Original Paper

Detection of circulating CEA-IgM complexes in early stage gastric cancer

Taiki Kojima¹⁾, Takanori Matsui²⁾, Yasuhiro Kodera³⁾, Hiroshi Kojima²⁾,
Chubu Clinical Oncology Group (CCOG)

¹⁾ Kojima family clinic, ²⁾ Department of Gastroenterological Surgery, Aichi Cancer Center Aichi Hospital,

³⁾ Department of Surgery II, Nagoya University Graduate School of Medicine

Abstract

Background: The early detection of gastric cancer is important issue for improving the survival rate. As a serum marker, Carcinoembryonic antigen (CEA) and CA19-9 in serum is widely used. But their limited sensitivity is not suitable for screen method.

Methods: In this study, titer of CEA-IgM complexes was measured in serum from 86 patients with primary gastric cancer who underwent surgery. 48 of the tumors (56%) were Stage IA, 10 (12%) were Stage IB, 6 (7%) were Stage II, 11 (13%) were Stage III, 11 (13%) were Stage IV. Serum samples from 23 patients found to have no tumors with gastritis were analyzed as non-gastric cancer group. In each serum sample, CEA and CEA-IgM complexes were measured.

Results: By CEA-IgM tests, overall sensitivity was 20% (17/86), Stage IA 19% (9/48), Stage IB 30% (3/10), Stage II 0% (0/6), Stage III 17% (3/11) and Stage IV 2% (2/11), while false positive rate was 9% (2/23). By conventional CEA test, overall sensitivity was 5% (4/86), with Stage IA, IB 2% (1/58). Moreover, CEA-IgM complexes detected differentiated adenocarcinoma with overall sensitivity 38% (11/29), more than undifferentiated adenocarcinoma of gastric cancer.

Conclusion: CEA-IgM might open a new possibility of detecting gastric cancer by serum in early stages, especially of differentiated adenocarcinoma.

Key Words: gastric cancer, tumor marker, CEA, CEA-IgM complexes

(Received October 22, 2010; Accepted November 9, 2010)

Introduction

Gastric cancer is the most common malignancy of the gastrointestinal tract among the Japanese and Far Eastern populations and the second most common cancer worldwide¹⁾. The outcome of patients with advanced or metastatic cancer remains poor, even after curative resection²⁾.

In Japan, the mortality rate of gastric cancer is showing a decreasing trend, reflecting advances in medical technology and social mass-screening system, such as early detection with upper gastrointestinal series or endoscope. In fact, currently, more than half of gastric cancer goes into therapy in Stage I³⁾. On the other hand, in some countries, early detection of gastric cancer remained in the rate of about 30%⁴⁾.

Early detection of gastric cancer by serum marker has long been a dream, which has not been accomplished yet. Since measurement of tumor markers using serum, is not invasive nor costing, the use of these tumor markers is expected to be more widely accepted for the screening of early gastric cancers. New serum marker for diagnosis of

gastric cancer needs improving in the point of sensitivity, specificity.

One of the conventional tumor marker, CEA, is elevated in advanced stages of incurable cancer but low in early, curable stages. Another marker, CA19-9, is the same with sensitivity. Their limited sensitivity is not suitable for screen method⁵⁾. It serves, at most, in monitoring of response to systemic chemotherapy in patients with advanced gastric cancer in restricted cases⁶⁾.

In this report, we tested CEA-IgM complexes in gastric cancer, which showed overall sensitivity of 20% in gastric cancer, and 38% in gastric cancer of differentiated adenocarcinoma, without compromising the detection specificity.

Patients and Methods

Patients

86 patients with primary gastric cancer who underwent surgery at the Department of Gastroenterological Surgery, Aichi Cancer Center Aichi Hospital, Japan, between December 2005 and May 2007 were enrolled in this study. All operated patients were not enrolled. Written informed consent was obtained from each patient. No patients had received preoperative radiotherapy or chemotherapy. They were 47 (55%) male and 39 (45%)

Correspondence and reprint request to: Taiki Kojima, M.D. Kojima Family Clinic, Miyamae17-1, Hegoshi-cho, Okazaki-shi, Aichi-ken, 444-0902, Japan. TEL: +81-564-32-8852, FAX: +81-564-32-3752, e-mail: koji3744@hotmail.com

Table 1. General information for the analyzed patients

	Histological classification					
	Stage IA	Stage IB	Stage II	Stage III	Stage IV	gastritis
Sample number	48	10	6	11	11	23
Male/Female	total 47/39					9/14
Age (mean)	total 38-84 (66.0)					40-71(54.1)
Differentiated/ Undifferentiated	20/28	2/8	3/3	4/7	0/11	-
H.Pyrori status	NT	NT	NT	NT	NT	11 positive
Peritoneal seeding	0	0	0	0	11	-

female patients, with an average age of 66.0 years (range, 38 – 84 years). Tumors were staged according to the International Tumor-Node-Metastasis (TNM) staging system and histological grade was assessed according to the World Health Organization (WHO) criteria. 48 of the tumors (56%) were Stage IA, 10 (12%) were Stage IB, 6 (7%) were Stage II, 11 (12%) were Stage III, and 11 (12%) were Stage IV. 29 tumors (34%) were well differentiated, 57 (66%) were undifferentiated.

Serum samples from 23 patients underwent gastric endoscope examination and found to have no tumors with gastritis were analyzed as non-gastric cancer group (control). Written informed consent was also obtained from these patients. They were 9 (39%) male and 14 (61%) female patients, with an average age of 54.1 years (range, 40-71 years).

Patients' background is summarized in Table 1.

Serum samples

Serum samples were collected from each patient before surgery or gastric endoscope examination. Samples were stored at -80°C until they were assayed.

Enzyme immunoassay for serum CEA-IgM complexes

Serum CEA-IgM complexes levels were assessed by the colon-IC ELISA Kit (Xeptagen, Pozzuoli, Italy). In brief, in 96 well ELISA plates, coated with anti-human CEA antibody, 100 µl of serially diluted reference standard or serum samples in dilution buffer were incubated for one hour at room temperature. After washing, the CEA-IgM complexes was incubated with enzyme-conjugated anti-human IgM for one hour at room temperature, and developed and assessed by measuring absorption at 405 m, using ELISA plate reader. Levels of CEA-IgM complexes were determined from a calibration curve constructed from the reference standard. The cutoff value was 120 AU/ml based on ROC curve analysis.

CEA assays

Serum CEA concentrations were measured with

Table 2. Sensitivity of CEA and CEA-IgM complexes titer (Overall patients)

	Gastric cancer						control (n=23)
	Overall (n=86)	Stage IA (n=48)	Stage IB (n=10)	Stage II (n=6)	Stage III (n=11)	Stage IV (n=11)	
CEA positive	4(5%)	0(0%)	1(10%)	1(17%)	1(1%)	1(1%)	0(0%)
CEA-IgM Complexes Positive	17(20%)	9(13%)	3(30%)	0(0%)	3(27%)	2(18%)	2(9%)
CEA and CEA-IgM Positive	20(23%)	9(13%)	4(40%)	1(17%)	3(27%)	3(27%)	2(9%)

*cutoff value CEA: 5ng/ml

CEA-IgM complexes: 120AU/ml

Chemiluminescent Immuno assay, using ARCHITECT CEA (Abbott Japan, Tokyo, Japan). The cutoff value of CEA was 5 ng/ml according to the manufacturers.

Statistical analysis

The areas under the ROC curves were calculated using software R⁷⁾.

Results

86 serum from patients suffering from gastric cancers and 22 serum from non-gastric cancer (with gastritis) taken as control group were analyzed in parallel by CEA and CEA-IgM complexes assays.

The sensitivity of CEA and CEA-IgM complex is summarized in Table 2. Overall sensitivity is only 5% by conventional CEA assay, while CEA-IgM improved the sensitivity, especially in early stage of gastric cancer.

This is more apparent in cases of differentiated adenocarcinoma (Table 3), compared with in cases undifferentiated adenocarcinoma (Table 4). ROC curves compared with overall patients and patients of differentiated adenocarcinoma also showed this tendency (Fig. 1). The diagnostic accuracy, represented as the areas under the ROC curves, improves in gastric cancer of differentiated adenocarcinoma compared with overall gastric cancer (value, 0.532 vs. 0.418).

By CEA-IgM tests, overall sensitivity was 20% (17/86), Stage IA 19% (9/48), Stage IB 30% (3/10), Stage II 0% (0/6), Stage III 17% (3/11) and Stage IV 2% (2/11), while false positive rate was 9% (2/23). Moreover, CEA-IgM complexes detected differentiated adenocarcinoma with overall sensitivity 38% (11/29), more than undifferentiated adenocarcinoma of gastric cancer.

Discussion

Recently, new type of serum marker is reported to overcome the limitation of sensitivity, for detecting early stage gastric cancer.

Table 3. Sensitivity of CEA and CEA-IgM complexes titer (differentiated adenocarcinoma)

	Gastric cancer						control (n=23)
	Overall (n=29)	Stage IA (n=20)	Stage IB (n=2)	Stage II (n=3)	Stage III (n=4)	Stage IV (n=0)	
CEA positive	2(7%)	0(0%)	0(0%)	1(33%)	1(25%)	-	0(0%)
CEA-IgM Complexes Positive	11(38%)	6(30%)	2(100%)	0(0%)	3(75%)	-	2(9%)
CEA and CEA-IgM Positive	12(41%)	6(30%)	2(100%)	1(33%)	3(75%)	-	2(9%)

*cutoff value CEA: 5ng/ml
CEA-IgM complexes: 120AU/ml

One approach for detecting new biomarker is employed by SELDI-TOF-MS^{4, 8, 9)}. They reported good sensitivity and specificity, but its reproductivity and availability (requiring SELDI-TOF-MS equipment) seems to be remained as problem.

In this report, we have chosen CEA-IgM complexes, by conventional ELISA method, for the first time in gastric cancer, described by Fassina G, et al¹⁰⁾, first reported long ago¹¹⁾, showing some usefulness in detecting colon cancer, especially if it combines with CEA^{10, 12)}.

CEA-IgM is especially useful for detecting differentiated adenocarcinoma compared with undifferentiated adenocarcinoma (Table 2, 3, Fig. 1). In gastric cancer of differentiated adenocarcinoma, 38% was detected, which is almost equivalent with detection of colon cancer^{10, 12)}.

It is worth emphasizing that the 68% of tested serum here are derived from Stage I, where they can hardly be detected by conventional tumor marker such as CEA or CA19-9.

Recently, it was reported that p53 autoantibody detected 15% of gastric cancer^{13, 14)}, but it could detect only about 10% of stage I gastric cancer. In this study, CEA-IgM complex detected 20% of gastric cancer, 21% of stage I gastric cancer, and 36% of stage I gastric cancer of differentiated adenocarcinoma.

The sensitivity of CEA-IgM was inferior to pepsinogen (about 55%) but the specificity was retained compared with pepsinogen (about 75%)^{15, 16)}.

Theoretically, there remain various arguments about CEA-IgM. How about IgG? What is the rate of free antigen, free antibody and immune complexes? What is the physiological role of such an immune complexes? Here, we show several implications.

Some authors propose the existence of natural antibodies, instead of acquired antibodies, to cancer cells, which are restricted to the isotype IgM and sometimes induce apoptosis of cancer cells. According to them, the target of such IgM comight be modified carbohydrate epitopes post-transcriptionally in cancer cells¹⁷⁾.

We have observed that many serum from rheumatoid

Table 4. Sensitivity of CEA and CEA-IgM complexes titer (undifferentiated adenocarcinoma)

	Gastric cancer						control (n=23)
	Overall (n=57)	Stage IA (n=28)	Stage IB (n=8)	Stage II (n=3)	Stage III (n=7)	Stage IV (n=11)	
CEA positive	2(4%)	0(0%)	1(13%)	0(0%)	0(0%)	1(9%)	0(0%)
CEA-IgM Complexes Positive	6(11%)	3(11%)	1(13%)	0(0%)	0(0%)	2(19%)	2(9%)
CEA and CEA-IgM Positive	8(14%)	3(11%)	2(25%)	0(0%)	0(0%)	3(27%)	2(9%)

*cutoff value CEA: 5ng/ml
CEA-IgM complexes: 120AU/ml

Fig. 1 ROC curves comparing the distribution of the serum levels of CEA-IgM with overall gastric cancer (n=86) and with gastric cancer of differentiated adenocarcinoma (n=29) vs. the control group (n=23).

arthritis have unspecifically high titer of CEA-IgM (data not shown). On the other hand, it is reported that, in rheumatoid arthritis patients, colorectal and gastric cancer was detected less than the expected numbers of patients¹⁸⁾. High titer of CEA-IgM may have some relations on this.

Although these are examples of questions to be answered in future study¹⁹⁾, it might be said that CEA-IgM opened a new possibility of detecting gastric cancer in early stages by serum.

Acknowledgement

This work was supervised and supported by Chubu Clinical Oncology Group (CCOG) and also supported, in part, by a non-profit organization Epidemiological & Clinical Research Information Network (ECRIN).

References

- 1) Parkin, D.M, Pisani, P, Ferlay, J. Estimates of the worldwide inci-

- dence of 25 major cancers in 1990. *Int J Cancer* 1999; 80: 827-41.
- 2) Yamazaki, H., Oshima, A., Murakami, R., Endoh, S., Ubukata, T. A long-term follow-up study of patients with gastric cancer detected by mass screening. *Cancer* 1989; 63: 613-7.
 - 3) Japanese Gastric Cancer Association Registration Committee. Gastric cancer treated in 1991 in Japan: data analysis of nationwide registry. *Gastric Cancer* 2006; 9: 51-66.
 - 4) Yahui, S., Jing, S., Huachong, M., Jiafu, J., Hong, M., Longhua, M., Weihua, Z., Ling, M., Zhenfu, L., Jian, W., Genglin, J., Jianzhi, Z., et al. Diagnosis of gastric cancer using decision tree classification of mass spectra data. *Cancer Sci* 2007; 98: 37-43.
 - 5) Kodera, Y., Yamamura, Y., Torii, A., Uesaka, K., Hirai, T., Yasui, K., et al. The prognostic value of preoperative serum levels of CEA and CA19-9 in patients with gastric cancer. *Am J Gastroenterol* 1996; 91: 49-53.
 - 6) Yamao, T., Kai, S., Koizumi, K., Handa, T., Takemoto, N., Maruyama, M. Tumor marker CEA, CA19-9 and CA125 in monitoring of response to systemic chemotherapy in patients with advanced gastric cancer. *Jpn J Clin Oncol* 1999; 29: 550-5.
 - 7) Peter, Dalgaard. *Introductory statistics with R*. Springer-Verlag New York Inc. 2002.
 - 8) Ren, H., Du, N., Liu, G., Hu, H.T., Tian, W., Deng, Z.P., Shi, J.S. Analysis of variabilities of serum proteomic spectra in patients with gastric cancer before and after operation. *W J Gastroenterol* 2006; 7: 2789-2792.
 - 9) Kojima, T., Yoshikawa, K., Matsui, T., Kodera, Y., Kojima, H. Detection of elevated proteins in peritoneal dissemination of gastric cancer by analyzing mass spectra data of serum proteins. *J Surg Res* 2009; 155: 13-17.
 - 10) Castaldi, F., Marino, M., Belluco, C., De Marchi, F., Mammano, E., Nitti, D., Lise, M., Fassina, G. Detection of circulating CEA-IgM complexes in early stage colorectal cancer. *Int J Biol Markers* 2005; 20: 204-208.
 - 11) Christian, F., Friedel, K., Peter, K., Suse, H., Wolfram, J., Jochen, R.K., CEA-containing immune complexes in sera of patients with colorectal and breast cancer: analysis of complexed immunoglobulin classes. *Cancer Immunol Immunother* 1988; 26: 180-184.
 - 12) Kojima, T., Yoshikawa, K., Matsui, T., Kodera, Y., Kojima, H. Titration of serum CEA, p53 antibodies and CEA-IgM complexes in patients with colorectal cancer. *Mol Med Reports* 2009; 2: 477-480.
 - 13) Shimada, H., Ochiai, T., Nomura, F.; Japan p53 Antibody Research Group. Titration of serum p53 antibodies in 1085 patients with various types of malignant tumors: a multiinstitutional analysis by the Japan p53 Antibody Research Group. *Cancer* 2003; 97: 682-689.
 - 14) Shimizu, K., Ueda, Y., Yamagishi, H. Titration of serum p53 antibodies in patients with gastric cancer: a single-institute study of 40 patients. *Gastric Cancer* 2005; 8: 214-219.
 - 15) Kodai, A., Yoshihara, M., Sumii, K., Haruma, K., Kajiyama, G. Serum pepsinogen in screening for gastric cancer. *J Gastroenterol* 1995; 30: 452-460.
 - 16) Miki, K. Gastric cancer screening using the serum pepsinogen test method. *Gastric Cancer* 2006; 9: 245-253.
 - 17) Vollmers, H.P., Brandlein, S. Natural antibodies and cancer. *J Autoimmunity* 2007; 29: 295-302.
 - 18) Thomas, E., Brewster, D.H., Black, R.J., Macfarlane, G.J. Risk of malignancy among patients with rheumatic conditions. *Int J Cancer* 2000; 88: 497-502.
 - 19) Saif, M.W., Zalonis, Syrigos, K. The clinical significance of autoantibodies in gastrointestinal malignancies: an overview. *Expert Opin Ther* 2007; 7: 493-507.