

• RAPID COMMUNICATION •

Peritoneal lavage cytology and carcinoembryonic antigen determination in predicting peritoneal metastasis and prognosis of gastric cancer

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Abstract

AIM: To evaluate the role of peritoneal lavage cytology (PLC) and carcinoembryonic antigen (CEA) determination of peritoneal washes (pCEA) in predicting the peritoneal metastasis and prognosis after curative resection of gastric cancer.

METHODS: PLC and radioimmunoassay of CEA were performed in peritoneal washes from 64 patients with gastric cancer and 8 patients with benign diseases.

RESULTS: The positive rate of pCEA (40.6%) was significantly higher than that of PLC (23.4%) ($P < 0.05$). The positive rates of PLC and pCEA correlated with the depth of tumor invasion and lymph node metastasis ($P < 0.05$). pCEA was found to have a higher sensitivity and a lower false-positive rate in predicting peritoneal metastasis after curative resection of gastric cancer as compared to PLC. The 1-, 3-, and 5-year survival rates of patients with positive cytologic findings or positive pCEA results were significantly lower than those of patients with negative cytologic findings or negative pCEA results ($P < 0.05$). Multivariate analysis indicated that pCEA was an independent prognostic factor for the survival of patients with gastric cancer.

CONCLUSION: Intraoperative pCEA is a more sensitive and reliable predictor of peritoneal metastasis as well as prognosis in patients with gastric cancer as compared to PLC method.

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Key words: Stomach neoplasm; CEA protein; Peritoneal metastasis; Prognosis

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INTRODUCTION

Peritoneal metastasis is the most common mode of relapse of gastric cancer after surgery and is the most frequent cause of death in patients with gastric cancer^[1-3]. Peritoneal recurrence develops from micrometastasis originating from peritoneal free cancer cells. Therefore, it is very important to examine the presence or absence of free cancer cells in the peritoneal cavity at the time of surgery^[4,5]. PLC is the gold standard for assessing the presence of peritoneal dissemination of gastric cancer, but its sensitivity is relatively low, ranging 14-21% in gastric cancer involving the serosa^[6-8]. Recently, several new methods for detecting micrometastasis, including immunohistochemical and biological methods have been developed^[9-11]. However, these diagnostic techniques are time-consuming and laborious compared to conventional cytological method. Therefore, a new and more sensitive method for the early detection of peritoneal metastasis is required.

In this paper, we have reported the clinical significance of a new method to detect peritoneal micrometastasis in combination with cytological method and measurement of pCEA level in peritoneal washes.

MATERIALS AND METHODS

Patients

Between December 1995 and December 1997, 64 patients with histologically confirmed gastric cancer underwent surgery at the Department of General Surgery of First Affiliated People's Hospital of Shanghai Jiaotong University. All patients underwent either a total or a partial gastrectomy with lymph node dissection and received no preoperative chemotherapy or radiotherapy before the surgery. These 64 cases included 42 male and 22 female patients with an average age of 59 (range 34-84 years) years. All specimens were histologically examined by HE staining according to the general rules of the Japanese Classification of Gastric Carcinoma^[12]. Seven patients with

the maximum depth of tumor invasion at mucosal or submucosal level, were diagnosed as early gastric cancer. The other 57 cases were diagnosed as advanced gastric cancer with invasion deep into the gastric wall. In this study, eight patients with benign diseases such as peptic ulcer or cholecystolithiasis served as controls. The follow-up period ranged 9-74 mo and the median follow-up period was 39 mo.

Examination of peritoneal washes

The study consisted of 64 patients with gastric cancer and 8 patients with benign disease. After laparotomy, 50 mL physiological saline was introduced into the right upper quadrant or the Douglas pouch immediately and then 20 mL fluid was collected. The fluid sample was immediately centrifuged for 5 min at 1 500 r/min. The sediment of each fluid sample was smeared on a glass slide. The slide was fixed in 99% alcohol, stained with HE and examined for the presence of cancer cells by an experienced pathologist. The supernatant of each sample was concentrated by ultrafiltration. CEA levels of the concentrated supernatant (pCEA) were measured with a radiometric immunoassay kit (Delfia CEA kit, Wallac Oy, Turku, Finland) and expressed as ng/g of protein. The cut-off level was set according to Takayuki method (pCEA level ≥ 100 ng/g of protein was defined as positive)^[13].

Statistical analysis

All the statistical analyses were done with SPSS statistical software. The χ^2 test was used to compare the positive results in CEA level and cytological examination. The survival rate was calculated by the Kaplan-Meier method and statistical difference was evaluated by the long-rank test. A Cox proportional hazard model was established to identify the independent prognostic factors.

RESULTS

Correlation between PLC or pCEA and clinicopathologic parameters

All the eight patients with benign diseases had a negative PLC finding or a pCEA level below the positive standard. Fifteen of sixty-four patients with gastric cancer had a positive PLC finding and an elevated pCEA level. Among the 49 patients with negative PLC findings, 11 had an elevated pCEA level. The overall pCEA positive rate in 64 patients with gastric cancer was 40.6% (26/64), which was significantly higher than that of PLC [23.4% (15/64), $P < 0.05$]. We analyzed the correlation between PLC or pCEA positive rate and various clinicopathologic factors. The PLC and pCEA positive rates were significantly associated with the depth of tumor invasion and lymph

node involvement. The patients with serosal invasion had a significantly higher PLC or pCEA positive rate than those without serosal invasion ($P < 0.01$). The pCEA positive rate in patients with lymph node involvement was significantly higher than that in patients without lymph node involvement ($P < 0.05$). Though the PLC positive rate in patients with lymph node involvement was also higher than that in patients without lymph node involvement, the difference did not reach statistical significance ($P > 0.05$, Table 1).

Predicting values of PLC and pCEA for postoperative peritoneal recurrence of gastric cancer

Thirty-seven of sixty-four patients with gastric cancer had various postoperative recurrences (57.8%). Among the 37 patients with recurrence, 19 had peritoneal recurrence within 2 years after the surgery. The peritoneal recurrence rate was 29.7%. The accuracy, sensitivity, specificity, positive and negative predictive values, false negative and positive rates of PLC or pCEA in predicting peritoneal recurrence are shown in Table 2.

Correlation between PLC or pCEA and prognosis

The 1-, 3-, and 5-year survival rates were 53.3%, 13.3%, and 0.0%, respectively in patients with positive PLC findings, which were significantly lower than 87.8%, 71.4%, and 55.1% in those with negative PLC findings ($P < 0.05$). Similarly, a significant difference in survival rates between pCEA-positive group and pCEA-negative group was observed. The 1-, 3-, and 5-year survival rates were 46.2%, 23.1%, and 15.4% in pCEA positive group and 89.5%, 73.7%, and 60.5% in pCEA negative group ($P < 0.05$). Multivariate Cox survival analysis showed that PLC was not an independent prognostic factor, but pCEA was an independent prognostic factor with a relative risk rate of 9.046 ($P = 0.0092$).

Table 1 Correlation between the results of peritoneal washes and clinicopathological factors

Clinicopathological factors	N	PLC(+)		P	pCEA(+)		P
		n	%		n	%	
Tumor size							
≤4 cm	28	4	14.3	>0.05	9	32.1	>0.05
>4 cm	36	11	30.6		17	47.2	
Histological type							
Differentiated	30	5	16.7	>0.05	10	33.3	>0.05
Undifferentiated	34	10	29.4		16	47.1	
Serosal invasion							
Negative	27	2	7.4	<0.01	5	18.5	<0.01
Positive	37	13	35.1		21	56.8	
Lymph node involvement							
Negative	16	1	6.3	>0.05	3	18.8	<0.05
Positive	48	14	29.2		23	47.9	

Table 2 Results of PLC and pCEA in predicting peritoneal recurrence of gastric cancer after surgery (%)

	Accuracy	Sensitivity	Specificity	Positive predictive value	Negative predictive value	False negative rate	False positive rate
PLC	90.6	73.7	97.8	93.3	89.8	26.3	2.2
pCEA	85.9	94.7	82.2	69.2	97.4	5.3	17.8

DISCUSSION

Though radical surgery is routinely practised, tumor recurrence is frequent in patients with gastric cancer. Peritoneal recurrence of gastric cancer is one of the most common patterns of recurrence and predicts a very poor prognosis for patients with gastric cancer^[2,3,14,15]. Peritoneal dissemination was also prevalent in our current prospective study, which was observed in 19 of 64 patients (29.7%) following a potentially curative surgery. Peritoneal recurrence develops from peritoneal free cancer cells originating from primary lesion or metastatic lymph nodes^[6,14,15]. However, it is difficult to identify these free cancer cells in the peritoneal cavity before or during the surgery^[16]. In order to prevent postoperative peritoneal recurrence and increase the survival rate, it is important to find a more sensitive, accurate, and convenient method to detect the presence of free cancer cells in the peritoneal cavity and to eliminate these cells with effective measures such as adjuvant intra-peritoneal chemotherapy^[17,18].

PLC is the gold standard for assessing the presence of free gastric cancer cells in the peritoneal cavity^[6-8,19]. Cytology-positive patients are classified as stage IV of Union International Contrele Cancer (UICC) gastric cancer classification and curative surgery is impossible in such cases. The present study revealed that the PLC positive rate in patients with serosal invasion (35.1%) was significantly higher than that in patients without serosal invasion (7.4%), indicating that the PLC positive rate increases with the invasion of gastric serosa and the chance of peritoneal dissemination increases in case, the gastric serosa is infiltrated. However, 14 of 19 patients with postoperative peritoneal recurrence had a positive PLC finding and the other five cases were PLC negative. PLC showed an accuracy of 90.6%, a sensitivity of 73.7%, and a specificity of 97.8% in predicting peritoneal recurrence of gastric cancer. The positive and negative predicting value, the false negative and positive rates of PLC were 93.3%, 89.8%, 26.3%, and 2.2%, respectively, suggesting that PLC is a very useful method for predicting peritoneal recurrence of gastric cancer with a high accuracy and a high specificity. However, PLC lacks sensitivity and has a relatively high false negative rate. Some patients with negative PLC results may have recurrence in the form of peritoneal dissemination.

CEA is generally accepted as a specific marker of gastrointestinal tumor^[20,21]. Recently, measurement of CEA level and CEA RT-PCR assay in peritoneal washes have been used to detect the existence of free cancer cells in the peritoneal cavity^[10,22,23]. It was reported that RT-PCR based assay has a relatively high sensitivity but is time-consuming, relatively laborious and less practical^[10,22]. In the present study, pCEA levels in peritoneal washes were measured in order to detect the presence of free cancer cells in 64 patients with gastric cancer. The results showed that all the 15 patients with PLC positive findings were pCEA positive. However, 11 of 49 patients with PLC negative findings were also pCEA positive. The total pCEA positive rate was 40.6%, which was significantly

correlated with the presence of serosal invasion and lymph node metastasis. Thus, as a useful method for detecting the presence of free cancer cells in the peritoneal cavity and for clinical staging of gastric cancer, pCEA level determination is considered to be superior to PLC. In our study, 18 of 19 patients with postoperative peritoneal recurrence of gastric cancer were pCEA positive and only one patient who died of postoperative peritoneal recurrence was pCEA negative, suggesting that pCEA assay is a highly sensitive method to predict postoperative peritoneal recurrence with a relatively high accuracy and specificity as compared to conventional PLC method.

In addition, our results showed that the positive rates of PLC and pCEA assay were significantly correlated with survival. Patients with negative results in PLC or pCEA assay survived significantly longer than those with positive findings in PLC or pCEA assay. In a multivariate analysis, pCEA level was found to be an independent prognostic factor even when all the clinicopathological variables were considered.

In conclusion, pCEA is a potential predictor of peritoneal recurrence as well as poor prognosis in patients with gastric cancer. Intra-operative pCEA assay can be considered as a reliable method for patients with gastric cancer.

REFERENCES

- 1 **Boku T**, Nakane Y, Minoura T, Takada H, Yamamura M, Hioki K, Yamamoto M. Prognostic significance of serosal invasion and free intraperitoneal cancer cells in gastric cancer. *Br J Surg* 1990; **77**: 436-439
- 2 **Nakamura K**, Ueyama T, Yao T, Xuan ZX, Ambe K, Adachi Y, Yakeishi Y, Matsukuma A, Enjoji M. Pathology and prognosis of gastric carcinoma. Findings in 10,000 patients who underwent primary gastrectomy. *Cancer* 1992; **70**: 1030-1037
- 3 **Kodera Y**, Yamamura Y, Torii A, Uesaka K, Hirai T, Yasui K, Morimoto T, Kato T, Kito T. Postoperative staging of gastric carcinoma. A comparison between the UICC stage classification and the 12th edition of the Japanese General Rules for Gastric Cancer Study. *Scand J Gastroenterol* 1996; **31**: 476-480
- 4 **Bando E**, Yonemura Y, Takeshita Y, Taniguchi K, Yasui T, Yoshimitsu Y, Fushida S, Fujimura T, Nishimura G, Miwa K. Intraoperative lavage for cytological examination in 1,297 patients with gastric carcinoma. *Am J Surg* 1999; **178**: 256-262
- 5 **Kodera Y**, Yamamura Y, Shimizu Y, Torii A, Hirai T, Yasui K, Morimoto T, Kato T. Peritoneal washing cytology: prognostic value of positive findings in patients with gastric carcinoma undergoing a potentially curative resection. *J Surg Oncol* 1999; **72**: 60-64; discussion 64-65
- 6 **Juhl H**, Stritzel M, Wroblewski A, Henne-Bruns D, Kremer B, Schmiegell W, Neumaier M, Wagener C, Schreiber HW, Kalthoff H. Immunocytological detection of micrometastatic cells: comparative evaluation of findings in the peritoneal cavity and the bone marrow of gastric, colorectal and pancreatic cancer patients. *Int J Cancer* 1994; **57**: 330-335
- 7 **Wu CC**, Chen JT, Chang MC, Ho WL, Chen CY, Yeh DC, Liu TJ, P'eng FK. Optimal surgical strategy for potentially curable serosa-involved gastric carcinoma with intraperitoneal free cancer cells. *J Am Coll Surg* 1997; **184**: 611-617
- 8 **Benevolo M**, Mottolese M, Cosimelli M, Tedesco M, Giannarelli D, Vasselli S, Carlini M, Garofalo A, Natali PG. Diagnostic and prognostic value of peritoneal immunocytology in gastric

- cancer. *J Clin Oncol* 1998; **16**: 3406-3411
- 9 **Nekarda H**, Gess C, Stark M, Mueller JD, Fink U, Schenck U, Siewert JR. Immunocytochemically detected free peritoneal tumour cells (FPTC) are a strong prognostic factor in gastric carcinoma. *Br J Cancer* 1999; **79**: 611-619
 - 10 **Kodera Y**, Nakanishi H, Ito S, Yamamura Y, Kanemitsu Y, Shimizu Y, Hirai T, Yasui K, Kato T, Tatematsu M. Quantitative detection of disseminated free cancer cells in peritoneal washes with real-time reverse transcriptase-polymerase chain reaction: a sensitive predictor of outcome for patients with gastric carcinoma. *Ann Surg* 2002; **235**: 499-506
 - 11 **Sakakura C**, Takemura M, Hagiwara A, Shimomura K, Miyagawa K, Nakashima S, Yoshikawa T, Takagi T, Kin S, Nakase Y, Fujiyama J, Hayasizaki Y, Okazaki Y, Yamagishi H. Overexpression of dopa decarboxylase in peritoneal dissemination of gastric cancer and its potential as a novel marker for the detection of peritoneal micrometastases with real-time RT-PCR. *Br J Cancer* 2004; **90**: 665-671
 - 12 Japanese Classification of Gastric Carcinoma - 2nd English Edition - *Gastric Cancer* 1998; **1**: 10-24
 - 13 **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
 - 14 **Yoo CH**, Noh SH, Shin DW, Choi SH, Min JS. Recurrence following curative resection for gastric carcinoma. *Br J Surg* 2000; **87**: 236-242
 - 15 **Sakakura C**, Hagiwara A, Nakanishi M, Shimomura K, Takagi T, Yasuoka R, Fujita Y, Abe T, Ichikawa Y, Takahashi S, Ishikawa T, Nishizuka I, Morita T, Shimada H, Okazaki Y, Hayashizaki Y, Yamagishi H. Differential gene expression profiles of gastric cancer cells established from primary tumour and malignant ascites. *Br J Cancer* 2002; **87**: 1153-1161
 - 16 **Fujimura T**, Kinami S, Ninomiya I, Kitagawa H, Fushida S, Nishimura G, Kayahara M, Shimizu K, Ohta T, Miwa K. Diagnostic laparoscopy, serum CA125, and peritoneal metastasis in gastric cancer. *Endoscopy* 2002; **34**: 569-574
 - 17 **Fujimoto S**, Takahashi M, Mutou T, Kobayashi K, Toyosawa T. Successful intraperitoneal hyperthermic chemoperfusion for the prevention of postoperative peritoneal recurrence in patients with advanced gastric carcinoma. *Cancer* 1999; **85**: 529-534
 - 18 **Ceelen WP**, Hesse U, de Hemptinne B, Pattyn P. Hyperthermic intraperitoneal chemoperfusion in the treatment of locally advanced intra-abdominal cancer. *Br J Surg* 2000; **87**: 1006-1015
 - 19 **Hayes N**, Wayman J, Wadehra V, Scott DJ, Raimes SA, Griffin SM. Peritoneal cytology in the surgical evaluation of gastric carcinoma. *Br J Cancer* 1999; **79**: 520-524
 - 20 **Holyoke ED**, Chu TM, Murphy GP. CEA as a monitor of gastrointestinal malignancy. *Cancer* 1975; **35**: 830-836
 - 21 **Korenaga D**, Funahashi S, Yano K, Maekawa S, Ikeda T, Sugimachi K. Relationship between peritoneal collagen type IV concentrations and the presence of disseminated metastases in gastric cancer. *Arch Surg* 1995; **130**: 769-773
 - 22 **Abe N**, Watanabe T, Toda H, Machida H, Suzuki K, Masaki T, Mori T, Sugiyama M, Atomi Y, Nakaya Y. Prognostic significance of carcinoembryonic antigen levels in peritoneal washes in patients with gastric cancer. *Am J Surg* 2001; **181**: 356-361
 - 23 **To EM**, Chan WY, Chow C, Ng EK, Chung SC. Gastric cancer cell detection in peritoneal washing: cytology versus RT-PCR for CEA transcripts. *Diagn Mol Pathol* 2003; **12**: 88-95