CA 72-4 Measurement of Tumor-associated Glycoprotein 72 (TAG-72) as a Serum Marker in the Management of Gastric Carcinoma

Fiorella Guadagni, Mario Roselli, Teresa Amato, Maurizio Cosimelli, Pasquale Perri, Vincenzo Casale, Massimo Carlini, Eugenio Santoro, Renato Cavaliere, John W. Greiner, and Jeffrey Schlom

I Department of Surgery [M. Co., P. P., R. C.], Department of Digestive Endoscopy [V. C.], and II Department of Surgery [M. Ca., E. S.], Regina Elena Cancer Institute [F. G., T. A.], and Department of Surgery, II University of Rome, School of Medicine [M. R.], Rome, Italy, and Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892 [J. W. G., J. S.]

ABSTRACT

The presence of three distinct serum markers of carcinoma, tumor-associated glycoprotein 72 (TAG-72; as measured by the CA 72-4 assay), CA 19-9, and carcinoembryonic antigen (CEA), was evaluated in 194 patients diagnosed with either malignant (n = 94) or benign (n = 100) gastric disease. Of the 94 patients diagnosed with gastric carcinoma, the percentage of patients whose serum samples were positive for TAG-72, CA 19-9, or CEA was 42.6, 31.9, and 20.2%, respectively. Furthermore, fewer false positive samples were observed for TAG-72 than either CA 19-9 or CEA. The analysis of serum TAG-72, CA 19-9, and CEA levels in patients diagnosed with early (stage I and II) versus advanced (stage III and IV) disease revealed a significantly higher level of TAG-72 and CA 19-9 in the serum of patients with advanced stage gastric carcinoma. The serum samples were also analyzed to determine whether any advantage might be gained by simultaneously measuring two or more of the tumor markers. The data clearly indicate that the measurement of TAG-72 with CA 19-9 significantly increased the percentage of gastric carcinoma patients with positive serum levels of either antigen. This advantage was achieved with no significant increase in the number of false positives. Twenty-one patients were followed postsurgically for up to 3 years to determine whether the appearance or reappearance of TAG-72, CA 19-9, or CEA accurately predicted disease recurrence. Positive serum TAG-72 levels correlated with disease recurrence in 7 of 10 patients, compared with 5 and 2 patients for CA 19-9 and CEA, respectively. The findings suggest that serum TAG-72 as measured by the CA 72-4 assay may be a useful marker for late stage gastric carcinoma and its measurement alone or in combination with CA 19-9 may have utility in the clinical management of gastric carcinoma.

INTRODUCTION

Although the incidence and death rate from gastric cancer in the United States have declined in past decades, it remains a common cause of cancer-related death (1, 2). The highest incidence rates of gastric carcinoma are found in certain Mediterranean countries, in Eastern Europe, and in the Pacific Rim, with Japan having the highest incidence worldwide (3–5). In Italy, the incidence of gastric carcinoma is 25.1/100,000 for males and 16.1/100,000 for females, representing the second most common cause of cancer-related deaths (4,500/year, in the past 5 years) (3).

The early stage of gastric carcinoma is often complicated and extremely difficult to diagnose due to presentation with vague, nonspecific symptoms which are sometimes associated with nonmalignant diseases (6, 7). The development of additional methods for this diagnosis includes the desire for efficient, noninvasive diagnostic procedures such as the identification of serum markers specifically associated with a high percentage of patients diagnosed with gastric carcinoma. Among the serum markers currently available for the diagnosis of gastric carcinoma are CEA (8) and CA 19-9 (9,10). CEA, a M,180,000 glycoprotein, and CA 19-9, a sialylated Lewis antigen, are distinct tumor markers expressed by human gastric carcinomas (9, 14). An analysis of the data shows that of all patients diagnosed with gastric carcinoma, 20.6% had positive serum CEA levels. Of those patients with advanced stage (stage IV) gastric carcinoma, measurable serum CEA was found in 37% (13). CA 19-9 has also been evaluated as a possible serum marker for gastric cancer. Elevated serum levels of this tumor antigen were found in 26% (15) to 72% (11) of the patients with gastric carcinoma, while 7% of the patients diagnosed with benign gastric disease had positive serum CA 19-9 levels (11). The studies revealed some potential utility as well as limitations for monitoring CEA and/or CA 19-9 serum levels in patients diagnosed with gastric carcinoma. The data also suggest the need to evaluate other serum tumor markers for their potential role in the management of gastric cancer.

For several years, the potential utility of a novel serum antigen, TAG-72, has been investigated (16, 17). TAG-72 was originally identified and characterized by MAb B72.3 (18–22) and the B72.3 epitope has, subsequently, been identified as a sialosyl-2–»6 α-N-acetylgalactosaminyl epitope (23). A series of "second generation" MAbs were produced by immunization with purified TAG-72 (24). One such MAb is CC49 which has a higher affinity for TAG-72 than B72.3 and also recognizes an epitope on TAG-72 which is distinctive from that recognized by B72.3. CC49 was used in combination with B72.3 to develop a double-determinant immunoradiometric assay, designated CA 72-4 (25, 26), which detects TAG-72 in sera or body fluids of carcinoma patients. Using the CA 72-4 assay, several groups have studied the presence of TAG-72 in the serum of patients diagnosed with gastrointestinal malignancies (27–30). The studies revealed that a significant percentage of patients diagnosed with gastrointestinal carcinoma whose serum CEA levels were negative had elevated TAG-72 (>6 units/ml), indicating a complementarity between the two tumor antigens. Moreover, the measurement of serum TAG-72 in patients during postsurgical follow-up was predictive of the appearance of recurrent disease (30).

The present study evaluates the preoperative serum levels of TAG-72, CEA, and CA 19-9, alone or in combination, in patients diagnosed with primary gastric carcinoma or benign gastric disease. The findings suggest the potential utility of using the CA 72-4 assay to detect serum TAG-72, either alone or in combination with CA 19-9, for the diagnosis of gastric carcinoma. In addition, a longitudinal follow-up of gastric carcinoma patients also revealed the potential utility of the CA 72-4 assay alone, or in combination with CA 19-9, as part of

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2 To whom requests for reprints should be addressed, at Laboratory of Tumor Immunology and Biology, Building 10, Room 8807, National Cancer Institute, NIH, Bethesda, MD 20892.

3 The abbreviations used are: CEA, carcinoembryonic antigen; TAG-72, tumor-associated glycoprotein 72; MAb, monoclonal antibody.
the clinical management of postsurgical gastric carcinoma patients.

MATERIALS AND METHODS

Patient Information. One hundred ninety-four patients, 94 with histologically diagnosed primary gastric adenocarcinoma [50 males, 44 females; mean age, 58 ± 1.3 (SE) years, ranging from 30 to 80 years old], and 100 patients with histologically confirmed benign [gastritis, ulcer, adenomas, polyps] gastric disease [54 males, 46 females; mean age, 53 ± 1.9 years] were evaluated. All patients with malignant disease underwent surgery for their primary tumor at the Department of Surgery, while patients diagnosed with benign gastric disease underwent endoscopic examination at the Department of Digestive Endoscopy of the Regina Elena National Cancer Institute, Rome, Italy. Malignant gastric disease was pathologically staged according to the tumor-nodes-metastasis classification (Union International Contre le Cancer tumor-nodes-metastasis classification of malignant tumors, 1983): Stage I (n = 14); Stage II (n = 16); Stage III (n = 36); and Stage IV (n = 28).

Serum samples from patients with benign disease were drawn at the time of endoscopy. All samples were aliquoted, coded, and stored at −20°C until assays were performed.

CA 72-4, CEA, and CA 19-9 Radioimmunoassays. Serum TAG-72 antigen levels were determined by a double-determinant immunoradiometric assay kit, CA 72-4, supplied by Centocor (Malvern, PA), as described previously (25). Samples and TAG-72 standards were assayed in duplicate. Briefly, 100 μl of specimen in the presence of 100 μl of phosphate buffer were incubated at 37°C for 4 h with beads coated with MAb CC49. The beads were washed 3 times with distilled water and incubated with 125I-B72.3 for 18 to 20 h at 4°C. After 3 washes with distilled water, bound radioactivity was measured in a gamma counter.

TAG-72 levels, expressed as units/ml, were determined by converting cpm to concentration values using a concurrently obtained standard curve. The cutoff limit for this assay was set at 6 units/ml as suggested (25). CEA serum levels were determined using a CEA RIA MAb kit (Abbott Laboratories, Inc., Chicago, IL). Several different cutoff limits, ranging from 2.5 to 10.0 ng/ml, have been used for the analysis of CEA serum levels (31–33). In the present study, we used a cutoff limit of 5.0 ng/ml for better specificity. CA 19-9 serum levels were determined as previously described using the suggested cutoff limit of 37 units/ml (12). Measurement of serum TAG-72, CEA, and CA 19-9 was done without any prior knowledge of the clinical diagnosis. A significant increase of the serum marker levels was considered, either when negative serum levels became positive or when there was an increase of >50% over the mean of previous positive levels.

Statistical Analysis. A Student t test (STATVIEW softwear package) was used to evaluate statistical differences among the serum tumor markers.

RESULTS

Sera from 94 patients with primary gastric carcinoma and 100 patients with benign gastric disease were evaluated for the presence of TAG-72 using the CA 72-4 assay, for 19-9 using the CA 19-9 assay, and for CEA using the CEA-radioimmunoassay-monoclonal antibody assay. As shown in Table 1, 42.6% of the sera from patients diagnosed with primary gastric carcinoma had elevated TAG-72 levels (>6.0 units/ml). In contrast, only 1 of 100 of the sera from patients with benign gastric disease had positive TAG-72 levels. CA 19-9 serum levels were elevated (>37.0 units/ml) in 31.9 and 7% in patients with malignant and benign disease, respectively. Serum CEA was elevated (>5.0 ng/ml) in 20.2% of sera from patients with gastric carcinoma and 9% of sera from patients with benign disease.

The relationships between serum TAG-72, CA 19-9, and CEA levels and the clinical staging of the patients with gastric carcinoma were also evaluated (Table 2). In particular, positive serum TAG-72 levels were found in patients diagnosed with advanced stage gastric carcinoma. Of the 40 patients which had positive serum TAG-72 levels, 37 were diagnosed with either stage III or stage IV gastric carcinoma. Likewise, elevated serum CA 19-9 and CEA were also found predominantly in sera samples from patients with advanced stage gastric carcinoma (Table 2).

No statistical difference was observed for differences in serum CEA levels between those patients diagnosed with early and advanced gastric carcinoma. Likewise, elevated serum CA 19-9 levels were found in patients diagnosed with early and advanced gastric carcinoma. A statistically significant difference between serum CA 19-9 levels in patients diagnosed with early and advanced gastric carcinoma was also observed. As shown, the mean serum CA 19-9 level from patients with stage I or II gastric carcinoma was 9.2 ± 2.5 compared with 23.2 ± 4.0 from patients diagnosed with stage I or II disease (P < 0.05) (Fig. 1B). No statistical difference was observed for differences in serum CEA levels between those patients diagnosed with early and advanced disease.

Studies were then conducted to determine if there was any advantage in the use of combinations of the CA 72-4, CA 19-9, and CEA assays. Fig. 2 illustrates the presence of TAG-72 and/or CA 19-9 in the sera of the 94 patients diagnosed with...
gastric carcinoma. As shown in Table 1, serum samples from 30 of the 94 patients have positive CA 19-9 levels. Of those 30, 17 serum samples also had positive TAG-72 levels. Of the remaining 64 samples which were negative for CA 19-9, 23 (35.9%) had positive titers of TAG-72. Therefore, combining the measurement of TAG-72 with CA 19-9, 53 of the 94 (56.4%) serum samples were positive for either tumor marker. Similar analyses were done with TAG-72 versus CEA (Fig. 3) and CEA versus CA 19-9 (Fig. 4). As shown in Fig. 3, of 94 patients serum samples 8 (8.5%) were CEA positive, and 11 (11.7%) were positive for both CEA and TAG-72. Of the

Fig. 1. Comparison of the presence of TAG-72 (A), CA 19-9 (B) and CEA (C) in the serum of patients diagnosed with early (stage I and II) or advanced (stage III and IV) gastric carcinoma. ——, serum cutoff values for TAG-72 (6 units/ml) and CA 19-9 (37 units/ml); n, number of patients in each quadrant (i.e., 23 patients were TAG 72 positive and CA 19-9 negative).

Fig. 2. Serum TAG-72 and CA 19-9 levels in patients diagnosed with gastric carcinoma. ——, serum cutoff values for TAG-72 (6 units/ml) and CA 19-9 (37 units/ml); n, number of patients in each quadrant (i.e., 23 patients were TAG 72 positive and CA 19-9 negative).

Fig. 3. Serum TAG-72 and CEA levels in patients diagnosed with gastric carcinoma. See Fig. 2 for explanation of symbols.

Fig. 4. Serum CA 19-9 and CEA levels in patients diagnosed with gastric carcinoma. n, number of patients grouped according to the presence/absence of the respective serum marker as analyzed with the indicated cutoff values (——).
remaining 75 patients whose serum samples were CEA negative, 38.7% were TAG-72 positive. When combining the measurement of serum CEA with TAG-72, serum samples from 48 of the 94 (51.1%) patients were positive. The relationship between serum CEA and CA 19-9 in the serum samples from the 94 (51.1%) patients were positive. The relationship between serum CEA and CA 19-9 in the serum samples from these patients was also investigated (Fig. 4). Serum from 9 of the 94 patients was positive for CEA, and serum samples from 10 patients contained positive levels of both CEA and CA 19-9. Of the 75 patients whose serum CEA levels were negative, 26.7% had positive CA 19-9 serum levels. Combining the measurement of both tumor markers revealed that 41.5% of serum samples were positive for either CEA or CA 19-9.

Table 3 summarizes the percentage of patients diagnosed with gastric carcinoma (n = 94) or with benign gastric disease (n = 100) whose serum samples contained either TAG-72, CA 19-9, and/or CEA. Serum TAG-72 (42.6%) alone was found in a higher percentage of patients with gastric carcinoma than either CEA (20.2%) or CA 19-9 (31.9%) alone. Furthermore, combining serum TAG-72 measurement with either CA 19-9 or CEA increased the percentage of serum positive to 56.4 and 51.1%, respectively. It should be noted that a concomitant increase in the percentage of false-positive serum levels was not observed when TAG-72 measurement was combined with either CA 19-9 or CEA. Sixty % of gastric cancer sera were positive when using all three markers.

Table 4 Summary of longitudinal evaluation of TAG-72 serum levels in gastric patients

Serum TAG-72, CA 19-9, and CEA levels were followed longitudinally in 21 patients for up to 3 years postsurgery for primary gastric carcinoma or until the clinical diagnosis of recurrence of malignant disease (Table 4). Eleven of the 21 patients with no clinical evidence of disease had no detectable serum levels of any of the 3 tumor antigens. One patient (PF) without clinical evidence of disease had a positive serum CEA at 207 days postsurgery. Ten clinical recurrences were diagnosed in 9 patients (patient RA, 2 recurrences). Serum TAG-72 levels were elevated in 7 of the 10 clinical recurrences, whereas serum CA 19-9 and CEA levels were positive in 5 and 2 cases, respectively. In all cases, detectable serum levels of TAG-72 either occurred prior to or concomitant with the clinical diagnosis of recurrent disease. As an example, Fig. 5 illustrates the changes in serum TAG-72 (panel A), CA 19-9 (panel B), and CEA (panel C) in patient DSA who was diagnosed with stage II gastric carcinoma and was followed for approximately 2 years after surgical resection of the primary tumor. Prior to surgery, serum samples from patient DSA were positive for CEA and negative for TAG-72 and CA 19-9. During the postsurgical follow-up, positive TAG-72 serum was detected, whereas serum CA 19-9 and CEA remained negative. Elevation of serum TAG-72 was detected at 297 days prior to clinical recurrence.

DISCUSSION

The present study indicates that the measurement of serum TAG-72 levels may be an important component in the diagnosis and clinical follow-up of patients with malignant gastric disease. When compared with other serum tumor markers that are currently used for the diagnosis and monitoring of patients for gastric carcinoma, i.e., CA 19-9 and CEA, a higher percentage of patients with gastric cancer had positive TAG-72 serum levels. In addition, the specificity of serum TAG-72 for differentiating carcinoma versus benign gastric disease was better than for either CA 19-9 or CEA. The 94 patients diagnosed with gastric carcinoma were also staged according to tumor-nodes-metastasis classification and the presence of serum TAG-
Arrow, time of surgery; , serum cutoff values for each of the tumor antigens. Observations suggest that the presence in the serum of TAG-72 (A), and CEA (C) in patients diagnosed with stage II gastric adenocarcinoma.

Roles that those tumor markers play in the biology of human gastric carcinoma. Additional studies with higher numbers of samples are needed to further investigate whether the analysis of multiple serum tumor markers may be advantageous in the diagnosis of gastric carcinoma.

One of the important applications of any serum marker is the ability to use the measurement of the serum tumor antigen in predicting the clinical course of the malignant disease, particularly, the diagnosis of disease recurrence. In the present study, 21 patients diagnosed with primary gastric cancer were followed postsurgery for up to 3 years or to time of disease recurrence. As seen in Table 4, none of the 12 patients with no clinical evidence of disease had positive TAG-72 levels. Six of 9 patients [1 patient (RA) had 2 recurrences], however, with clinically confirmed disease recurrence had elevated serum TAG-72 levels. In 5 of the 6 patients with recurrent disease, positive serum TAG-72 levels were detected prior to the clinical diagnosis (Table 4) and, in some patients, positive serum TAG-72 levels preceded clinical evidence of disease by 100 to 300 days. Monitoring both serum CA 19-9 as well as CEA did not correlate as well as TAG-72 with the onset of recurrent disease. While additional studies with larger population groups and different population bases are needed, these findings indicate that the measurement of serum TAG-72 levels may be useful in the clinical diagnosis of primary and recurrent gastric cancer.

REFERENCES


Fig. 5. Longitudinal postsurgery evaluation of serum TAG-72 (A), CA 19-9 (B), and CEA (C) in patients diagnosed with stage II gastric adenocarcinoma. Arrow, time of surgery; , serum cutoff values for each of the tumor antigens.

72, CA 19-9, and CEA in the different stages were evaluated. Serum levels for both TAG-72 and CA 19-9 were highly correlated with the advanced stages of gastric carcinoma. These observations suggest that the presence in the serum of TAG-72 and CA 19-9 closely reflects the clinical staging of the disease and that additional studies are needed which may elucidate the roles that those tumor markers play in the biology of human gastric carcinoma.

It is generally agreed that the sensitivity as well as the specificity of a single serum tumor marker for the diagnosis of primary and recurrent carcinoma is limited. No one serum tumor marker will unfailingly predict the presence of malignant disease or differentiate between benign versus malignant disease. The present data clearly indicate that serum TAG-72, CA 19-9, and CEA appear in 42.6, 31.9, and 20.2%, respectively, of the serum of patients diagnosed with gastric carcinoma (Table 1). Therefore, a majority of patients (i.e., >50%) with malignant gastric disease do not contain measurable serum levels of any of these three tumor markers if analyzed separately. Recent findings have suggested a complementarity among various serum tumor markers which may be exploited in the diagnosis of gastrointestinal carcinoma (30). Ideally, one would envision that the complementarity might increase the sensitivity with respect to clinical diagnosis with little change in the specificity (i.e., little increase in false positives). For example, the present data indicate that combining the measurement of serum TAG-72 with CA 19-9 increased the percentage of patients diagnosed with gastric carcinoma who also had measurable serum titers of either tumor antigen. At the same time, there was no change in the number of false positive serum samples (i.e., patients with benign gastric disease), suggesting that the simultaneous measurement of TAG-72 and CA 19-9 may selectively identify a higher percentage of patients with gastric cancer. Additional studies with higher numbers of samples are needed to further investigate whether the analysis of multiple serum tumor markers may be advantageous in the diagnosis of gastric carcinoma.
CA 72-4 MEASUREMENT OF TAG-72 AS SERUM MARKER

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