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Comparative analysis of cancer-associated antigen CA-195, CA19-9 and carcinoembryonic antigen in diagnosis, follow-up and monitoring of response to chemotherapy in patients with gastrointestinal cancer

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Summary. To establish further the clinical significance of the CA-195 tandem immunoradiometric assay in gastrointestinal malignancies, the sera of a total of 222 subjects have been analysed and compared with assays of the "classical gastrointestinal tumour markers", CA19-9 and carcinoembryonic antigen (CEA). CA-195 elevations above normal (>10 U/ml) were noted in $^{51}/_{72}$ (70.8%) colorectal, $^{15}/_{15}$ (100%) pancreatic, and in $^{6}/_{12}$ (50%) gastric cancer patients. Whereas CA19-9 was increased (>37 U/ml) in 65%, 93%, and 42% of cases, only 54% colorectal, 45% pancreatic, and 42% gastric cancer patients had pathologically elevated serum CEA levels (>5 ng/ml). No abnormal increase of both CA-195 and CA19-9 was found in healthy volunteers, whereas $^{3}/_{20}$ (smoking) individuals had CEA levels slightly above normal. With a 29% false-positive rate noted among 103 patients with benign gastroinestinal disorders, the specifity of CA-195 was superior to that of CA19-9 (58%) and comparable with that of CEA (31%). A significant correlation between CA-195 levels and the clinical/pathological stage of disease was noted in colorectal ($P<0.01$) and pancreatic cancer patients ($P<0.007$). Preliminary results of serial measurements of CA-195 in colorectal cancer suggest that this new marker protein, which has no cross-reactivity with CEA, may be useful as a non-invasive test for postoperative surveillance of patients to detect disease recurrence, and serve to complement (though certainly not replace) standard clinical measurements of response to chemotherapy.

Key words: Cancer-associated antigen CA-195 – Carcinoembryonic antigen – CA19-9 – Gastrointestinal cancer

Introduction

In recent years a variety of tumour-associated antigens have been described and investigated with regard to their value for diagnosis and follow-up studies in cancer patients (Holyoke et al. 1985). In malignancies of the digestive tract, carcinoembryonic antigen (CEA), α-fetoprotein and the "gastrointestinal-related antigen" CA19-9 are commonly used. Some of them appear helpful in determining diagnosis, prognosis, and monitoring of therapeutic response; their clinical specifity, however, is compromised because the serum concentration can be influenced by cigarette smoking, the presence of liver- and numerous other non-malignant disorders, or they simply lack sensitivity (Holyoke et al. 1985; Moore et al. 1989).

CA-195 is a circulating tumour-associated antigen recently identified in high concentrations in the serum of patients with gastrointestinal malignancies (Bray et al. 1987). It is defined by the monoclonal antibody CC3C195, which recognizes both sialylated and non-sialylated forms of the Lewis A blood-group antigen (Fukura et al. 1987; Koda et al. 1987). Since the CA-195 antigen is immunologically and biochemically distinct from CEA, there is no cross-reactivity between these assays.

Although several recent studies have suggested the clinical utility of CA-195 in colorectal and pancreatic cancers (Bhargava et al. 1989; Gupta et al. 1987; Sundaram et al. 1987), its precise role has not been firmly established. The present study was undertaken to confirm the high sensitivity and specifity of CA-195 in these tumours, and prospectively to define its correlation with clinical stage in order to determine what part this tumour marker may play in the follow-up of patients with tumours amenable to surgical cure as well as in the monitoring of response to chemotherapy in those with metastatic disease.

Abbreviation. CEA, carcinoembryonic antigen

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Materials and methods

Specimens. CA-195 was measured in the serum of 72 patients with colorectal, 15 with pancreatic, and 12 with gastric cancers, as well as in 103 cases with benign gastrointestinal disorders, and 20 healthy volunteers (Table 1). The diagnosis of cancer was based on intraoperative and histological findings and tumour stage, and diagnosis of benign gastrointestinal disorders was based on clinical examination, laboratory tests, endoscopy, radiography, sonography, and computed tomography scan. Peripheral blood specimens were obtained from the patients after overnight fasting. Sera were promptly separated, stored at −20° C, and examined within 2 weeks. In 39 colorectal cancer patients who had undergone potential curative surgery, CA-195 and CEA levels were followed at 3-monthly intervals. Serial measurements of these marker proteins were obtained in an additional 22 patients with metastatic disease at 4-week intervals and when therapeutic response to cytotoxic chemotherapy was assessed.

Assay procedures. CA-195 was measured by a solid-phase, two-site immunoradiometric assay (Tandem-R CA-195, Hybritech Europe S.A., Liege, Belgium). In brief, samples containing CA-195 are reacted with a plastic bead (solid phase) coated with monoclonal antibody directed against the CA-195 antigen and simultaneously with radiolabeled monoclonal antibody of the same specifity. Following the formation of an isosandwich, the bead is washed to remove unbound labelled antibody. The reactivity bound to the solid phase is measured in a gamma counter. The amount of radioactivity measured is directly proportional to the concentration of CA-195 present in the test sample, which is determined form a standard curve. All samples were run in duplicate; the mean coefficient of variation between assays was 8%. In this study abnormally high levels of CA-195 in the serum were defined as those of more than 10 U/ml (Koda et al. 1987). For comparative purposes, simultaneous testing for CA19-9 (Biomedica GmbH, Vienna, Austria) and CEA was performed using standard (immunoradiometric) procedures (Abbot Diagnostic Division 1982; DelVillano et al. 1983). Cut-off values of 37 U/ml and 5 ng/ml were used for CA19-9 and CEA respectively.

Levels of tumour markers in different stages of malignant tumours were compared by analysis of variance. Statistical significance was taken at the $P<0.05$ level.

Results

CA-195 Levels in patients with gastrointestinal cancer

Levels of CA-195 greater than 10 U/ml were found in 72.7% of cases; 31% of patients had a value greater than 100 U/ml and 17% had a value greater than 1000 U/ml. The average serum level was 686.9 U/ml, and the median value (37.4 U/ml) was almost four times the cut-off value.

The results according to the primary tumour are shown in Table 1. Sensitivity was excellent in pancreatic cancer (100%) and high in colon cancer (70.8%). On the other hand, sensitivity (50%) and serum values (median = 4.3 U/ml) were low in stomach cancer. Among colorectal cancer patients no significant difference was found between either the CA-195 mean or the sensitivity when results were analysed according to histological grade. Examination of the data for a possible correlation with the surgical/pathological stage of disease revealed a significant relationship with Dukes' stage ($P<0.01$). Whereas a clear correlation with the extent of tumour was also noted among patients with (locally advanced versus disseminated) pancreatic cancer ($P<0.007$), the predominance of metastatic disease in the case of stomach cancer (75%) was considered not to allow a meaningful statistical analysis.

Other tumour markers and their combined use with CA-195

Synchronous serum specimens of the 99 patients with gastrointestinal malignancies were also assayed for levels of carcinoembryonic and CA19-9 tumour-associated

Table 1. Analysis of the serum values of CA-195 in normal subjects and patients with benign or malignant gastrointestinal disorders ($n=222$)

Diagnosis	No. of cases	CA-195 >10 U/ml *n* (%)	Mean value (U/ml)	Median value (U/ml)
Normal controls	20	0 (0)	2.8	2.3
Benign gastrointestinal (GI) disorders	103	30 (29)	23.6	3.5
Liver	48	19 (40)	16.5	8.8
Colorectum	33	8 (24)	4.9	1.0
Pancreas	15	3 (20)	10.4	5.1
Upper GI	7	0 (0)	0.5	0.0
Gastrointestinal cancers	99	72 (73)	686.9	37.4
Colorectum	72	51 (71)	704.0	24.8
Dukes A	3	1 (33)	5.3	0.6
Dukes B	21	11 (52)	11.6	2.4
Dukes C	15	12 (80)	189.4	7.9
Dukes D	33	27 (81)	1057.2	73.7
Pancreas	15	15 (100)	6477.9	89.6
Locally advanced	6	6 (100)	47.4	34.3
Metastatic	9	9 (100)	10152.4	90.8
Stomach	12	6 (50)	2129.7	5.5
Locally advanced	3	1 (33)	69.3	8.0
Metastatic	9	5 (56)	2816.5	21.8

CA-195		CEA		Detection rate
>10 U/ml	<10 U/ml	>5 ng/ml	<5 ng/ml	
•		•		53%
•			•	17%
	•	•		11%
	•		•	19%
			Total	81%

Fig. 1. Sensitivity of the combined use of CA-195 and carcinoembryonic antigen (*CEA*) serum levels in 72 patients with colorectal cancer

antigen. Whereas 65% colorectal, 93% pancreatic, and 42% gastric cancer patients had pathologically elevated CA19-9 levels (>37 U/ml), CEA was increased (>5 ng/ml) in only 54%, 45%, and 42% of cases.

The results of the combined use of CA-195 and CEA are summarized in Fig. 1. Since, according to a lack of cross-reactivity, 17% of colorectal cancer patients fell into the $CA\text{-}195^+/CEA^-$ category and 11% in the $CA\text{-}195^-/CEA^+$ category, the overall detection rate for this tumour could be improved to 81%. In gastric cancer the combined use of CA-195 and CEA resulted in only a modest improvement, i.e., a 58% detection rate. The sensitivity of CA-195 was not enhanced by using CA19-9 as an additional marker for the gastrointestinal cancers.

Follow-up studies in patients with colorectal cancer

For 22 patients who initially presented with malignant colorectal tumours not amenable to surgical cure and pathologically elevated CA-195 levels, it was possible to carry out follow-up examinations and to take additional samples monthly, and when therapeutic response to cytotoxic chemotherapy with either 5-fluorouracil, folinic acid, and cisplatin ($n=14$) or amonafide ($n=8$), a new synthetic imide antineoplastic agent (Scheithauer et al.), was established. A 68% overall concordance rate was found; concordance was greater in the case of progressive disease or in the case of response to treatment, and small in the case of stable disease. For 8 of the 11 patients (73%) exhibiting clinically progressive disease, the CA-195 serum level increased by more than 50%; it remained stable in 1 patient (9%), and it decreased in 2 (18%). In one case the drop was linked to an acute liver failure. A comparable strong concordance (75%) was noted in the 4 patients who achieved a partial response to treatment. When the tumour had been considered clinically stable (7 patients), some conflicting results were found in CA-195 variations: increase in 2 (29%), decrease in 1 (14%) and stabilization in only 4 (57%).

Synchronous evaluation of the specimens for variation of CEA and CA19-9 levels was performed in 18 patients. Results were comparable: for CEA, there was a 78%, 66%, and 50% concordance rate in the case of progressive disease (9 cases), partial response (3 cases) and stable disease (6 cases). The respective values for CA19/9 were 67%, 66%, and 50%.

Only preliminary follow-up data are available for patients who had undergone potential curative surgery. The postoperative period in these 39 patients varies between 3 and 15 months. There have been 3 deaths (1 was unrelated to tumour) and 2 patients with recurrence are alive. Of the patients who were operated on for cure, 38 had a postoperative CA-195 level that was normal. Of 4 recurrences, 3 were accompanied ($n=1$) or preceded ($n=2$) by a rise in serum CA-195. All of the 3 patients have had increased levels preoperatively. The lead time between serum CA-195 elevation and clinically manifest recurrence was 0,3 and 6 months, respectively. It seems noteworthy that only 2 of the 4 patients with recurrent colorectal cancer demonstrated a rise in the serum CEA.

Normal controls and patients with benign gastrointestinal disorders

Among 20 healthy volunteers, none had elevated levels of both CA-195 and CA19-9. Marginal elevations of CEA were noted in 3 (smoking) individuals. In the group of 103 patients admitted to the Department of Gastroenterology II because of non-malignant GI disorders, 30 patients (29.1%) had a value of CA-195 higher than 10 U/ml ($^{19}/_{48}$ liver, $^{3}/_{15}$ pancreas, $^{8}/_{33}$ colorectum, and $^{0}/_{7}$ upper gastrointestinal tract). The mean value in this series of 30 patients was 23.6 U/ml. The maximum value of 132 U/ml occurred in a female patient who was in hospital for decompensated liver cirrhosis with ascites. The incidence of false positives from benign gastrointestinal disorders was 58% for CA19-9 and 31% for CEA.

Discussion

An ideal tumour marker should not only signal the presence of tumour, but also should define the site of tumour and the morphological type of neoplasm. It should be sensitive in order to allow the diagnosis of small tumours and should be useful to monitor the evolution of cancer under the effect of treatment and to identify any recurrence early. Unfortunately, most markers available now have not obtained a high enough degree of sensitivity or they simply lack specifity in order to be useful for these functions.

Bray and coworkers (1987) have recently developed a monocolonal antibody designated as CC3C195 by immunization of Balb/c mice with a partially purified membrane preparation from the liver metastases of a human colon tumour. This antibody reacts with an epitope that consists of both Lewis A and sialylated Lewis A blood-group antigen (Fukuta et al. 1987; Koda et al. 1987). Serum CA-195 levels have been determined previously in healthy controls and in patients with carcinomas of various organ systems; elevated levels have been shown to be relatively specific for pancreatic and colorectal cancer (Bhargava et al. 1989; Gupta et al. 1987; Sundaram et al. 1987). The purpose of the present study was to define further the principal role of CA-195 in gastrointestinal cancer, to compare it with the established markers CEA

and CA19-9 and, especially, to assess its potential usefulness as a monitor of tumour burden in patients with resected or advanced disease while receiving cytotoxic chemotherapy.

The levels among healthy volunteers, patients with benign disease and different digestive-tract malignancies correlate well with the results from other laboratories (Bhargava et al. 1989; Gupta et al. 1987; Sundaram et al. 1987) and suggest an excellent sensitivity of CA-195 for pancreatic as well as colorectal cancer. With a detection rate of 100% and 71%, in fact, this new marker seems superior to both CA19-9 and CEA in these tumour types. It should be noted, however, that most of our patients presented with advanced stages of cancer, and that despite significantly higher levels of CA-195 in patients with gastrointestinal cancer than in those with non-malignant disease, the degree of overlap seems such as to preclude a definitive value for early diagnosis in most individuals. The limited potential of CEA for screening and diagnostic purposes, which has demonstrated a comparable rate of false positives in the present series, has been well recognized (Zamcheck et al. 1980). Similar considerations apply to CA19-9 (Holyoke et al. 1985; Moore et al. 1989), which was actually increased in more than half of our patients with non-malignant gastrointestinal disease.

For patients with metastatic colorectal cancer the CA-195 serum level appears to be useful to complement standard clinical measurements of tumour response and to provide an index of the effectiveness of chemotherapy. As with CEA and other marker proteins, however, instances of discordance between the objectively measured change in tumour size and corresponding serum levels have occurred (Aabo et al. 1986; Shani et al. 1978). The CA-195 tumour marker assay can therefore also not be accepted as an alternative to standard criteria of response assessment.

Following a complete surgical resection, normalization of elevated preoperative CA-195 concentrations within several weeks was generally observed. Regular and sequential assays of serum CA-195 in these patients revealed increased levels before tumour recurrence could be detected by conventional clinical or other diagnostic techniques in two of four instances. In one additional patient verification of local tumour recurrence was accompanied by a rise in serum CA-195. Although interpretation of our data is hampered by the relatively small number of patients and short duration of follow-up, the assay may represent a new non-invasive test for the postoperative surveillance of patients to detect localized or disseminated recurrence of colorectal cancer. Whether CA-195 is equally effective or even offers an advantage over CEA, or whether the combined use of the two marker proteins that do not correlate will prove superior, is currently being investigated by accrual and follow-up of additional patients.

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