

# Peritoneal Lavage CEA mRNA Levels Predict Conversion Gastrectomy Outcomes after Induction Chemotherapy with Intraperitoneal Paclitaxel in Gastric Cancer Patients with Peritoneal Metastasis

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## ABSTRACT

**Background.** The outcome of gastric cancer patients with peritoneal metastasis remains poor. We treated these patients with intraperitoneal and intravenous paclitaxel plus oral S-1 (tegafur/gimeracil/oteracil), followed by gastrectomy in responders. We evaluated the clinical significance of peritoneal lavage carcinoembryonic antigen (CEA) messenger RNA (mRNA) levels as a biomarker for indication of conversion gastrectomy.

**Methods.** The peritoneal lavage of 68 patients who received the above regimen as induction chemotherapy was repeatedly collected via intraperitoneal access ports. Gastrectomy was considered when improvement of peritoneal metastasis was confirmed by a second laparoscopic examination with negative peritoneal cytology. CEA and porphobilinogen deaminase mRNAs were chronologically quantified using the transcription reverse-transcription concerted reaction method. The CEA mRNA index (CmRI) was calculated as CEA mRNA/porphobilinogen deaminase mRNA  $\times$  10,000.

**Results.** Thirty-nine patients underwent gastrectomy and 29 patients did not (median survival time, 27.8 vs. 10.7 months, respectively;  $P < 0.001$ ). In gastrectomy-positive patients, the outcome largely differed according to CmRI values immediately prior to surgery. Patients with a preoperative CmRI value  $<100$  ( $n = 20$ ) were associated with a significantly longer survival than those with a preoperative CmRI value  $>100$  ( $n = 19$ ) (41.8 vs. 20.1 months, respectively;  $P < 0.001$ ). A preoperative CmRI value  $<100$  was confirmed as an independent predictor of survival for gastrectomy-positive patients in the multivariate analysis.

**Conclusions.** The CmRI reflects the response of peritoneal metastases to induction intraperitoneal chemotherapy. It may be a useful biomarker for indicating gastrectomy in gastric cancer patients with peritoneal metastasis.

Despite recent advances in chemotherapy agents, systemic chemotherapy alone has limited clinical effects on peritoneal metastases in gastric cancer patients.<sup>1–3</sup> Recent reports have revealed that the intraperitoneal administration of taxanes (e.g., paclitaxel and docetaxel) can exhibit significant effects on peritoneal metastases in gastric cancer patients.<sup>4,5</sup> Induction chemotherapy with intraperitoneal taxanes, followed by gastrectomy, has shown promise as a potential therapeutic strategy for this dismal disease. We and others have performed phase II studies targeting gastric cancer patients with peritoneal metastasis and have demonstrated a median survival time (MST) of 16.2–22.6 months and a 1-year overall survival (OS) rate of

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70.0–78.0%.<sup>6–9</sup> Additional retrospective analyses have shown that patients who undergo gastrectomy following a good response tend to exhibit a better outcome than those who do not undergo surgery.<sup>4,5</sup>

However, it remains to be determined whether gastrectomy contributes significantly to the survival benefit in such good responders. It also is unclear how and when gastrectomy should be performed. Hence, biomarkers are needed to predict the outcome of gastrectomy. We chronologically measured the relative expression of carcinoembryonic antigen (CEA) messenger RNA (mRNA) against an internal control [CEA mRNA index (CmRI)] in the peritoneal lavage of patients treated with induction intraperitoneal chemotherapy and evaluated the clinical significance of the CmRI in the decision to perform gastrectomy in these patients.

## PATIENTS AND METHODS

### *Patients and Treatments*

In total, 103 gastric cancer patients underwent staging laparoscopy under general anesthesia and peritoneal metastasis was confirmed at The University of Tokyo Hospital (Tokyo, Japan) between July 2009 and October 2013. Peritoneal access ports were implanted subcutaneously and patients received combination chemotherapy, including intraperitoneal paclitaxel, via the access port. Peritoneal lavage CEA mRNA levels were repeatedly quantified over time in 72 patients and the patients' clinical records were retrospectively reviewed. In four patients, CEA mRNA levels were not positively detected (calculated as 0), despite positive cytology. These patients were excluded from further analysis.

A chemotherapy regimen comprising intravenous and intraperitoneal paclitaxel plus oral S-1 (tegafur/gimeracil/oteracil) was administered as described previously.<sup>10</sup> Briefly, paclitaxel was administered intravenously (50 mg/m<sup>2</sup>) on Day 1 and intraperitoneally (20 mg/m<sup>2</sup>) on Day 8. Oral S-1 (80 mg/m<sup>2</sup>) was administered daily (Days 1–14 with a 1-week rest). Paclitaxel was diluted in 1.0 L of normal saline and administered to the peritoneal cavity through the implanted peritoneal access port. The treatment course was repeated every 3 weeks until disease progression or intolerable toxicity occurred.

Gastrectomy was considered when intraperitoneal chemotherapy exhibited a good response that facilitated macroscopically curative resection. The eligibility criteria for gastrectomy were as follows: no distant metastases detectable by computed tomography, except in the peritoneal cavity; cytologically negative peritoneal lavage; and the disappearance or marked shrinkage of peritoneal metastasis on second-look laparoscopy. Persistent peritoneal

white nodules or scar-like lesions were resected as much as possible.<sup>11</sup> In patients who did not undergo gastrectomy, the same combination chemotherapy regimen was continued until disease progression or intolerable toxicity occurred.

This study was approved by the Ethical Review Board Committee of The University of Tokyo (Tokyo, Japan). All participants provided informed, written consent, and research was conducted in accordance with the Declaration of Helsinki.

### *Peritoneal Lavage Sampling and the CEA mRNA Index*

At initial staging laparoscopy, 100 mL of saline was administered into the Douglas pouch and collected through a suction and irrigation device. Half of the lavage was used for cytological examinations and the remainder for measurements of CEA mRNA levels. Peritoneal lavage was repeatedly collected every 3 weeks on the first day of each treatment course via the peritoneal access port after the injection of 100 mL of saline into the intraperitoneal space. Peritoneal lavage also was collected at the time of second-look laparoscopy, using the same procedure as that performed at the time of the initial staging laparoscopy.

CEA mRNA levels were measured using the transcription reverse-transcription concerted reaction (TRCR) method as described previously.<sup>12,13</sup> Briefly, total cellular RNA was extracted from peritoneal lavage cell pellets using ISOGEN RNA extraction buffer (catalogue no.: #311-02501; Nippon Gene, Tokyo, Japan). The TRCRtest CEA-m (catalogue no.: #051009; Tosoh Corp., Tokyo, Japan) was used to measure CEA mRNA levels and the TRCRtest PBGD-m (catalogue no.: #051010; Tosoh Corp., Tokyo, Japan) was used to measure porphobilinogen deaminase (PBGD) mRNA levels as the internal control. The CmRI was calculated as CEA mRNA/PBGD mRNA × 10,000. The CmRI of each patient was measured 3–45 times. The CmRI of the samples taken 0–28 days before to surgery was adopted as the preoperative value in patients who had undergone conversion gastrectomy.

### *Statistical Analyses*

Chi square tests were used to determine the significance of associations between different clinicopathological variables. The Wilcoxon rank-sum test was used to analyze the relationship of the CmRI in the peritoneal lavage of gastric cancer patients with peritoneal metastasis. OS was calculated using the Kaplan–Meier method and compared using the log-rank test. A Cox proportional hazards regression model was used to determine the significance of prognostic factors. Variables with a  $P < 0.05$  in the univariate analysis were included in the multivariate analysis. Statistical analyses were conducted using JMP software version 12.2.0 (SAS Institute Inc., Cary, NC).

## RESULTS

### Patient Characteristics

Thirty-nine of the 68 gastric cancer patients with peritoneal metastasis who were administered induction chemotherapy with intravenous and intraperitoneal paclitaxel plus oral S-1 met the criteria for surgery and underwent gastrectomy. The remaining 29 patients failed to meet the criteria ( $n = 20$ ) or refused surgery ( $n = 9$ ), even though they met the eligibility criteria.

The characteristics of gastrectomy-positive and gastrectomy-negative patients at the time of the initial staging laparoscopy are summarized in Table 1. Statistically significant differences in Eastern Cooperative Oncology Group performance status, the presence or absence of malignant ascites, and serum cancer antigen-125 (CA-125) levels were observed between the groups. The Peritoneal Cancer Index scores of the gastrectomy-negative group tended to be higher than those of the gastrectomy-positive group, although this could not be confirmed statistically, due to a lack of available data on seven patients in the gastrectomy-negative group.

### Peritoneal Lavage CEA mRNA Index Values

Peritoneal lavage CmRI values of gastrectomy-positive and gastrectomy-negative patients at the time of the initial staging laparoscopy, prior to induction chemotherapy, and the CmRI values of gastrectomy-positive patients for the preoperative period after induction chemotherapy are displayed in Fig. 1. Initial peritoneal lavage CmRI values were not statistically significant between the groups, although a trend was observed for higher CmRI values in the gastrectomy-negative group ( $P > 0.05$ ). In the majority of the gastrectomy-positive patients ( $n = 39$ ), preoperative CmRI values were reduced compared to those at the time of the initial staging laparoscopy ( $P < 0.0001$ ).

### Perioperative Characteristics of Conversion Gastrectomy Patients

The perioperative characteristics of the 39 patients who underwent conversion gastrectomy are summarized in Table 2. The median and maximum number of preoperative chemotherapy cycles was 6 and 31, respectively. In the majority of patients, serum levels of tumor markers reduced to within normal limits, even in patients whose values were elevated before induction chemotherapy. In fact, the levels of CEA, cancer antigen 19-9 (CA19-9), and CA-125 were normalized in 4 of 7 (57.1%), 4 of 12 (33.3%), and 11 of 17 (64.7%) patients who had exhibited elevated levels of each

**TABLE 1** Patients' clinical characteristics

Characteristic	G <sup>+</sup> ( $n = 39$ )	G <sup>-</sup> ( $n = 29$ )	<i>P</i> value
Gender, <i>n</i> (%)			
M	21 (53.8)	15 (51.7)	
F	18 (46.2)	14 (48.3)	0.860
Age (years), median (range)	59 (28–72)	55 (29–75)	0.450
ECOG PS, <i>n</i> (%)			
0	33 (84.6)	17 (58.6)	
1	6 (15.4)	11 (37.9)	
2	0 (0.0)	1 (3.5)	0.040*
Macroscopic type, <i>n</i> (%)			
Type 4	26 (66.7)	21 (72.4)	
Other	13 (33.3)	8 (27.6)	0.610
Histological type, <i>n</i> (%)			
Intestinal	3 (7.7)	2 (6.9)	
Diffuse	36 (92.3)	27 (93.1)	0.900
Malignant ascites, <i>n</i> (%)			
Present	20 (51.3)	27 (93.1)	
Absent	19 (48.7)	2 (6.9)	<0.001*
Serum CEA level, <i>n</i> (%)			
Normal	32 (82.1)	23 (79.3)	
Elevated	7 (17.9)	6 (20.7)	0.780
Serum CA19-9 level, <i>n</i> (%)			
Normal	27 (69.2)	15 (51.7)	
Elevated	12 (30.8)	14 (48.3)	0.140
Serum CA-125 level, <i>n</i> (%)			
Normal	22 (56.4)	8 (27.6)	
Elevated	17 (43.6)	21 (72.4)	0.017*
PCI score at staging laparoscopy, <i>n</i> (%)			
Median (range)	11 (1–27)	23 (7–39)	
1–9	18 (46.2)	3 (10.4)	
10–19	15 (38.4)	5 (17.2)	
≥20	6 (15.4)	14 (48.3)	
N/A	0 (0.0)	7 (24.1)	–

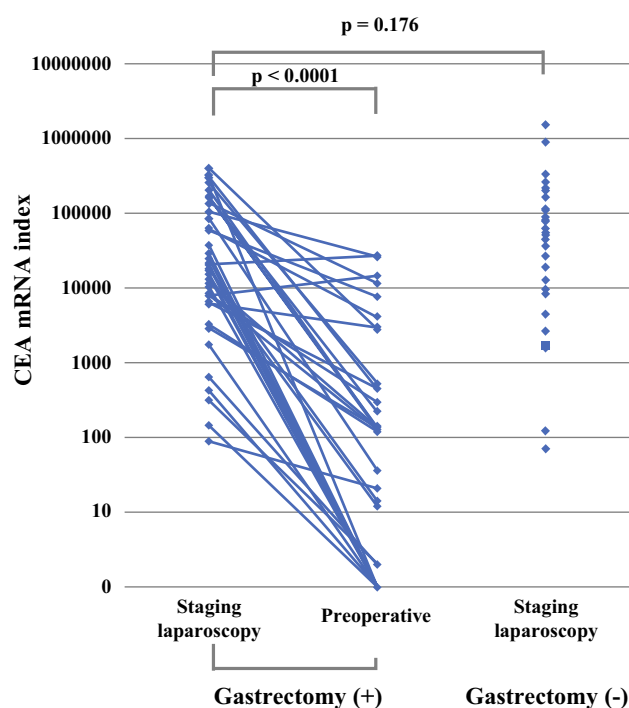
CA-125 cancer antigen 125, CA19-9 cancer antigen 19-9, CEA carcinoembryonic antigen, ECOG Eastern Cooperative Oncology Group, F female, G gastrectomy, M male, N/A not available, PCI Peritoneal Cancer Index, PS performance status

\*  $P < 0.05$

marker, respectively. A histological response of Grade IA was observed in 25 patients after induction chemotherapy, while Grade III was only observed in 1 patient.

### Overall Survival and Preoperative CEA mRNA Index Values

Gastrectomy-positive patients ( $n = 39$ ) exhibited a significantly better outcome than gastrectomy-negative patients ( $n = 29$ ) (MST, 27.8 vs. 10.7 months, respectively;  $P < 0.001$ ; Fig. 2a). In the gastrectomy-positive



**FIG. 1** Carcinoembryonic antigen (CEA) messenger RNA (mRNA) index values at initial staging laparoscopy in patients who received induction chemotherapy with intravenous and intraperitoneal paclitaxel plus oral S-1 (tegafur/gimeracil/oteracil) ( $n = 68$ ). Preoperative CEA mRNA index values are also expressed for patients who underwent conversion gastrectomy ( $n = 39$ )

group, preoperative CmRI values were  $<100$  in 20 patients and  $>100$  in 19 patients. The MST of the former 20 patients was 41.8 months versus 20.1 months for the latter 19 patients. A significant difference in OS was observed between the groups ( $P < 0.01$ ; Fig. 2b).

In the univariate analysis, OS was not associated with any factors of preinduction chemotherapy but was significantly associated with preoperative serum CA19-9 ( $P < 0.05$ ) and CA-125 ( $P < 0.05$ ) levels and CmRI values ( $P < 0.01$ ). In the multivariate analysis of prognostic factors, preoperative serum CA19-9 levels ( $P < 0.05$ ) and CmRI values of  $<100$  ( $P < 0.05$ ) were confirmed as independent risk factors for OS (Table 3).

#### Overall Survival and CEA mRNA Index Values during Induction Chemotherapy

Changes in CmRI values during induction chemotherapy were subsequently examined in each patient. Among patients with preoperative CmRI values  $<100$  ( $n = 20$ ), the CmRI value was initially reduced to  $<100$  at 22–161 days after commencing induction chemotherapy (Supplementary Fig. S1a). Moreover, among patients with preoperative CmRI values  $>100$  ( $n = 19$ ), the CmRI value was reduced to  $<100$  on at least one occasion at 28–221 days in 12

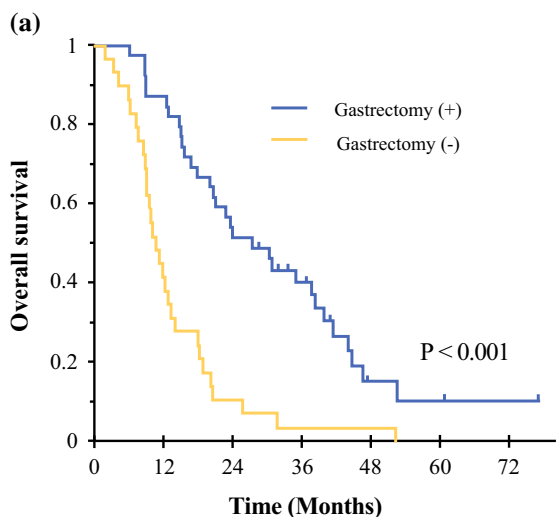
**TABLE 2** Perioperative characteristics of conversion gastrectomy patients ( $n = 39$ )

Characteristic	Patients, $n$ (%)
No. of cycles of preoperative chemotherapy	
2–5	14 (35.9)
6–9	19 (48.7)
$\geq 10$	6 (15.4)
Serum CEA level	
Normal	25 (64.1)
Elevated	14 (35.9)
Serum CA19-9 level	
Normal	30 (76.9)
Elevated	9 (23.1)
Serum CA-125 level	
Normal	31 (79.5)
Elevated	8 (20.5)
Gastrectomy type	
Total	36 (92.3)
Distal	3 (7.7)
Surgical resection	
R0	23 (59.0)
R1	4 (10.2)
R2	12 (30.8)
Histological effects	
Grade IA	25 (64.1)
Grade IB	8 (20.5)
Grade II	5 (12.8)
Grade III	1 (2.6)

CA-125 cancer antigen 125, CA19-9 cancer antigen 19-9, CEA carcinoembryonic antigen

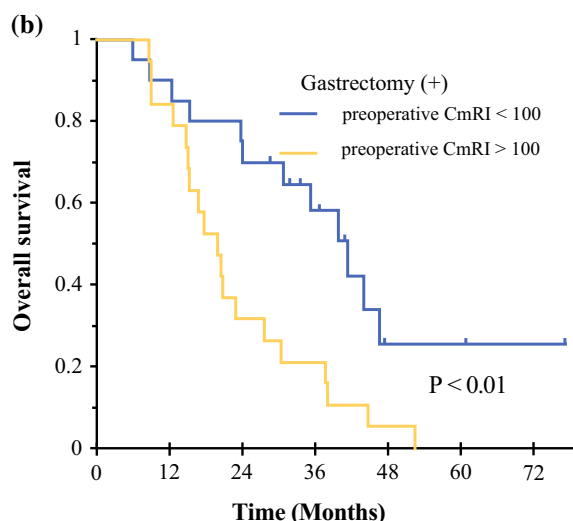
patients, whilst never reducing to  $<100$  in the remaining seven patients (Supplementary Fig. S1b). Supplementary Fig. S1c displays the tentative graph of CmRI values until the shift to second-line treatment in patients who did not undergo gastrectomy ( $n = 29$ ). During induction chemotherapy, CmRI values of  $>100$  were maintained in 17 patients. In the remaining 12 patients, CmRI values were reduced to  $<100$  on at least one occasion.

Finally, the outcome of patients stratified according to CmRI values of  $<100$  or  $>100$  were compared. In the former patients who experienced CmRI values of  $<100$  during induction chemotherapy, the MST of the gastrectomy-positive group ( $n = 32$ ) was significantly longer than that of the gastrectomy-negative group ( $n = 12$ ) (35.5 vs. 13.0 months, respectively;  $P < 0.001$ ; Fig. 2c). In contrast, in the latter patients who did not experience CmRI values of  $<100$ , a survival benefit was not apparent in the gastrectomy-positive group [MST, 15.0 ( $n = 7$ ) vs. 9.0 ( $n = 17$ ) months, respectively;  $P > 0.05$ ; Fig. 2d].



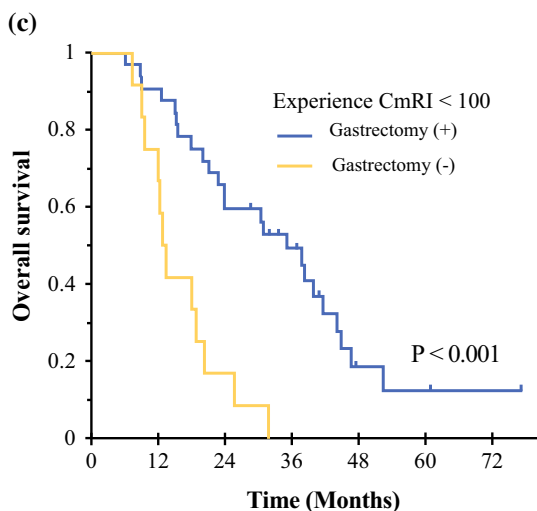
Number at risk

Gastrectomy (+)	39	34	20	13	3	2	1
Gastrectomy (-)	29	12	3	1	1	0	0



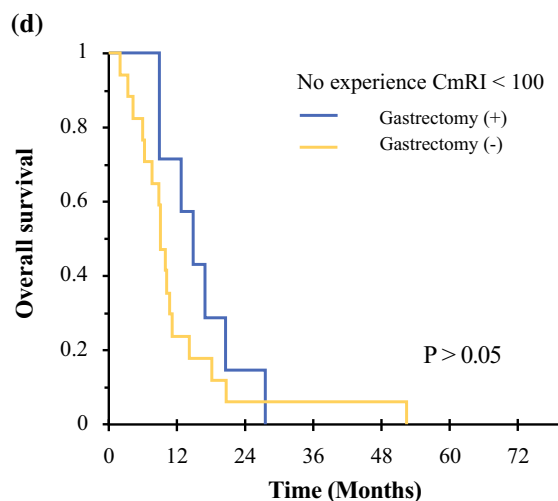
Number at risk

CmRI <math>< 100</math>	20	18	14	9	2	2	1
CmRI >math>100</math>	19	16	6	4	1	0	0



Number at risk

Gastrectomy (+)	32	29	19	13	3	2	1
Gastrectomy (-)	12	8	2	1	1	0	0



Number at risk

Gastrectomy (+)	7	5	1	0	0	0	0
Gastrectomy (-)	17	4	1	1	1	0	0

**FIG. 2** Kaplan–Meier curves of overall survival. **a** Patients were stratified according to those who underwent conversion gastrectomy ( $n = 39$ ) versus those who did not ( $n = 29$ ). **b** Patients who underwent conversion gastrectomy were stratified according to a preoperative carcinoembryonic antigen messenger RNA index (CmRI) value of  $<100$  ( $n = 20$ ) versus a preoperative CmRI value  $>100$  ( $n = 19$ ). **c** Patients who experienced CmRI values  $<100$  on at least one occasion during induction chemotherapy ( $n = 44$ ) were

stratified according to those who underwent conversion gastrectomy versus those who did not. **d** Patients with CmRI values  $>100$  during induction chemotherapy ( $n = 24$ ) were stratified according to those who underwent conversion gastrectomy versus those who did not.  $P$  values were calculated using the log-rank test

**DISCUSSION**

Generally, intraperitoneal taxanes are highly effective for peritoneal metastasis, although they appear to be less effective for primary tumors and other metastases.<sup>4–6</sup> In fact, the histological response of the resected gastric tumor

was Grade IA in 25 of 39 patients, with a complete response observed in only one patient, although there were no residual tumor cells in the majority of the resected peritoneal metastases. Therefore, our rationale for performing gastrectomy after induction chemotherapy was to remove the primary lesion to prevent the development of

**TABLE 3** Univariate and multivariate analyses of clinical factors influencing overall survival in conversion gastrectomy patients

Clinical factor	Univariate analysis	Multivariate analysis	
	<i>P</i> value	HR (95.0% CI)	<i>P</i> value
<b>Preinduction chemotherapy</b>			
Gender (M vs. F)	0.485	–	–
Age (years) ( $\geq 60$ vs. $<60$ )	0.597	–	–
ECOG PS (1 vs. 0)	0.817	–	–
Macroscopic type (type 4 vs. other)	0.168	–	–
Pretreatment serum CEA level (elevated vs. normal)	0.316	–	–
Pretreatment serum CA19-9 level (elevated vs. normal)	0.480	–	–
Pretreatment serum CA-125 level (elevated vs. normal)	0.241	–	–
Peritoneal cytology (CY0 vs. CY1)	0.803	–	–
Malignant ascites (present vs. absent)	0.650	–	–
PCI score at staging laparoscopy ( $\geq 10$ vs. $<10$ )	0.530	–	–
CmRI at staging laparoscopy ( $\geq 20,000$ vs. $<20,000$ )	0.623	–	–
<b>Postinduction chemotherapy</b>			
No. of cycles of preoperative chemotherapy ( $\geq 6$ vs. $<6$ )	0.443	–	–
Preoperative CmRI ( $\geq 100$ vs. $<100$ )	0.004*	2.22 (1.03–4.94)	0.042*
Preoperative serum CEA level (elevated vs. normal)	0.740	–	–
Preoperative serum CA19-9 level (elevated vs. normal)	0.013*	2.95 (1.20–6.81)	0.019*
Preoperative serum CA-125 level (elevated vs. normal)	0.030*	2.46 (0.98–5.74)	0.056
Surgical resection (R0 vs. R1–2)	0.572	–	–
Histological effects (Grade IA vs. Grade IB–III)	0.054	–	–

CA-125 cancer antigen 125; CA19-9 cancer antigen 19-9; CEA carcinoembryonic antigen; CI confidence interval, CmRI CEA mRNA Index; ECOG Eastern Cooperative Oncology Group; F female; HR hazard ratio; M male; mRNA messenger RNA, PCI Peritoneal Cancer Index; PS performance status

\*  $P < 0.05$

new metastases and refractory hemorrhagic or stenotic complications caused by the regrowth of the primary tumor. However, open surgery frequently results in the progression of peritoneal micrometastases because of the perioperative pausing of induction chemotherapy and immunosuppression accompanying surgical intervention. Therefore, it is best to perform gastrectomy when peritoneal metastases are adequately controlled.

Peritoneal cytology is widely used to detect tumor cells in the abdominal cavity, which reportedly reflects the response of peritoneal metastasis to induction chemotherapy. Thus, we considered negative cytology to be one of the criteria for gastrectomy. However, previous studies have suggested that conventional cytological examinations of peritoneal lavage specimens lack sensitivity.<sup>14–16</sup> Moreover, the molecular detection of cancer-specific peritoneal lavage CEA mRNA levels using reverse transcription polymerase chain reaction has superior prognostic value for postoperative peritoneal recurrence in patients with advanced gastric cancer.

In the present study, we calculated the CmRI as the relative expression of CEA mRNA levels by normalizing

to PBGD mRNA. We subsequently evaluated whether CmRI values could serve as a biomarker to determine the outcomes of gastrectomy in a cohort of 68 gastric cancer patients with peritoneal metastasis. We applied a cutoff value of 100 for the CmRI based on the findings of a previous study.<sup>13</sup> Among patients ( $n = 39$ ) who underwent conversion gastrectomy, we found that the OS of patients with a preoperative CmRI value  $<100$  was significantly better than that of patients with a preoperative CmRI value  $>100$ . Multivariate analysis identified preoperative serum CA19-9 levels and a CmRI value  $<100$  as independent predictors of a better OS in patients who underwent conversion gastrectomy. Furthermore, conversion gastrectomy significantly improved the OS of patients with CmRI values that had once reduced to  $<100$ . However, it did not improve the OS of patients with CmRI values that had never reduced to  $<100$ . These findings indicate that CmRI values more accurately reflect the response of peritoneal metastasis to induction chemotherapy than conventional cytological examinations and suggest that CmRI values during induction chemotherapy could predict the benefit of conversion gastrectomy.



Interestingly, initial CmRI values were not associated with the OS of conversion gastrectomy patients. Additionally, OS was not associated with the presence of malignant ascites, Peritoneal Cancer Index scores, or tumor marker CEA, CA19-9, and CA-125 levels at the time of the initial staging laparoscopy prior to induction chemotherapy. Conversely, OS was positively associated with preoperative serum CA19-9 and CA-125 levels, as well as, CmRI values after induction chemotherapy. These findings suggest that patient outcomes are not affected by the initial tumor burden of the peritoneal metastases but are largely dependent on the tumor response to induction chemotherapy.

In a previous study, we reported that the sensitivity of serum CEA levels for the detection of peritoneal metastasis in gastric cancer patients was only 18.6%, whereas that of serum CA-125 levels was 46.1%.<sup>17</sup> In the present study, we measured the CEA mRNA levels of epithelial cells in peritoneal lavage to detect carcinoma cells. According to the Spearman's rank correlation test, there was no correlation between preoperative serum CEA levels and preoperative CmRI values of peritoneal lavage in patients ( $n = 39$ ) who underwent conversion gastrectomy ( $\rho = 0.21$ ). There also was no correlation between these variables at the time of the initial staging laparoscopy in patients ( $n = 68$ ) who underwent induction chemotherapy ( $\rho = 0.17$ ).

One possible limitation of our study is the variability in CEA mRNA expression levels among gastric cancer cells. In fact, CmRI values at the time of the initial staging laparoscopy did not correlate with Peritoneal Cancer Index scores and CEA mRNA levels were not detected in four patients, despite positive peritoneal lavage cytology. Given that CmRI values did not exceed 100, tumor cells in the peritoneal cavity were thought to express insufficient quantities of CEA mRNA. Previous studies have identified peritoneal lavage cytokeratin 20, matrix metalloproteinase-7, cluster of differentiation 44 antigen, and insulin-like growth factor 2 mRNA-binding protein 3 mRNA levels as being clinically useful for the detection of peritoneal micrometastases.<sup>18-21</sup> Genetic detection of these components with the same methodology may be necessary for these patients.

In conclusion, our retrospective data suggest that the CmRI in peritoneal fluids is a reliable biomarker for predicting gastrectomy outcomes after induction chemotherapy in gastric cancer patients with peritoneal metastasis. Conversion gastrectomy should be recommended if peritoneal lavage CmRI values are reduced to  $<100$ .

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