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CA72-4 Compared with Carcinoembryonic Antigen as a Tumour Marker for Gastric Cancer

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Serum levels of a newly identified, tumour-associated antigen, CA72-4, were measured in 86 patients with histologically proven gastric carcinoma. Preoperative levels of CA72-4 in serum tended to be higher with increased dissemination of the cancer. Elevated levels of CA72-4 (above 5.0 U/ml) were significantly more frequent than those of carcinoembryonic antigen (CEA) (above 5.6 ng/ml) in patients with stage III or IV ($P < 0.01$) carcinoma, in patients with Borrmann type 4 ($P < 0.01$), and in patients with peritoneal metastasis ($P < 0.01$). No correlation was seen between serum levels of CA72-4 and those of CEA. Serum levels of CA72-4 were lower 1 month after gastrectomy in 25 of 39 patients with resected cancers. In each of 4 patients with recurrence, lower levels of CA72-4 after gastrectomy were replaced by elevated levels on detection of the recurrence of cancer. These results indicate that CA72-4 is highly specific to gastric cancer and may be more reliable as a tumour marker than CEA for gastric cancer.
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INTRODUCTION

WITH THE development of hybridoma technology, many monoclonal antibodies (Mabs) have been prepared against human tumour-associated antigens, and some of them have been exploited clinically as serum tumour markers. The Mab B72.3, which was prepared by immunising mice with a membrane-enriched fraction from human metastatic breast carcinoma [1], was recently shown to bind a high molecular weight tumour-associated glycoprotein (TAG-72) [2]. TAG-72 can be detected immunohistochemically in epithelial malignant tumours, such as cancers of the breast, colon, ovary, endometrium, stomach and lung [3–6]. The antigen is rarely detected in the corresponding normal cells [3, 7], so it is considered to be an antigen that is highly specific for malignant epithelial tumours. The Mab CC-49 was prepared by immunising mice with the TAG-72 antigen purified from LS-174T cells, a colon cancer cell line [2]. CA72-4 is an antigen that is recognised by both the B72.3 and CC-49 Mabs, and it is considered to be an antigenic determinant of the TAG-72 antigen. Elevation of serum levels of CA72-4 has been reported frequently in cases of cancer of the breast, colon and ovary [8, 9], but few reports on serum levels of CA72-4 in patients with gastric cancer have been published. In the present study, we evaluated CA72-4 as a serum tumour marker for gastric cancer, and we compared its utility in this regard with that of carcinoembryonic antigen (CEA).

PATIENTS AND METHODS

86 patients with primary gastric carcinoma, which had been proven histologically, were assayed for serum levels of CA72-4 and CEA. The group was composed of 77 newly diagnosed patients and 9 patients with recurrent cancer. Laparotomy was performed in all the 77 newly diagnosed patients; 72 had resectable tumours, and the remaining 5 patients had non-resectable tumours. Macroscopic classification, histological progression, histological staging, and histopathological grading of each gastric cancer were determined in accordance with the rules set forth by the Japanese Research Society for Gastric Cancer [10] and the Japanese Research Society Committee on Histological Classification of Gastric Cancer [11]. Serum levels of these markers were also measured in 12 patients with benign gastrointestinal diseases (gastric ulcer, 5 patients; cholelithiasis, 7 patients).

Samples of blood were collected preoperatively from patients with benign diseases, newly diagnosed cancer and immediately after the detection of relapse from patients with recurrent cancer. In 39 cases of resected cancer, samples of blood were also collected 1, 2, 3 or 12 months after surgery. The serum was stored at −40°C until the levels of CA72-4 and CEA were measured. A two-site radioimmunometric assay kit, using the two Mabs B72.3 and CC-49 (CA72-4 RIA kit; Centocor Inc., Malvern, Pennsylvania, USA), was used for determinations of levels of CA72-4. CEA was measured by enzyme-immunoassay using a Glaozyme CEA Kit (Sanyo Chemical Ltd, Kyoto, Japan). The upper cut-off value for CA72-4 was taken as 5.0 U/ml, according to Kimura *et al.* [12]. The upper cut-off value for CEA was taken as 5.6 ng/ml, corresponding approximately to the mean value plus the double standard deviation of 264 normal healthy adults in our hospital.

The statistical significance of the differences between the various groups was evaluated by t and χ^2 tests.

RESULTS

Pretreatment levels of CA72-4 in serum are shown in Fig. 1. The mean (S.D.) pretreatment serum levels of CA72-4 in patients with benign diseases were 3.5(0.7) U/ml. In patients with resectable gastric cancer, the preoperative levels of CA72-4 in serum were 3.6(0.8) U/ml for stage I, 3.0 U/ml for stage II, 9.7(21.3) U/ml for stage III, and 56.0(126.2) U/ml for stage IV cancer. In cases of non-resectable cancer, preoperative levels were 35.6(54.0) U/ml. In cases of recurrent cancer, levels of CA72-4 immediately after the detection of recurrence were 7.2(3.8) U/ml. The serum levels of CA72-4 tended to be higher with higher tumour load, but there were no significant differences between the groups.

In benign diseases, CA72-4 was elevated above the normal

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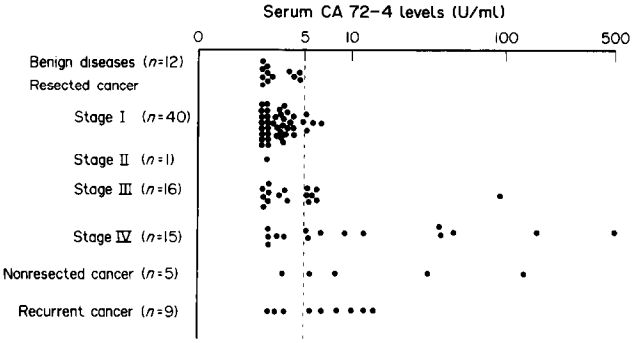

Fig. 1. Pretreatment serum levels of CA72-4 in 86 patients with newly diagnosed or recurrents carcinoma of the stomach and in 12 patients with benign gastrointestinal diseases. The dotted line represents the chosen upper limit normal value of CA72-4 in serum.

range in none of the patients while an elevated CEA value was found in 1/12 patients. Specificity was higher for CA72-4 (100%) than for CEA (92%). In gastric cancer, 31 patients (36%) had an elevated CA72-4 level while 14 patients (16%) had an elevated CEA level. Sensitivity was significantly higher for CA72-4 than for CEA in all patients of gastric cancer ($P < 0.01$). Sensitivity was slightly elevated to 40% by the combined assay of CA72-4 and CEA. The rates of detection of elevated levels of CA72-4 in cases of Borrmann type 2, type 3 and type 4 were higher than those of CEA. In particular, rates for type 4 were significantly higher than those of CEA ($P < 0.01$), as shown in Table 1. Rates of elevated levels of CA72-4 and CEA in serum, above the chosen upper limits and classified by cancer stage, are shown in Table 2. In stage III or IV cancer, significant differences were observed between frequencies of elevated levels of CA72-4 and CEA ($P < 0.01$). The frequencies of elevated levels of these markers according to histologic extent of disease in cases of resected gastric cancer are shown in Table 3. Frequencies of elevated levels of CA72-4 were significantly higher in patients with serosal invasion by the cancer, lymph node metastasis, or invasion of cancer into lymph vessels or into the vein within the stomach wall than in patients without these factors ($P < 0.001$, $P < 0.02$, $P < 0.001$, $P < 0.001$, respectively). Furthermore, the frequency of elevated levels of CA72-4 in patients with these factors was significantly higher than that in the case of CEA ($P < 0.01$, $P < 0.05$, $P < 0.04$, $P < 0.01$, respectively). The

Table 1. Frequencies of elevated levels (above chosen cut-off values) of CA72-4 and CEA in serum as they relate to macroscopic classification in 72 patients with resected gastric cancer

Macroscopic classification (type)	No. of patients	No. of patients with the elevated levels	
		CA72-4	CEA
0	37	4(11%)	1 (3%)
1	0	—	—
2	4	3(75%)	2(50%)
3	19	8(42%)	3(16%)
4	9	6(67%)	1(11%)*
5	3	0 (0%)	1(33%)

Type 0, superficial cancer; Types 1, 2, 3 and 4 correspond to Borrmann's classification; Type 5, the others. *$P < 0.01$.

Table 2. Frequency of elevated levels of CA72-4 and CEA in serum from 86 patients with newly diagnosed or recurrent carcinoma of the stomach

	No. of patients	No. of patients with the elevated levels		
		CA72-4	CEA	CA72-4 and/or CEA
Resected cancer				
stage I or II	41	4(10%)	2 (5%)	5(12%)
stage III or IV	31	17(55%)	6(19%)*	18(58%)
Non-resected cancer	5	4(80%)	2(40%)	4(80%)
Recurrent cancer	9	6(67%)	4(44%)	7(78%)
Total	86	31(36%)	14(16%)*	34(40%)

*$P < 0.01$.

Table 3. Frequency of elevated levels of CA72-4 and CEA in serum as they relate to histologic extent of cancer in 72 patients with resected gastric cancer

Histologic extent of gastric cancer		No. of patients	No. of patients with the elevated levels	
			CA72-4 (%)	CEA (%)
Serosal invasion	s(−)	42	4(10)	2 (5)
	s(+)	30	17(57)	6(20)*
Lymph node involvement	n(−)	44	8(18)	2 (5)
	n(+)	28	13(46)	6(21)‡
Invasion into lymph	ly(−)	41	5(12)	2 (5)
vessels of the stomach wall	ly(+)	31	16(52)	8(26)†
Invasion into the veins of	v(−)	39	4(10)	1 (3)
the stomach wall	v(+)	33	17(52)	7(21)*

*$P < 0.01$; †$P < 0.04$; ‡$P < 0.05$.

frequency of elevated levels of CA72-4 in serum from patients with resected gastric cancer exhibited no significant variation with the histopathological grading of differentiation (Table 4). However, in patients with poorly differentiated adenocarcinoma, the frequency of elevated levels of CA72-4 in serum was significantly higher than that of CEA ($P < 0.01$).

Table 4. Frequency of elevated levels of CA72-4 and CEA in serum as they relate to histopathological grade of differentiation in 72 patients with resected gastric cancer

Histopathological grading	No. of patients	No. of patients with the elevated levels	
		CA72-4 (%)	CEA (%)
Well	21	5(24)	3(14)
Moderately	15	3(20)	12 (3)
Poorly	36	13(36)	3(8)*

Well, well-differentiated adenocarcinoma; moderately, moderately differentiated adenocarcinoma; poorly, poorly differentiated adenocarcinoma. *$P < 0.01$.

Table 5. Frequency of elevated levels of CA72-4 and CEA in serum as they relate to patterns of metastasis in 29 patients with stage IV, non-resected or recurrent gastric cancer

Patterns of metastasis	No. of patients	No. of patients with the elevated levels CA72-4 (%)	CEA (%)
Liver metastasis (H) with or without P, N or L	7	5(71)	4(57)
Disseminated peritoneal metastasis (P) without H and with or without N, L	13	9(69)	3(23)*
Lymph node metastsis (N) or local recurrence (L) without H or P	9	6(67)	3(33)

*$P < 0.01$.

Metastatic lesions in 29 patients with stage IV, non-resected or recurrent gastric cancer were divided into three groups, classified by the main pattern of metastasis, as shown in Table 5. Frequencies of elevated levels of CA72-4 in serum did not differ between these groups. In cases of disseminated peritoneal metastasis, the frequency of elevated levels of CA72-4 was significantly higher than that of CEA ($P < 0.01$). Pretreatment levels of CA72-4 in serum were hardly correlated with those of CEA ($r = -0.024$, $n = 86$, $0.5 < P < 1.0$) in serum from 86 patients with gastric cancer.

Levels of CA72-4 in serum, 1 month after gastrectomy, decreased in 25 patients but increased slightly in 2 of 39 patients with resected cancer. In all 4 patients who had recurrence of cancer, serum levels of CA72-4, which had shown a decrease 1 month after gastrectomy, were found to have increased again at the time of detection of the recurrence, as shown in Fig. 2.

DISCUSSION

In immunohistochemical studies, the Mabs B72.3 and CC-49 have both been reported to bind efficiently to gastric cancers and to some lesser extent to benign lesions such as adenomas accompanied by intestinal metaplasia, hyperplastic polyps or atypical epithelium, but these Mabs have been reported not to bind to normal gastric mucosa at all [6, 7, 13]. It is possible that CA72-4 accumulates as a result of insufficient synthesis of carbohydrate chains concomitant with malignant transformation.

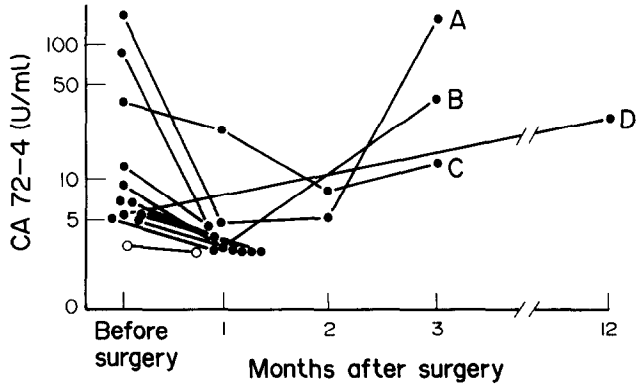

Fig. 2. Postoperative follow-up study with measurements of serum levels of CA72-4 from patients with resectable gastric cancer. Open circles represent the mean levels of CA72-4 in 28 patients who had normal levels of CA72-4 in serum preoperatively. (A) Metastasis to the lung; (B) metastasis to the liver; (C) disseminated peritoneal metastasis; (D) disseminated pleural metastasis.

It has been reported that elevated serum levels of CA72-4 antigen are associated with very low false-positive rates in benign diseases [8, 9, 14], and that the antigen is highly specific to cancer. The frequency of elevated levels of CA72-4 in serum in cases of gastric cancer was reported by Ohuchi *et al.* [15] as 52% ($n = 82$) who used 4.0 U/ml as the cut-off level. Byrne *et al.* [16] found a rate of 94% ($n = 33$) using 4.4 U/ml as the cut-off level, and Konishi *et al.* [13] found a rate of 53% ($n = 30$) using 5.6 U/ml as the cut-off level. In our study, the frequency of elevated levels of CA72-4 was 36%, with a cut-off level of 5.0 U/ml [12], which was a lower frequency than in other reports. The low frequency in our series is probably due to the fact that 47% of the patients had early gastric cancer (stage I). A comparison of levels of CA72-4 with the pathological findings from specimens of resected gastric cancers showed that the frequency of elevated levels of CA72-4 was significantly higher when tumour invasion of the serosa, lymph node metastasis, or invasion into the vein or the lymph vessels within the gastric wall was present. Konishi *et al.* [13] reported similar findings. However, in cases of gastric cancer, the localisation of CA72-4 in cancer cells has been confirmed histochemically not only in advanced cancers but also in 80 or 100% of early cancers [6, 7, 13]. Therefore, it appears that the CA72-4 antigen in cancer cells is not released into the blood during the early stage of the cancer but may enter the circulation as the lymph vessels or veins are invaded with the increasing spread of the cancer. Therefore, measurement of the serum level of CA72-4 seems to be useful for assessing the extent of gastric cancer before surgery, but it is inadequate as a screening test.

CEA has achieved widespread acceptance clinically as a useful tumour marker in gastric cancer with a relatively high specificity [17, 18]. However, in cases of Borrmann type 4 gastric cancer, in cases of adenocarcinomas that are poorly differentiated histologically, and in cases of disseminated peritoneal metastasis, levels of CEA are rarely elevated beyond the upper cut-off limit [17–20]. The frequency of elevated levels of CA72-4 was significantly higher than those of CEA in cases of Borrmann type 4, in cases of poorly differentiated adenocarcinomas, and in cases of peritoneal metastasis of the gastric cancer. These findings indicate that CA72-4 may be a more reliable tumour marker than CEA for gastric cancer. The antigenic determinants of CA72-4 and CEA are considered to be clearly different, in terms of their immunohistochemical reactions [6, 7]. In the present study, hardly any correlation was seen between the serum levels of CA72-4 and CEA.

The most important role of serum tumour markers is to reflect the tumour burden that remains in the body, allowing a prediction to be made of the probability of recurrence of cancer. The serum levels of CA72-4 decreased rapidly after excision of the primary lesion, and they increased again at the time of the recurrence of cancer. Therefore, it appears that the serum levels of CA72-4 accurately reflect the tumour burden.

In conclusion, our data indicate that the serum levels of CA72-4 may become a useful index for the staging of gastric cancer, for judgement of the tumour's response to therapy, and for the prediction of the recurrence of gastric cancer.

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Feasibility of Measuring Oxygen Tension in Uterine Cervix Carcinoma

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Cellular hypoxia is a cause of radioresistance. The oxygen tension (pO_2) in normal tissues and in tumours can be measured by polarography. In this feasibility study we have measured the tissue pO_2 of 10 patients suffering from uterine cervix carcinoma, using the Eppendorf histograph. The measurements were performed at the time of the brachytherapy after external radiotherapy. The machine was found to be reliable and no adverse effect was noted. The mean pO_2 values in tumours were lower than those of normal tissues.
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Introduction

EXPERIMENTAL SOLID tumours often have hypoxic regions, and laboratory studies indicate that cellular hypoxia is one of the parameters which may influence the response to chemo- or radiotherapy [1,2]. Aerobic cells are about three times more sensitive to low LET radiations than are hypoxic cells and the regrowth of these hypoxic cells after fractionated radiotherapy could explain some local treatment failures [3, 4]. Considerable effort has been devoted to developing techniques able to detect such cells, and to overcoming their radioresistance [1]; non-invasive methods with radioactively-labelled radiosensitisers or magnetic resonance spectroscopy have been used to detect hypoxia [1]. Until the 1980s there had been few direct measurements of oxygen tension (pO_2) in human tumours by polarography [5–8]. Gatenby founded a correlation between tumour pO_2 levels and the response to radiotherapy in 1988 [7]. However, none of these polarographic techniques was easily reproducible.

A computerised polarographic histograph (Kimoc 6650-

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