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Use of serum and peritoneal CEA and CA19-9 in prediction of peritoneal dissemination and survival of gastric adenocarcinoma patients: are they prognostic factors?

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ABSTRACT

INTRODUCTION To evaluate the impact of serum and peritoneal levels of tumour markers on peritoneal carcinomatosis and survival in gastric adenocarcinoma.

MATERIALS AND METHODS Patients with gastric adenocarcinoma were evaluated with regard to serum and peritoneal carcinoembryonic antigen (CEA) and CA19-9. Numeric values and groupings based on serum and peritoneal cutoff values were used. Development of peritoneal carcinomatosis, including positive washing cytology, was regarded as main outcome. Gastric cancer outcomes as disease free and overall survival were analysed.

RESULTS There were 67 patients with a mean age of 60 ± 11 years. Positive peritoneal washing cytology was significantly associated with serum CA19-9 and high serum CA 19–9 group ($P = 0.033$ and $P = 0.011$, respectively). High peritoneal CEA was shown to be significantly associated with peritoneal carcinomatosis ($P = 0.032$). After a median follow up of 17 months, 48 patients (71.7%) were alive. Patients with peritoneal carcinomatosis showed significant poorer prognosis as shown by overall survival rate of 28.6%. Only serum CEA was significantly associated with lower disease free and overall survival ($P = 0.002$ and $P = 0.001$, respectively).

DISCUSSION AND CONCLUSION Serum CEA is shown to be significantly associated with poor prognosis for gastric cancer patients. Serum level of CA19-9 and high peritoneal CEA levels are significant predictors for positive peritoneal washing cytology and the development of peritoneal carcinomatosis, respectively. Therefore, the possible impact of serum and peritoneal tumor markers especially on the staging and prognosis of gastric cancer remains to be clarified by future studies.

KEYWORDS
Gastric adenocarcinoma – Carcinoembryonic antigen – CA19-9 – Survival

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Introduction

It has been known that peritoneal dissemination is the most frequent pattern of recurrence even after curative surgery for gastric adenocarcinoma.[1–5] Despite the many diagnostic modalities including computed tomography, magnetic resonance imaging and position emission tomography, none has demonstrated a high predictive value and it still remains difficult to reach a precise diagnosis of peritoneal dissemination.[1,2] Staging laparoscopy has also been employed to identify peritoneal seeding that cannot be found using any other radiological modality.[1] Although intraoperative peritoneal washing cytology has been regarded as the golden standard to detect peritoneal carcinomatosis, there were many reports with regard to its low sensitivity lying in the 14–21% range for gastric cancer with serosal invasion.[4–6] Therefore, early suspicion and diagnosis of peritoneal dissemination is thought to be an important measure to improve the staging and treatment of gastric cancer.

Carcinoembryonic antigen (CEA) and CA19-9 have been the most commonly used tumour markers for gastric cancer.[1,2,7,8] There have been many conflicting reports with regard to the association of the levels of serum and peritoneal tumour markers and peritoneal carcinomatosis in patients with gastric cancer before and after the curative surgery.[1]

It has been thought that the analysis of preoperative tumour markers in serum and peritoneal lavage fluid of the

patients with gastric cancer may result in an improved accuracy for the prediction of peritoneal metastasis.[1,2,9,10] In Yamamoto's studies,[2,11] the level of CEA in peritoneal fluid has been shown to be a reliable marker for early stage of peritoneal metastasis. In addition to the levels of CEA in peritoneal fluid, serum CEA has been shown to be associated with haematogenous and lymphatic metastasis. However, they have had low sensitivity and high false positive rates as independent risk factors.[7,12,13] Therefore, a reliable approach using combinations of markers for pre-, intra- or postoperative early detection of occult micro metastasis or recurrences in the peritoneum of the patients with gastric cancer is necessary to modify individualised treatment plans in these patients.[6,14–16]

This study aimed to evaluate the association between the levels of tumour markers in serum and peritoneal fluid of the gastric adenocarcinoma patients and clinicopathological features and to determine their impact to detect peritoneal carcinomatosis, recurrences and survival.

Materials and methods

Patients

From April 2015 to December 2016, all consecutive patients with gastric or gastro-oesophageal adenocarcinoma confirmed by histopathologically and surgically treated with a curative intent were included into this prospective observational study. Patients were not excluded if they had received preoperative neoadjuvant chemotherapy which was planned for resectable but locally advanced disease. Exclusion criteria were the lesions located 3 cm above the gastro-oesophageal junction, surgery with a palliative intent and metastatic disease and the patients who did not accept to be included in the study. The study was approved by the local Ethical Committee (71306642-050-01-04) and registered to Clinical Trials with an identifier number of NCT02801955.[17] The study complied with the principles of the Declaration of Helsinki and all patients gave the written informed consent. Demographics including age and gender, neoadjuvant treatment status and clinicopathological features were recorded using a prospective database.

During the preoperative preparation, serum CEA (sCEA) and serum CA19-9 (sCA19-9) were measured for all patients. Normal levels of sCEA and sCA19-9 were ≤ 5 ng/ml and ≤ 37 u/ml, respectively. In patients with neoadjuvant chemotherapy, the serum levels of tumour markers were studied before the induction of chemotherapy.

Neoadjuvant treatment

The decision of neoadjuvant chemotherapy and its protocol were determined by the multidisciplinary tumour board based on the tumour stage and patient ability to tolerate therapy. The 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX) regimen was administered to all patients as the neoadjuvant treatment. The median number of the cycles was three. Surgical treatment was performed after the treatment within one month.

Surgical procedures

Immediately after laparotomy, the surgical tumour stage was carefully examined. In the absence of overt peritoneal dissemination, 200 ml physiological saline was administered into the abdominal cavity. After gentle stirring, at least one-third was aspirated from several regions of the peritoneal cavity, including near the primary tumour, the left and right sub-phrenic areas and the pouch of Douglas with suction tubes to a clean bottle for both cytological examination and for the measurement of peritoneal tumour markers (pCEA and pCA19-9).

The fluid sample for tumour marker was immediately centrifuged for five minutes at 3000 rpm and the supernatant was concentrated by ultra-filtration; pCEA and pCA19-9 measurements were performed with chemiluminescent enzyme immunoassay kits. The actual levels of tumour markers in peritoneal fluid were studied and expressed as ng/ml for CEA and u/ml for CA19-9.

Standard radical subtotal or total gastrectomy with D2 lymph node were performed according to the Japanese Classification of Gastric Carcinoma (third English edition) to those patients with lesions located in the antropyloric region or in the medium and proximal third of the stomach.[18] Splenectomy or distal pancreatectomy was performed in selected cases in which gross involvement of the splenic hilus or local invasion of the pancreatic tail.

Pathology

All specimens were sent immediately to the pathology laboratory. Haematoxylin and eosin staining were used for evaluation of the paraffin blocks from each patient to examine tumour size, histological type, lymphatic and vascular invasion, grade and tumour, node, metastasis (TNM) staging according to the seventh American Joint Committee on Cancer/International Union Against Cancer system.[19] For grading the tumours, patients with undifferentiated, signet ring cell and mucinous tumours were regarded as undifferentiated histology.

Peritoneal washing lavage fluid was centrifuged at 1500 rpm for 10 minutes to collect intact cells. The remaining precipitate was smeared on to four slides, fixed with acetone and stained with conventional haematoxylin and eosin. An experienced cytopathologist interpreted the samples. Detection of free tumour cells after the histopathological examination of the peritoneal washing fluid was accepted as positive cytology.

Follow-up

The decision of adjuvant chemotherapy and its protocol were also determined by the multidisciplinary tumour board based on the stage and comorbidities of the patients. 5-fluorouracil-based adjuvant or FOLFOX regimens were administered to all patients with stage III or high-risk stage II cancers. All patients were followed-up with physical examination combined with laboratory and imaging techniques every three months during the first two years and every six months during the following years. The site of recurrence

and causes of death were carefully recorded to the database. The type of recurrence was classified as peritoneal recurrence, local recurrence or hepatic and other distant metastasis. The last follow-up date for the study was the end of March 2017 or until their death. Thus, the median follow-up duration for all patients and the patients who survived was 17 months (ranging from 2 to 34 months) and 19 months (ranging from 5 to 34 months), respectively.

Peritoneal carcinomatosis or recurrence was diagnosed based on the presence of clinical symptoms, radiological findings including ascites, thickening of the bowel walls and increased density of peritoneal fat. The other types of recurrences, including local and hepatic metastasis and other distant metastasis, were diagnosed according to the imaging findings.

Statistical analysis

Development of peritoneal carcinomatosis during the follow up of the patients including the detection of positive cytology in the pathological analysis of the peritoneal fluid just after surgery was regarded as the main outcome. All statistics were performed using SPSS version 20.0 for Windows. Normally distributed continuous variables were expressed as mean plus or minus standard deviation (SD). Categorical variables were expressed as frequencies and percentages.

The patients with peritoneal carcinomatosis including positive cytology were group PC (+)/CY (+). The patients without peritoneal carcinomatosis were group PC (–). Patient demographics, tumour characteristics, including the maximum tumour diameter, grade of differentiation, T and N stages, vascular, lymphatic and neuronal invasion and TNM stage, were analysed based on these groups. One-way analysis of variance was performed to identify the impact of independent risk factors on peritoneal carcinomatosis. The patients were analysed based on sCEA and sCA19-9 as low (≤ 5 ng/ml and ≤ 37 u/ml, respectively) or high (> 5 ng/ml and > 37 u/ml, respectively).

The analysis of the receiver operating characteristic (ROC) curve associated with area under curve (AUC) was used to discover the optimal cut-off values of the levels of the peritoneal tumour markers (pCEA and pCA19-9) to predict the development of peritoneal carcinomatosis. Definition of peritoneal carcinomatosis in this context included positive cytology during the surgery or development of peritoneal carcinomatosis during the subsequent follow-up period. Patients were then analysed into the low or high group according to cut-off values of peritoneal tumour markers to detect their impact on the development of peritoneal carcinomatosis.

Gastric cancer outcomes (i.e. disease-free survival and overall survival) were analysed using the Cox regression analysis and Kaplan–Meier curves and a log rank test was used for comparison of the groups according to the survival rates. All tests were two-sided and *P* value greater than 0.05 was considered statistically significant.

Results

Patient demographics and tumour characteristics

There were 67 patients with a mean age of 60 ± 11.1 years included in the study. Of the total, 51 patients (76.1%) were male, and the remaining 16 (23.9%) were female. Neoadjuvant treatment was used in 17 patients (25.4%). Tumour markers sCEA and sCA19-9 were found to be increased in 6 (9%) and 15 patients (22.4%), respectively. Distal subtotal and total gastrectomy with D2 lymph node dissection were performed in 25 (37.3%) and 42 patients (62.7%), respectively. Positive peritoneal washing cytology was detected in eight patients (12%). T4 (37.3%) and T3 (34.3%) were the most common tumour stages. Although 15 patients (22.4%) were N0, N3 was the most commonly detected N stage in 25 patients (37.3%). Patient demographics, serum tumour marker levels, TNM stages and other tumour characteristics are shown in Table 1.

Factors affecting positive cytology

There were eight patients (12%) with positive cytology. All tumours with positive cytology were T4 and N3. Owing to positive peritoneal cytology, TNM stage was 4 for all patients. There was no significant impact of age, gender, neoadjuvant treatment and sCEA on the development of positive peritoneal cytology ($P > 0.05$; Table 2). However, besides T ($P = 0.001$), N ($P = 0.001$) and TNM ($P = 0.0001$) stages, sCA19-9 levels ($P = 0.033$) and tumour diameter ($P = 0.015$) were shown to be significantly associated with positive peritoneal cytology.

The analysis based on the serum levels of CEA and CA19-9 as low or high revealed that high sCA19-9 groups were significantly associated with positive cytology ($P = 0.011$), unlike with high sCEA levels ($P = 0.549$).

Factors affecting the development of peritoneal carcinomatosis

There were 21 patients (31.3%) with peritoneal carcinomatosis including positive cytology in 8 patients (group PC (+)/CY (+)). The patients with and without peritoneal carcinomatosis were similar except the tumour diameter ($P = 0.002$), T ($P = 0.0001$), N ($P = 0.0001$) and TNM stages ($P = 0.0001$), the presence of lymphatic and neuronal invasion ($P = 0.032$ for both; Table 1). Although there were higher levels of sCEA in patients with peritoneal carcinomatosis (12.6 ± 33 ng/ml vs 3.9 ± 10.5 ng/ml), it did not reach to statistical significance ($P = 0.378$). One-way analysis of variance revealed that larger tumour diameter ($P = 0.01$), higher stages of T, N and TNM ($P = 0.0001$ for all) and presence of lymphatic and neuronal invasion ($P = 0.025$ for both) were significantly associated with the development of peritoneal carcinomatosis including positive cytology. The analysis according to the low and high levels of sCEA and sCA19-9 also revealed that there was no significant impact of high sCEA and sCA19-9 on the development of peritoneal carcinomatosis ($P = 0.072$ and $P = 0.207$, respectively).

Table 1 Patient demographics, serum tumour marker levels, tumour, node, metastasis stages and other tumour characteristics.

Feature	Overall		Group PC (–)[a]		Group PC (+)/CY (+)[b]		*P*-value
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	
Patients	67		46	68.7	21	31.3	
Age (years)	60 ± 11.1		59.8 ± 10.7		60.6 ± 12.0		0.626
Sex:							
Male	51	76.1	33	71.7	18	86	0.354
Male	16	23.9	13	28.3	3	14	
Neoadjuvant treatment	17	25.4	10	21.7	7	33.3	0.370
sCEA (ng/ml)	6.7 ± 20.5		3.9 ± 10.5		12.6 ± 33		0.378
sCA19-9 (u/ml)	101.5 ± 421.9		92.9 ± 451.3		120.3 ± 358.7		0.323
Surgery:							
Distal subtotal gastrectomy	25	37.3	16	34.8	9	42.9	0.591
Total gastrectomy	42	62.7	30	65.2	12	57.1	
Positive peritoneal cytology	8	11.94	0	0	8	38.1	0.0001
Tumour diameter (mm)	54.6 ± 31.4		46.3 ± 23.9		72.5 ± 38.5		0.002
Grade:							
Well differentiated	6	9	4	8.7	2	9.5	0.807
Moderately	19	28.4	12	26.1	7	33.3	
Undifferentiated	42	62.7	30	65.2	12	57.1	
Vascular invasion	22	32.8	12	26.0	10	47.6	0.099
Lymphatic invasion	41	61.2	24	52.2	17	81	0.032
Neuronal invasion	41	61.2	24	52.2	17	81	0.032
T stage:							
1	11	16.4	11	23.9	0	0	0.0001
2	8	11.94	7	15.2	1	4.8	
3	23	34.3	18	39.1	5	23.8	
4	25	37.3	10	21.7	15	71.4	
N stage:							
0	15	22.4	15	32.6	0	0	0.0001
1	11	16.4	9	19.6	2	9.5	
2	16	23.9	12	26.1	4	19	
3	25	37.3	10	21.7	15	71.4	
TNM stage:							
1a	10	14.9	10	21.7	0		0.0001
1b	2	3	2	4.3	0		
2a	6	9	6	13	0		
2b	11	16.4	8	17.4	3	14.3	
3a	10	14.9	8	17.4	2	9.5	
3b	9	13.4	7	15.2	2	9.5	
3c	11	16.4	5	10.9	6	28.6	
4	8	11.9	0	0	8	38.1	

sCA19-9, serum CA19-9; sCEA, serum carcinoembryonic antigen; TNM, tumour, node, metastasis.
[a] Patients without peritoneal carcinomatosis.
[b] Patients with peritoneal carcinomatosis including positive cytology.

Serum and peritoneal tumour markers

Based on the cut-off values of sCEA (≤ 5 ng/ml) and sCA19-9 (≤ 37 u/ml), there were 6 (9%) and 15 patients (22.4%) with higher sCEA and sCA19-9 in serum of the patients, respectively. Only in one patient (1.5%), both sCEA and sCA19-9 were detected as higher than the cut-off values. There was no significant association between the serum levels of tumour markers (sCEA and sCA19-9) and demographic data and tumour features ($P > 0.05$ for all). In addition, low or high sCEA grouping also showed no significant association

Table 2 Comparison of the patients with low and high serum tumour markers.

Feature	Overall		High sCEA		Low sCEA		*P*-value	High sCA19-9		Low sCA19-9		*P*-value
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)		(*n*)	(%)	(*n*)	(%)	
Patients	67		6	9	61	91		15	22.4	52	7.6	
Age (years)	60 ± 11		59.5 ± 8.3		60.1 ± 11		0.676	60.3 ± 9		59.9 ± 12		0.839
Sex:												
Male	51	76.1	5	83.3	46	75.4	1.0	13	86.7	38	73.1	0.492
Male	16	23.9	1	16.7	15	24.6		2	13.3	14	26.9	
Neoadjuvant treatment	17	25.4	0	0	17	27.9	0.325	5	33.3	12	23.1	0.504
Positive peritoneal cytology	8	11.94	1	16.7	7	11.5	0.549	5	33.3	3	5.8	0.011
Tumour diameter (mm)	54.6 ± 31		87.0 ± 58		51.4 ± 26		0.085	76.3 ± 43		48.4 ± 25		0.004
Grade:												
Well differentiated	6	9	0	0	6	9.8	0.717	1	6.7	5	9.6	0.276
Moderately	19	28.4	2	33.3	17	27.9		2	13.3	17	32.7	
Undifferentiated	42	62.7	4	66.7	38	62.3		12	80	30	57.5	
Vascular invasion	22	32.8	3	50	19	31.3	0.386	5	33.3	17	32.7	1.0
Lymphatic invasion	41	61.2	4	66.7	37	60.7	1.0	9	60	32	61.5	1.0
Neuronal invasion	41	61.2	6	100	35	57.4	0.074	10	66.7	31	59.6	0.767
T stage:												
1	11	16.4	0	0	11	18	0.174	1	6.7	10	19.2	0.014
2	8	11.94	1	16.7	7	11.5		1	6.7	7	13.5	
3	23	34.3	1	16.7	22	36.1		3	20	20	38.5	
4	25	37.3	4	66.7	21	34.4		10	66.7	15	28.8	
N stage:												
0	15	22.4	0	0	15	24.6	0.118	2	13.3	13	25	0.079
1	11	16.4	1	16.7	10	16.4		2	13.3	9	17.3	
2	16	23.9	1	16.7	15	24.6		2	13.3	14	26.9	
3	25	37.3	4	66.7	21	34.4		9	60	16	30.8	
TNM stage:												
1a	10	14.9	0	0	10	16.4	0.124	1	6.7	9	17.3	0.014
1b	2	3	0	0	2	3.3		0	0	2	3.8	
2a	6	9	1	16.7	5	8.2		1	6.7	5	9.6	
2b	11	16.4	0	0	11	18		2	13.3	9	17.3	
3a	10	14.9	0	0	10	16.4		2	13.3	8	15.4	
3b	9	13.4	2	33.3	7	11.5		0	0	9	17.3	
3c	11	16.4	2	33.3	9	14.8		4	26.7	7	13.5	
4	8	11.9	1	16.7	7	11.5		5	33.3	3	5.8	

sCA19-9, serum CA19-9; sCEA, serum carcinoembryonic antigen; TNM, tumour, node, metastasis.

(Table 2). However, positive cytology (P = 0.011), tumour diameter (P = 0.004), T (P = 0.014) and TNM stages (P = 0.014) were shown to be positively associated with high sCA19-9 group.

Peritoneal tumour markers as pCEA and pCA19-9 were measured as 2.5 ± 4.3 ng/ml and 320.5 ± 2298 u/ml, respectively (Table 3). There were significant correlations between pCEA and pCA19-9 (Spearman's rank correlation 0.333, P = 0.006, at the level of 0.01) and between pCA19-9 and sCA19-9 (Spearman's rank correlation 0.538, P = 0.0001, at the level of 0.01). Considering peritoneal carcinomatosis including positive cytology, there was no significant differences in pCEA and pCA19-9 levels (Table 3).

To determine cut-off values, ROC analysis using the sensitivities and specificities based on the diagnosis of peritoneal carcinomatosis either made at surgery or during the follow-up period revealed that the optimal cut-off values for pCEA and pCA19-9 were 0.51 ng/ml and 5.75 u/ml, respectively. Their corresponding sensitivity and specificities under the optimal cut-off values are shown in Table 4 and Figure 1. Grouping based on the cut-off values revealed that there were 41 (61.2%) and 19 patients (28.4%) with high pCEA and pCA19-9 levels, respectively. Although there was no significant association between the grouping based on these cut-off levels of pCA19-9 and peritoneal carcinomatosis including positive cytology (P = 0.088), high pCEA was shown to be significantly associated with peritoneal carcinomatosis (P = 0.032). There were 17 patients with peritoneal carcinomatosis including positive cytology among patients with high pCEA with a rate of 41.5% compared with a rate of 15.4% in patients with low pCEA.

Table 4 ROC analysis revealing the optimal cut-off values of pCEA and pCA19-9 as 0.51 ng/ml and 5.75 u/ml, respectively.

	Peritoneal CEA	pCA19-9
Area under the curve	0.630	0.531
Value	0.51	5.75
P-value	0.09	0.685
Standard error	0.074	0.083
95% confidencve interval	0.485–0.775	0.369–0.693
Sensitivity	0.81	0.43
Specificity	0.48	0.78

CEA, carcinoembryonic antigen; pCA19-9, peritoneal CA19-9.

Survival

After a median follow up of 17 months, 48 patients (71.7%) were alive at the last follow-up. Postoperative adjuvant chemotherapy and radiotherapy were used in 46 (68.7%) and seven patients (10.4%), respectively. Although overall survival for all patients was 71.7%, the patients with peritoneal carcinomatosis including positive cytology showed a statistically significant poorer prognosis as shown by overall survival rate of 28.6% (log-rank, P = 0.0001 for disease-free survival; log-rank, P = 0.0001 for overall survival; Fig 2). The overall survival without peritoneal carcinomatosis including positive cytology was 91.3%.

Cox regression analysis revealed that only serum level of CEA (sCEA) was significantly associated with disease-free and overall survival (P = 0.002 and P = 0.001, respectively). The patients with positive CEA findings as shown by high sCEA (log-rank, P = 0.003 for disease-free survival; log-rank, P = 0.006 for overall survival) showed a significantly poorer prognosis than the patients with negative CEA findings (Fig 3). The overall survival rate for high sCEA patients (sCEA > 0.51 ng/ml) was 33.3% compared with that of the patients with low sCEA (as sCEA < 0.51 ng/ml), which was 75.4%. Although there was no significant association with pCEA and disease-free survival and overall survival at the Cox regression analysis (P = 0.878 and P = 0.928, respectively), the Kaplan–Meier analysis showed that there was a poorer survival in patients with high pCEA (log-rank, P = 0.0025 for disease-free survival; log-rank, P = 0.0028 for overall survival; Fig 4). The overall survival rates for patients with high and low pCEA were 61% and 88.5%, respectively.

Recurrence

During the follow-up period, there were 31 recurrences in 25 patients (37.3%). Development of peritoneal carcinomatosis was the most common recurrence as seen in 16 patients (23.9%). Lymph node and liver metastasis were detected in five (7.5%) and four patients (6%), respectively.

Table 3 Comparison of peritoneal tumour markers with and without peritoneal carcinomatosis including positive peritoneal cytology.

Feature	Overall		Peritoneal carcinomatosis (+)		Peritoneal carcinomatosis (–)		*P*-value
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	
Patients	67		21	31.3	46	68.7	–
pCEA	2.5 ± 4.3	1.09	3.4 ± 5.3	1.1	2.1 ± 3.7	0.56	0.086
pCA19-9	320.5 ± 2298	2	931 ± 4098	2	41.7 ± 169	2	0.669

pCA19-9, peritoneal CA19-9; pCEA, peritoneal carcinoembryonic antigen.

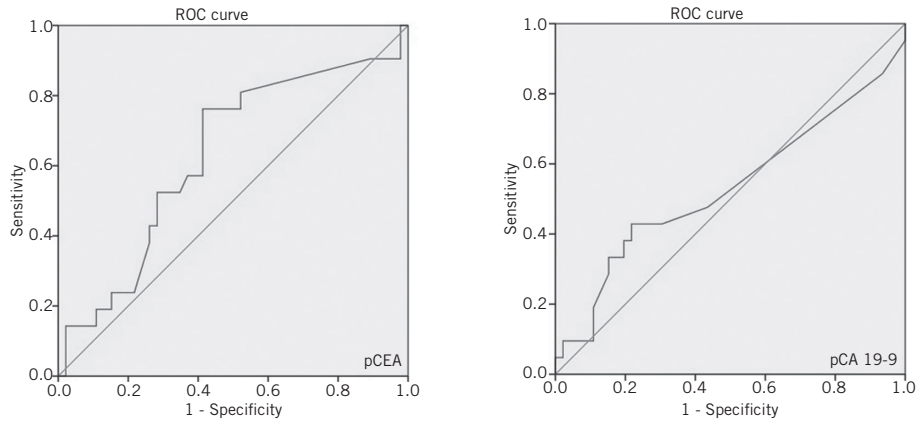

Figure 1 The receiver operating characteristic (ROC) curves for pCEA (left) and pCA19-9 (right). Area under curve (AUC) for pCEA and pCA19-9 were 0.630 and 0.531, respectively

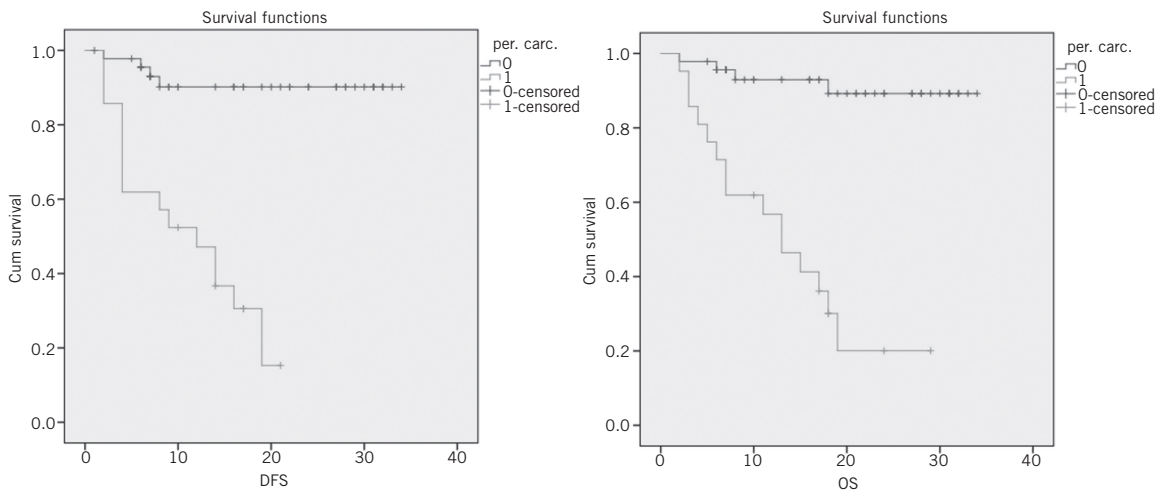

Figure 2 Disease free survival (DFS) (left) and overall survival (OS) (right) curves of the patients with and without peritoneal carcinomatosis including positive cytology

Other metastasis located at the lung, the pleura and the brain were seen in six patients (9%).

Discussion

It has been known that peritoneal carcinomatosis is the main reason for recurrences and consecutively mortality in patients with gastric cancer, even after the curative surgery.[20] Therefore, pre- or intraoperative prediction of peritoneal carcinomatosis may be an important preventive measure for future recurrences and mortality in these patients. Additionally, modification of adjuvant treatment should be considered in high risk patients.

Although there are several theories for development of peritoneal carcinomatosis, direct seeding of tumour cells through gastric wall, via blood vessels and perigastric lymphatic channels is thought to be the major route for spreading of gastric cancer through the peritoneum.[4] In previous studies, it was thought that direct cytology at or after surgical treatment was the gold standard for detection of peritoneal recurrences and carcinomatosis.[20] However, there have been many studies with variable sensitivities. In addition to cytology, tumour markers in serum or in the peritoneum may be used for more accurate reflection of peritoneal dissemination.[20–22]

Tumour markers such as CEA, CA19-9, CA72-4, CA125, neuron-specific enolase, CYFRA21-1 and tumour-specific

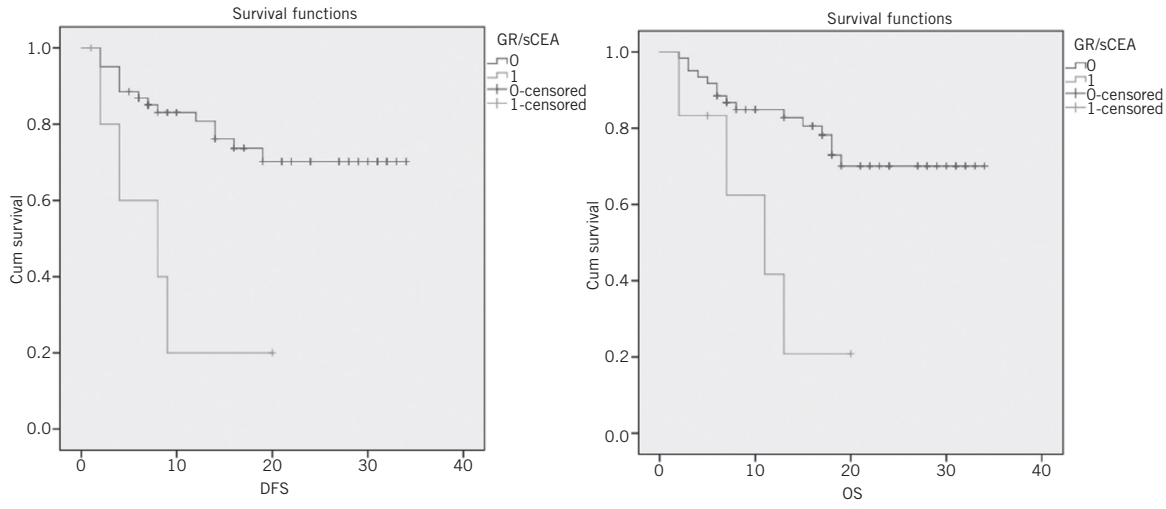

Figure 3 Disease-free survival (DFS; left) and overall survival (OS; right) curves of the patients with high and low serum carcinoembryonic antigen (CEA)

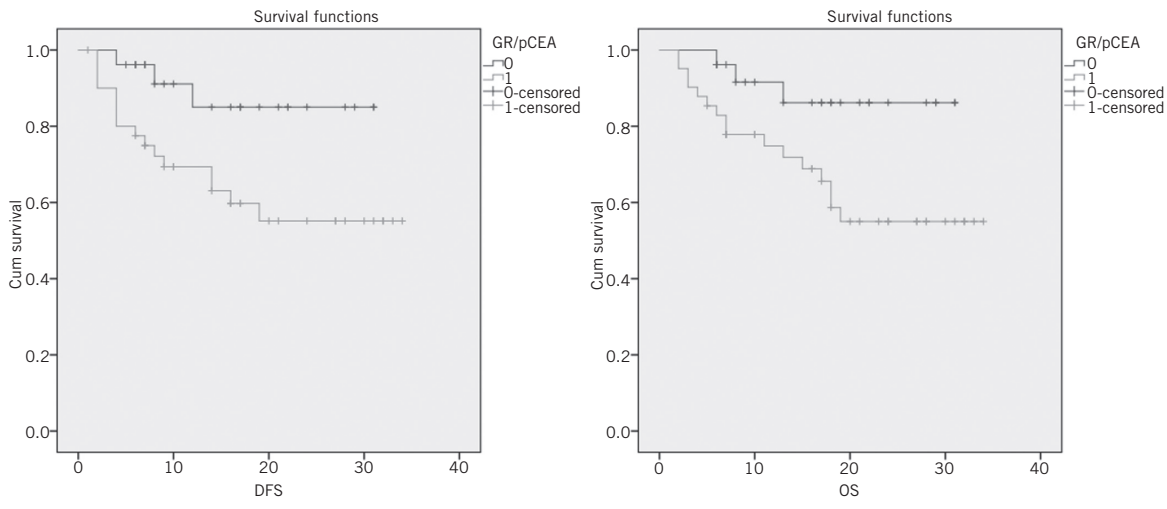

Figure 4 Disease free survival (DFS; left) and overall survival (OS; right) curves of the patients with high and low peritoneal carcinoembryonic antigen (CEA)

growth factor have been used in the evaluation of gastric cancer. The prognostic value of these markers for the survival of patients with gastric cancer is still disputed.[23] For gastric cancer, CEA and CA19-9 are the most commonly studied markers.[3] Serum and peritoneal levels of these two tumour markers were employed in this study.

There are many studies with contradictory results on the prognostic value of serum and peritoneal tumour markers in gastric cancer.[23] The underlying explanations for such inconsistencies are the limited number of eligible cases, limited statistical power of a single study, use of different cut-off values for peritoneal tumour markers and heterogeneity in designing the studies (i.e. follow-up periods and treatment protocols). However, there is a tendency for patients with gastric cancer who have high levels of tumour markers to have a higher risk of mortality compared with that of those patients with low levels.[23]

It was thought that these markers might have been auxiliary modalities for clinical diagnosis and prognostic evaluation for tumours.[1,2,24] It has been shown that these markers, either alone or in combination, increase the sensitivity of lymphatic and peritoneal metastasis in gastric cancer.[2,16,25]

In previous studies, it has been reported that there was a close relationship between tumoural features and sCEA or pCEA levels. As a conclusion, the authors proposed that sCEA and pCEA might be used potential predictors of peritoneal dissemination and consecutively poor prognosis.[20] In Lee's study,[7] tumour marker cut-off ratio for CEA, CA19-9 and CA72-4 has been studied and it has been shown that it could be a useful tool for the prediction of prognosis in gastric cancer. Additionally, sCA19-9 has been shown to be associated with advanced gastric cancer.[8,10,14] In a systematic review, it has been concluded that preoperative serum tumour markers (CEA, CA19-9, CA72-4) are significantly associate with tumour stage and patient survival.[3] In another meta-analysis, it has reported that high sCEA as an independent prognostic factor for gastric cancer doubled the risk of mortality.[23] In contrast to these studies, it has been also reported that sCEA and sCA19-9 do not show any association with TNM staging of gastric cancer.[14,26] In another study, it has been shown that CEA, CA19-9 and CA72-4 were not associated with peritoneal recurrences.[3] Preoperative positivity of these markers was regarded as the independent risk factor for haematogeneous recurrences. Owing to the small size of the study group, such analysis could not be performed in the present study. In this study, numeric values of sCA19-9 and high sCA19-9 based on the serum cut-off value were significantly associated with positive cytology. However, such a relationship was not present with sCEA. For the development of peritoneal carcinomatosis including positive cytology, there was a significant difference in patients with high pCEA. Although sCEA levels were higher in patients with peritoneal carcinomatosis including positive cytology, statistical significance could not be detected. In addition, high serum tumour markers and numeric values of peritoneal tumour markers were shown to have no significant impact on this issue. Thus, the use of tumour markers either in the serum or the peritoneal fluid may not help physicians to reach an accurate prediction and diagnosis of peritoneal carcinomatosis including positive cytology.Therefore, different results in previous studies necessitate large-scale prospective studies in different geographical areas to reach a generalised conclusion for this issue.

In cases in which peritoneal carcinomatosis cannot be diagnosed by conventional imaging techniques, it has been reported that pCEA has the higher sensitivity than the cytology to detect peritoneal carcinomatosis. In Yamamoto's study,[1] sensitivity and specificity of pCEA were reported to be 75.8% and 90.8%, respectively. In the present study, only sCA19-9 was shown to be associated with positive cytology. To understand the possible association between positive cytology and tumoural features, all tumours with positive cytology in the present study were T4 and N3 in accordance with the idea of exfoliation of neoplastic cells from lesions invading the serosa or lymphatic channels on the metastatic lymph nodes.[5] Large scale studies are still needed to clarify this possible association.

In a systematic review, it has been concluded that preoperative serum tumour markers (CEA, CA19-9, CA72-4) are significantly associate with tumour stage and patient survival.[3] In this review, overall positive rates for CEA and CA19-9 were 16–68% and 14–68% for CEA and CA19-9, respectively. However, these rates were found to be 9% and 22.4% in the present study. Compared with this review, the low rate of sCEA positivity remains controversial and it may be associated with the distribution of T and N stages.[7]

Compared with the elevated sCA19-9 and pCEA, pCA19-9 and sCEA have been thought to be more reliable markers for staging of gastric cancer.[5] In Kanetaka's study,[20] pCEA was shown to be a strong prognostic factor only in univariate analysis. With regard to the comparison of sCEA and pCEA, Kanetaka *et al.* also found that pCEA is a better biomarker for clinical utility of gastric cancer.[20] Contrary to this finding, our results showed that only sCEA is a significant prognostic factor for disease-free and overall survival of patients with gastric cancer.

It has been also speculated that elevated pCEA is independently associated with poor prognosis in patients with peritoneal dissemination.[27] Yamamoto *et al.* also showed the positive association between CEA level in peritoneal lavage and peritoneal dissemination.[1] The authors recommended that pCEA can be considered as a predictor of peritoneal dissemination including positive cytology. In the present study, although there was a poorer prognosis in patients with positive pCEA, it did not reach statistical significance. These findings would not provide additional benefit for use of pCEA as a predictor or prognostic factor for gastric cancer.

It has been shown that only serum tumour markers are associated with tumour diameter.[5] In the present study, univariate analysis of serum tumour markers also revealed that sCA19-9 was significantly associated with tumour diameter and TNM staging. However, such association could not be detected for sCEA. Contrary to these findings, prognosis has been shown to be significantly related with sCEA and high sCEA group. Therefore, controversial issues with regard to the impact of serum and peritoneal tumour markers and prognosis in gastric cancer patients remain to be solved.

The main limitations of the study were the small size of the study group, the use of only two tumor markers and the short follow-up period. In addition, inclusion of all patients with gastric adenocarcinoma as early or locally advanced might prevent more significant and reasonable results. Lack of cut-off values for the levels of peritoneal tumour markers and calculation of these values using the study group were other drawbacks of the study. Although use of a prospective data base and a standardised follow-up programme were the important issues for the accuracy of the study, more cases are needed to reach more meaningful results.

Conclusion

In conclusion, the use of serum and peritoneal tumour markers for diagnosis, staging and prognosis of gastric cancer remain to be still controversial. However, sCEA is shown to be significantly associated with poor prognosis for patients with gastric cancer as shown through disease-free and overall survival rates. In addition, serum level of CA19-9 is a significant predictor for positive peritoneal washing cytology. There is also a significant association between high peritoneal CEA levels and the development of

peritoneal carcinomatosis. Therefore, the possible impact of serum and peritoneal CEA and CA19-9, especially on the staging and prognosis of gastric cancer, should be evaluated by prospective large-scale studies.

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References

1. Yamamoto M, Baba H, Kakeji Y *et al*. Prognostic significance of tumour markers in peritoneal lavage in advanced gastric cancer. *Oncology* 2004; **67**: 19–26.
2. Yamamoto M, Yoshinaga K, Matsuyama A *et al*. CEA/CA72-4 levels in peritoneal lavage fluid are predictive factors in patients with gastric carcinoma. *J Cancer Res Clin Oncol* 2014; **140**: 607–612.
3. Shimada H, Noie T, Ohashi M *et al*. Clinical significance of serum tumour markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association. *Gastric Cancer* 2014; **17**: 26–33.
4. Hasbahceci M, Akcakaya A, Guler B *et al*. Use of peritoneal washing cytology for the detection of free peritoneal cancer cells before and after surgical treatment of gastric adenocarcinoma. *J Can Res Ther* 28 July 2016. http://www.cancerjournal.net/preprintarticle.asp?id=184518 (cited January 2018).
5. Crepaldi-Filho R, Palma RT, Giusti MF *et al*. Levels of carcinoembryonic antigen and CA19-9 in the sera and peritoneal washing of patients undergoing surgical treatment for gastric carcinoma. *Arq Gastroenterol* 2008; **45**: 219–224.
6. Deng K, Zhu H, Chen M *et al*. Prognostic significance of molecular analysis of peritoneal fluid for patients with gastric cancer: a meta-analysis. *PLoS One* 2016; **11**: e0151608.
7. Lee JC, Lee SY, Kim CY, Yang DH. Clinical utility of tumour marker cutoff ratio and a combination scoring system of preoperative carcinoembryonic antigen, carbohydrate antigen 19-9, carbohydrate antigen 72-4 levels in gastric cancer. *J Korean Surg Soc* 2013; **85**: 283–2839.
8. Sisik A, Kaya M, Bas G *et al*. CEA and CA19-9 are still valuable markers for the prognosis of colorectal and gastric cancer patients. *Asian Pac J Cancer Prev* 2013; **14**: 4,289–4,294.
9. Hamazoe R, Maeta M, Matsui T *et al*. CA72-4 compared with carcinoembryonic antigen as a tumour marker for gastric cancer. *Eur J Cancer* 1992; **28**: 1,351–1,354.
10. Han ES, Lee HH, Lee JS *et al*. At which stage of gastric cancer progression do levels of carcinoembryonic antigen and carbohydrate antigen 19-9 increase? Application in advanced gastric cancer treatment. *J Gastric Cancer* 2014; **14**:1,23–1,238.
11. Yamamoto M, Baba H, Toh Y *et al*. Peritoneal lavage CEA/CA125 is a prognostic factor for gastric cancer patients. *J Cancer Res Clin Oncol* 2007; **133**: 471–476.
12. Choi SR, Jang JS, Lee JH *et al*. Role of serum tumour markers in monitoring for recurrence of gastric cancer following radical gastrectomy. *Dig Dis Sci* 2006; **51**: 2,081–2,086.
13. Lai IR, Lee WJ, Huang MT, Lin HH. Comparison of serum CA72-4, CEA, TPA, CA19-9 and CA125 levels in gastric cancer patients and correlation with recurrence. *Hepatogastroenterology* 2002; **49**: 1,157–1,160.
14. Yu J, Zhang S, Zhao B. Differences and correlation of serum CEA, CA19-9 and CA72-4 in gastric cancer. *Mol Clin Oncol* 2016; **4**: 441–449.
15. Yang AP, Liu J, Lei HY *et al*. CA72-4 combined with CEA, CA125 and CAI9-9 improves the sensitivity for the early diagnosis of gastric cancer. *Clin Chim Acta* 2014; 437: 183–186.
16. Liu X, Qiu H, Liu J *et al*. Combined preoperative concentrations of CEA, CA19-9, and 72-4 for predicting outcomes in patients with gastric cancer after curative resection. *Oncotarget* 2016; **7**: 3,5446–3,5453.
17. Bezmialem Vakif University. Serum and Peritoneal CEA and CA 19-9 for Gastric Adenocarcinoma. clinicaltrials.gov NCT02801955. https://clinicaltrials.gov/ct2/show/NCT02801955 (cited January 2018).
18. Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinoma, 3rd ed. *Gastric Cancer* 2011; **14**: 101–112.
19. Zhang J, Niu Z, Zhou Y, Cao S. A comparison between the seventh and sixth editions of the American Joint Committee on Cancer/ International Union Against classification of gastric cancer. *Ann Surg* 2013; **257**: 81–86.
20. Kanetaka K, Ito S, Susumu S *et al*. Clinical significance of carcinoembryonic antigen in peritoneal lavage from patients with gastric cancer. *Surgery* 2013; **154**: 563–572.
21. Asao T, Fukuda T, Yazawa S, Nagamachi Y. Carcinoembryonic antigen levels in peritoneal washings can predict peritoneal recurrence after curative resection of gastric cancer. *Cancer* 1991; **68**: 44–47.
22. Abe N, Watanabe T, Toda H *et al*. Prognostic significance of carcinoembryonic antigen levels in peritoneal washes in patients with gastric cancer. *Am J Surg* 2001; **181**: 356–361.
23. Deng K, Yang L, Hu B *et al*. The prognostic significance of pretreatment serum CEA levels in gastric cancer: a meta-analysis including 14651 patients. *PLoS One* 2015; **10**: e0124151.
24. Yin LK, Sun XQ, Mou DZ. Value of combined detection of serum CEA, CA72-4, CA19-9 and TSGF in the diagnosis of gastric cancer. *Asian Pac J Cancer Prev* 2015; **16**: 3,867–3,870.
25. Lai H1, Jin Q, Lin Y *et al*. Combined use of lysyl oxidase, carcino-embryonic antigen, and carbohydrate antigens improves the sensitivity of biomarkers in predicting lymph node metastasis and peritoneal metastasis in gastric cancer. *Tumour Biol* 2014; **35**: 10547–10554.
26. Gwak HK, Lee JH, Park SG. Preliminary evaluation of clinical utility of CYFRA 21-1, CA 72-4, NSE, CA19-9 and CEA in stomach cancer. *Asian Pac J Cancer Prev* 2014; **15**: 4,933–4,938.
27. Jung M, Jeung HC, Lee SS *et al*. The clinical significance of ascitic fluid CEA in advanced gastric cancer with ascites. *J Cancer Res Clin Oncol* 2010; **136**: 517–526.