0.001   | 14.8                | 0.001   | 21.3                | 0.001   |
| 1-3                 | 16           | 31.3             | 0.001   | >0.05               | 0.001   | 31.3                | 0.001   |
| >3                  | 30           | 30.0             | 0.001   | 43.3                | 0.001   | 53.3                | 0.001   |
| Dukes stage         |              |                  |         |                     |         |                     |         |
| A                   | 8            | 12.5             | 0.001   | 0.0                 | 0.0     | 0.0                 | 0.0     |
| B                   | 53           | 20.8             | 0.001   | 18.9                | 0.001   | 24.5                | 0.001   |
| C                   | 33           | 39.4             | 0.001   | 30.3                | 0.001   | 39.4                | 0.001   |
| D                   | 13           | 53.8             | 0.001   | 53.8                | 0.001   | 69.2                | 0.001   |

*sm: superficial muscularis propria; dm: deep muscularis propria; s: serosa layer; sp: serosal penetration to neighboring organs

Table 2  Recurrence rates in patients with positive and negative serum levels of CEA, CA19-9 and CA72-4 who underwent curative resection

| Tumor markers | No. of cases | Recurrence rate (%) | P value |
|--------------|--------------|---------------------|---------|
| CEA positive | 24           | 45.8                | 0.102   |
| negative     | 62           | 27.4                |         |
| CA19-9 positive | 19         | 52.6                | 0.034   |
| negative     | 67           | 26.9                |         |
| CA72-4 positive | 24         | 54.2                | 0.008   |
| negative     | 62           | 24.2                |         |

Figure 1  Survival curves of serum CEA-positive and CEA-negative patients. The difference in survival rates was statistically significant (P = 0.042).
Zheng CX, et al. CEA, CA19-9 and CA72-4 in patients with colorectal cancer

Figure 2 Survival curves of serum CA19-9 positive and CA19-9-negative patients. The difference in survival rates was statistically significant ($P = 0.011$).

Figure 3 Survival curves of serum CA72-4 positive and CA72-4-negative patients. The difference in survival rates was statistically significant ($P = 0.003$).

DISCUSSION

Ideal tumor markers would be inexpensive screening and deliver early diagnosis in a large population at risk for cancer. Unfortunately, currently available serological tumor markers for gastrointestinal cancers have not been proven to be ideal. Although CEA, CA19-9 and CA72-4 are the tumor markers commonly used currently in colorectal cancer, all of them have low diagnostic sensitivity\cite{11-13,17,20,21}. In this study, the overall positive rates of serum CEA, CA19-9 and CA72-4 were 29.2%, 25.2% and 32.7%, respectively. For early (Dukes A and B) colorectal carcinoma, the positive rates of the above tumor markers were significantly lower than those with Dukes C and D (Table 1). The lack of sufficient sensitivity of serum tumor markers strongly restrict their applications as appropriate tools for screening and diagnosis\cite{22,23}. Nevertheless, measurement of these tumor markers is still of importance in early detection of recurrence\cite{24,26}. Careful examinations of abdominal-pelvic CT scan, abdominal ultrasonography, colonoscopy and endoscopic ultrasonography can be complementary\cite{27}.

In this study, we investigated the correlation between preoperative serum levels of tumor markers and pathological following. The main aim was to reveal whether the following pathologic lesions as histological type, depth of invasion, lymph node metastasis and staging of lesions, were related to the preoperative serum levels of tumor markers. Our data showed that the positive rate of serum CA72-4 was related to the histological type which was comparable with those reported by Ikeguchi et al\cite{28}, in which there was a close relationship between CA72-4 and proliferative activity of cancer cells. Although there was no significant correlation between these tumor markers and the size of the tumor or the morphology, serum CEA and CA19-9 correlated well with the depth of invasion and Dukes staging. In contrast to the study carried out by Sato et al\cite{29}, we found that serum CA19-9 was also influenced by the number of metastatic lymph nodes, whereas serum CA72-4 was related to the depth of invasion, lymph node metastasis and staging of the lesions.

An important consideration in assessing the clinical value of tumor markers is to find whether tumor marker assay can provide useful information on clinical outcome of the patients, in addition to the common prognostic factors. Previous reports demonstrated that elevated preoperative serum levels of CEA and CA19-9 were predictive of increased mortality in colorectal cancer\cite{30}. Increased levels of CEA and CA19-9 were also associated with venous and lymphatic spread\cite{31,32}. As shown in our study and the reports of Forones and Tanaka\cite{30}, preoperative serum level of CEA was not a perfect predictor for recurrence in patients undergoing curative resection, but CA19-9 was proven to be a better indicator for recurrence. Positive CA19-9 expression on tumor tissue, and its positive pre and post-operative serum level, were associated with poor survival rates\cite{33,34}. Our study showed that there was significant difference in 3-year survival curves between serum CEA-positive and CEA-negative patients, and also significant difference was found in serum CA19-9 positive and CA19-9 negative patients, which was similar to that reported by Sato et al\cite{29}. CA72-4 was confirmed to have better sensitivity and specificity than CEA and CA19-9\cite{35,36}. However, very few studies on the prognostic value of CA72-4 in colorectal cancer have been reported in the literature, except some concerning gastric cancer\cite{16,28,37}. In the current study, in addition to CEA and CA19-9, we also investigated the prognostic value of serum level of CA72-4 in preoperative patients with colorectal cancer. We found that patients with positive serum CA72-4 had higher recurrence rate than those with negative CA72-4 ($P = 0.008$), and the 3-year survival rate much lower than that in CA72-4 negative patients ($P = 0.003$). High serum levels of tumor markers are often associated with more aggressive cancer, which usually has short disease free interval and short survival period. Both CEA and CA19-9 are molecules involved in intercellular
adhesion[3,5], hence, the cells expressing these molecules on their surfaces would possess a greater metastatic potential[38]. It was shown that in experimental metastasis model of colorectal carcinoma in nude mice, a systemic injection of recombinant CEA enhanced hepatic colonization of tumor cell in vivo[39], presumably by their increased hepatic retention[40]. Other authors reported that CA19-9 serves as a specific ligand for ELAM-1 and plays a significant role in the ELAM-1-mediated binding of human cancer cells to activated endothelial cells, which indicates CA19-9 expressed on the surface of cancer cells may involve in the process of vascular invasion and metastasis[41]. Meanwhile, the function of TAG-72 (CA72-4) in the process of tumor progression remains unclear.

In conclusion, the measurement of preoperative serum levels of CEA, CA19-9 and CA72-4 will aid in predicting the prognosis of patients with colorectal cancer who have been treated surgically. Elevated serum levels of tumor markers indicate high risk of cancer recurrence and poor survival. Such patients should receive adjuvant therapy, at the earlier stages of this disease.

REFERENCES

1 Gold P. Freedman SO. Demonstration of tumor specific antigens in human colon carcinoma by immunological tolerance and absorption techniques. J Exp Med. 1965;121:439-462
2 Paxton RJ, Mooser G, Pande H, Lee TD, Shively JE. Sequence analysis of carcinoembryonic antigen: identification of glycosylation sites and homology with the immunoglobulin supergene family. Proc Natl Acad Sci USA. 1987;84:920-924
3 Benkelson S, Fukis A, Jothy S, Beauchemin N, Shirato K, Stanners CP. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell. 1989;57:327-334
4 Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P. Anti-carcinoembryonic antigen monoclonal antibody B72.3. Cancer Res. 1989;49:78-85
5 Pinedo HM, de Vries E, Blankenship S, Farhood M, van den Heuvel-Lanfermeijer E, van der Heijden GJ. Carcinoma associated antigen in serum of patients. J Surg Oncol. 1989;41:18-24
6 Berg EL, Magnani J, Warnock RA, Robinson MK, Butcher EC. Comparison of carbohydrate antigen CA19-9 with CA15-3 in the diagnosis of pancreatic cancer. World J Gastroenterol. 1995;1:227-231
7 Ueda T, Shimada E, Uraga T. The clinicopathologic features of serum CA19-9 in patients operated upon for cure. Hepatogastroenterology. 1995;42:510-514
8 Lokhnygina O, Custers GJ, van den Heuvel-Eibrink MM, van der Graaf Y, van der Schans CP, van Asseldonk A, Spinnewyn BA, Meijer DK, Lamberts SWJ, van der Kwast TH. Carcinoma-associated antigen (CA) 19-9 levels in colorectal cancer. J Surg Oncol. 1995;61:173-178
9 Koshida K, Nakagawa K, Iwasaki K, Takahashi K, Kuroki S, Kato K, Inaba T, Kitamura Y, Kuramochi Y, Nakamura K, Fuse H, Kato H, Tanaka H, Torikai Y, Sasaki Y, Tanioka Y. Prognostic significance of carbohydrate antigen CA19-9 in colorectal carcinoma. J Surg Oncol. 1995;63:475-484
10 Watanabe K, Yamamoto K, Nishioka M, Sato T, Harada Y, Tanaka Y, Ohtani T, Ohno R, Nakamura Y, Uehara Y, Hashimoto Y, Takahashi M, Nakamura T, Takeuchi T, Ohno H, Uchiyama S, Kamaya A, Nakanishi Y, Toyota N, Kameyama H, Sakurai H, Abe T, Furukawa T, Goto Y, Yamada T, Ohno S, Sakai Y, Takahashi K, Iwase T, Ohno H, Kato K, Kitamura Y, Koshida K, Nakagawa K, Iwasaki K, Takahashi K, Kuroki S, Kato K, Inaba T, Kitamura Y, Kuramochi Y, Nakamura K, Fuse H, Kato H, Tanaka H, Torikai Y, Sasaki Y, Tanioka Y. Prognostic significance of carbohydrate antigen CA19-9 in colorectal carcinoma. J Surg Oncol. 1995;63:475-484
11 Kornek GV, Depisch D, Rosen HR, Temsch EM, Scheithauer W. Com-