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Original article

Prognostic significance of intraperitoneal cancer cells in gastric carcinoma: detection of cytokeratin 20 mRNA in peritoneal washes, in addition to detection of carcinoembryonic antigen

Yasuhiro Kodera[1], Hayao Nakanishi[2], Seiji Ito[3], Yoshitaka Yamamura[3], Michitaka Fujiwara[1], Masahiko Koike[1], Kenji Hibi[1], Katsuki Ito[1], Masae Tatematsu[2], and Akimasa Nakao[1]

[1] Second Department of Surgery, Nagoya University Graduate School of Medicine, 65, Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[2] Laboratory of Pathology, Aichi Cancer Center Research Institute, Nagoya, Aichi, Japan
[3] Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya, Aichi, Japan

Abstract

Background. **In patients with gastric cancer, the presence of gastric cancer cells in the peritoneal cavity detected by cytologic examination, is a significant prognostic factor. A more sensitive, reverse transcriptase-polymerase chain reaction (RT-PCR) technique, amplifying carcinoembryonic antigen (CEA), was introduced as a new detection system, but produced some false-positive results. A search for other molecular markers is ongoing.**

Methods. **Peritoneal washes were obtained from 195 patients with gastric carcinoma during surgery. Cytokeratin 20 (CK20) mRNA levels were quantified, in addition to those of CEA, using the LightCycler, and the feasibility of CK20 as a target was evaluated.**

Results. **CK20 was limited, in terms of sensitivity, for detecting disseminated cancer cells (sensitivity, 63%; specificity, 91%; positive predictive value, 70%; and negative predictive value, 88%). Multimarker analysis was performed, in which samples positive for either CK20 or CEA mRNA were considered to be positive for cancer cells. Multivariate analysis identified the multimarker analysis as a significant independent prognostic determinant.**

Conclusion. **CK20 RT-PCR produced information that could add a significant impact to the knowledge obtained by CEA RT-PCR, although detection by CK20 alone was not sufficiently sensitive to replace CEA in the detection system.**

Key words Peritoneal carcinomatosis · Cytology · Micrometastasis · RT-PCR

Introduction

Peritoneal carcinomatosis represents a common pattern of failure following curative surgery for gastric carcinoma [1,2]. Recurrence with this pattern is most likely caused by the presence in the abdominal cavity of free cancer cells exfoliated from the serosal surfaces of the primary cancers [3], and these cells have been detected by the cytologic examination of peritoneal washes [4–8]. For this detection, however, microscopic examination following conventional Papanicolaou staining lacked sensitivity [9], and immunohistochemistry [10,11] and reverse-transcriptase polymerase chain reaction (RT-PCR) techniques have been introduced to improve the detection rates. The authors have reported on RT-PCR, using the LightCycler instrument (Roche Diagnostics, Mannheim, Germany), with carcinoembryonic antigen (CEA) as a target [12]; sensitivity (positive rate for CEA mRNA among patients who had peritoneal deposits at surgery, or a relapse, in the form of peritoneal carcinomatosis, during the follow up) of 80% was achieved, while that achieved by conventional Papanicolaou staining was 56% [13]. Meanwhile, several studies exploring other targets have been reported [14,15]. Nevertheless, false-negative results and, more frequently, false-positive results, were occasionally observed, and studies in search of a target mRNA with improved specificity and without loss of sensitivity are warranted.

In carcinoma of the gastrointestinal tract, cytokeratin 20 (CK20) has been suggested as a promising marker, due to its restricted expression pattern [16] and the lack of pseudogenes [17]. It was quantitated in a study detecting gastric carcinoma micrometastasis to the lymph nodes [18], and was positive in one patient with nodal metastasis that was detected by routine histopathologic examination but missed by CEA RT-PCR. It was one of five genes that were recently selected, first by a gene-screening strategy using microarray, and then by confirmation through RT-PCR analysis of the representative peritoneal washes [19], as potential markers for specifically detecting cancer cells in peritoneal washing samples. In the present study, the sensitivity and specificity of CK20 mRNA for detecting free cancer cells

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from peritoneal washes was tested, and compared with results obtained by a CEA RT-PCR.

Patients and methods

Patients

Between June 1995 and December 1999, peritoneal washing samples were collected from 230 patients who underwent surgery for histologically proven gastric carcinoma at Aichi Cancer Center Hospital. Some of these samples were lost or used in previous studies, while some patients were lost to follow up; thus, samples from 195 patients were available for the present analyses. All 195 patients were followed up for a median of 1843 days (range, 1011–2575 days) or until death. The depth of cancer invasion (pT category) was evaluated histologically according to the tumor-node-metastasis Classification [20]. There were 76 patients with pT1 cancer (cancer confined to the mucosa or invading as far as the submucosa), 41 patients with pT2 cancer (invasion beyond the submucosa but not as far as the serosa), 61 with pT3 cancer (serosal invasion), and 17 with pT4 cancer (invasion to adjacent tissues). The population included 26 patients with synchronous peritoneal metastasis. The study was approved by the institutional review board in 1995, and informed consent was obtained from all patients prior to collection of the samples.

Surgical procedure

After laparotomy, the abdominal cavity was thoroughly examined for tumor metastasis, and peritoneal deposits in particular. Samples of the latter were taken whenever they were observed and the diagnosis of cancer metastasis was histologically confirmed with frozen sections before the abdomen was closed. When potentially curative R0 resection [20] was planned, gastrectomy with D2 lymphadenectomy was the treatment of choice. Palliative resection was performed and chemotherapy given at the discretion of the surgeons for patients who were not treated with R0 resection. Gastrectomy was avoided for those with extensive invasion to the retroperitoneum and for those with extensive peritoneal dissemination.

Postoperative surveillance of patients

The follow-up program consisted of interim history, physical examination, hematology, and blood-chemistry panels, including tests for CEA and carbohydrate antigen (CA)19-9, performed every 3 months for the first postoperative year, and every 6 months thereafter. Either abdominal ultrasonography or computerized tomography was carried out every 6 months. Peritoneal recurrence, evident on the basis of clinical symptoms, digital examination, or physical and radiological findings of bowel obstruction and ascites, was confirmed by paracenthesis or laparotomy (performed at the discretion of the surgeon). Autopsy was performed at the discretion of the surgeon.

Peritoneal washes

At the beginning of each operation, 100 ml of saline was introduced into the Douglas and left subphrenic cavities and aspirated. After gentle stirring, the wash sample was centrifuged at 1800 rpm for 5 min to collect intact cells, rinsed with phosphate-bufferred saline (PBS), dissolved in Isogen RNA extraction buffer (Nippon Gene, Tokyo, Japan) and stored at –80°C until use. A portion of each peritoneal washing sample was examined cytopathologically, using conventional Papanicolaou and Giemsa staining.

Real-time RT-PCR

Real-time RT-PCR detection of CEA mRNA in the peritoneal washing samples was performed as described elsewhere [12]. In brief, total RNA was extracted, using a guanidinium isothiocyanate-phenol-chloroform method. Extracted total RNA was converted to first-strand cDNA and was immediately used for PCR amplification, done with a LightCycler (Roche Diagnostics). Real-time RT-PCR was performed by a single-step method (50 cycles), using hybridization probes. The primers for CEA, CK20, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed as described previously [18]. Both CEA and CK20 mRNA levels were normalized by the GAPDH mRNA level, and the CEA/GAPDH ratio and CK20/GAPDH ratio were calculated (CEA or CK20 mRNA level divided by GAPDH mRNA $\times 10^7$). A cutoff value of 30 for the CEA/GAPDH ratio had been established in our previous study [13], with reference to the receiver-operating characteristic curve. A multimarker analysis was performed in order to evaluate whether the combination of the CEA and CK20 RT-PCR would increase the accuracy of the diagnosis. In this analysis, a sample that was positive for either or both of the markers was determined to be positive for the RT-PCR.

Statistical analysis

Receiver-operating characteristic (ROC) curves were used to compare the accuracies of the CEA/GAPDH ratio and the CK20/GAPDH ratio for distinguishing between the patients who were positive and negative for

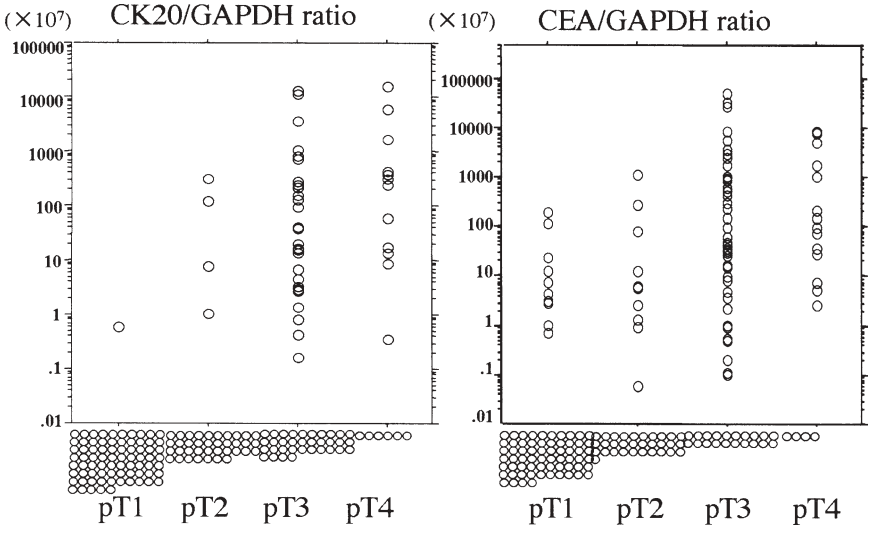

Fig. 1. Results of quantitative analysis for cytokeratin 20 (*CK20*)/glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) ratio (CK20 mRNA/GAPDH mRNA × 10^7) and carcinoembryonic antigen (*CEA*)/GAPDH ratio (CEA mRNA/GAPDH mRNA × 10^7), stratified according to the pathological T category. *Open circles* below the CEA or CK20/GAPDH ratios of 0.01 stand for samples in which CEA or CK20 mRNA was undetectable

intraperitoneal metastatic cancer cells. Being positive for intraperitoneal cancer cells in this analysis was arbitrarily defined as either having macroscopic dissemination at surgery or being diagnosed clinically as having peritoneal carcinomatosis during the 2 years of follow up [13]. ROC curves were constructed by plotting all possible sensitivity/specificity pairs for the two mRNA ratios, resulting from continuously varying the cutoff values over the entire range of results obtained. Sensitivity in this context was defined as the positive rate for CEA or CK20/GAPDH ratios in the peritoneal washes of patients who had synchronous peritoneal seeding or relapse, in the form of peritoneal carcinomatosis, within 2 years of postoperative surveillance. Specificity was the negative rate for the CEA or CK20/GAPDH ratios among the patients who were free of peritoneal metastasis at surgery, and without signs of peritoneal carcinomatosis thereafter. For both the CEA and CK20/GAPDH ratios, sensitivity (%) on the y axis was plotted against the false-positive fraction (100-specificity, as percentage) on the x axis, with various cutoff values ranging from 0 to several thousands. The sensitivity increases at the expense of specificity as the cutoff value is lowered, and a plot lying above and to the left of another plot indicates greater observed accuracy. Sensitivity/specificity pairs for conventional cytologic examination and the multimarker analysis were worked out and plotted for reference.

Survival analyses were made by Kaplan and Meier curves, with death as the endpoint. Independent prognostic factors were identified by multivariate analysis, using the Cox regression hazards model, through the analysis of results for 156 patients who had no concomitant peritoneal metastasis and were negative by the conventional cytologic examination. The prognostic significance of CEA RT-PCR, CK20 RT-PCR, and the multimarker analysis were evaluated separately in three independent multivariate analyses, with nodal metastasis and serosal invasion as other covariates.

Results

CK20 mRNA levels stratified according to the depth of tumor invasion

Results of quantitative analysis by the LightCycler for the CK20/GAPDH ratio (CK20 mRNA/GAPDH mRNA × 10^7), stratified according to the pT category, are summarized in Fig. 1. For individuals with samples from both the Douglas and left subphrenic cavities, the higher value was selected. It is clear, as in case of the CEA/GAPDH ratio, that the CK20/GAPDH ratios tended to take higher values as the pT stage progressed. The cutoff value for the CK20/GAPDH ratio was arbitrarily determined as any value above 0, so as to compensate for the lack of sensitivity compared with CEA, whereas a predetermined cutoff value of 30 was used for the CEA/GAPDH ratio [13]. Only five samples of peritoneal washes were available from patients with benign disease, and all these samples were negative for CEA and CK20 mRNA.

Accuracy of the CK20/GAPDH ratio for detecting intraperitoneal cancer cells

ROC curves for CEA/GAPDH and CK20/GAPDH are shown in Fig. 2. The maximum sensitivity of 63% was obtained for the CK20/GAPDH ratio by arbitrarily employing the smallest detectable value as a cutoff

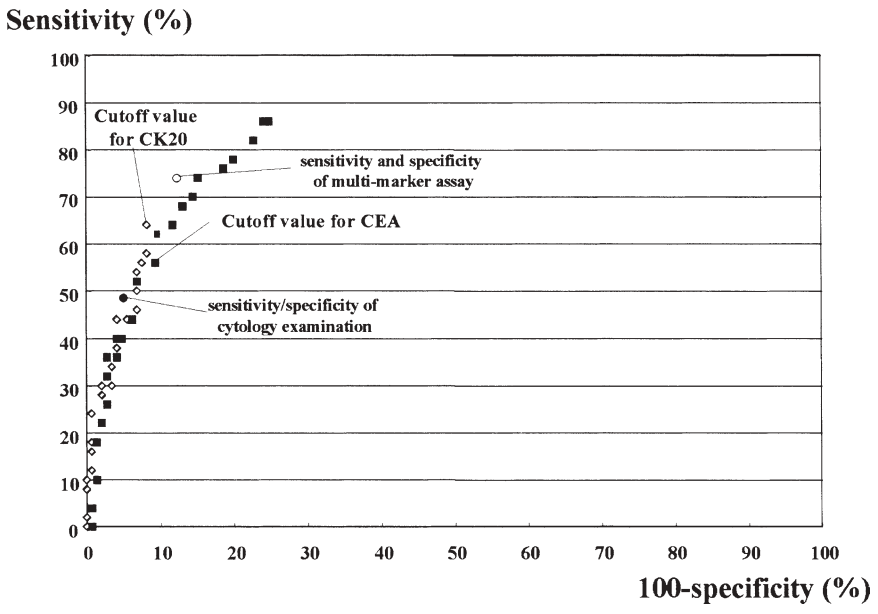

Fig. 2. Receiver-operation characteristic curves for the CK20/GAPDH ratio (*diamonds*) and CEA/GAPDH ratio (*closed squares*). The cutoff value for CEA was predetermined as 30, where sensitivity and specificity were 56% and 90%, respectively. The maximum sensitivity obtained by the CK20/GAPDH ratio was 63% (specificity, 91%). Sensitivity/specificity pairs for conventional cytology examination (*closed circle*) and combined diagnosis with the CEA/GAPDH and CK20/GAPDH ratios (multimarker assay; *open circle*) are also plotted

value (any value above 0). The specificity, positive predictive value, and negative predictive value at this point were 91%, 70%, and 88%, respectively. The sensitivity and specificity obtained by the CEA/GAPDH ratio at the predetermined cutoff value of 30[13] were 56% and 90%, respectively, but the sensitivity was improved substantially (up to 86%) by lowering the cutoff value. The sensitivity/specificity pairs obtained by the conventional cytologic examination (49% and 95%) and by the multimarker assay using data from CEA and CK20 RT-PCR (73% and 86%) are also plotted in Fig. 2. The sensitivity/specificity pair of the multimarker assay was found to have deviated above and to the left of the ROC curve for CEA and CK20, indicating that the multimarker assay had greater accuracy.

Results of cytology and real time RT-PCR and their relation to recurrence and survival

The 195 patients with gastric carcinoma were stratified into the following four groups, according to the results of cytology and CK20 RT-PCR: a group of 18 patients who were positive for both cytology and CK20/GAPDH (CY+ CK20+), a group of 13 patients who were positive for cytology but negative for CK20/GAPDH ratio (CY+ CK20–), a group of 26 patients who were positive for PCR but negative for cytology (CY– CK20+), and a group of 138 patients who were negative for both cytology and PCR (CY– CK20–). Survival curves for these four groups demonstrated that the prognosis of the CY– CK20+ group approached that of the CY+ groups, indicating that knowledge of the peritoneal fluid CK20 mRNA level has a place as a prognostic indicator, even after the result of cytology examination has been obtained (Fig. 3). An identical survival analysis was performed with CY+ CEA+ ($n = 19$), CY+ CEA– ($n = 12$), CY– CEA+ ($n = 23$), and CY– CEA– ($n = 141$) groups, and the result was similar to that obtained previously (Fig. 3). CK20 mRNA was detected from the samples of 11 patients from the CY– CEA– group, of whom 2 patients had concomitant peritoneal deposits at surgery and 3 had recurrences as peritoneal carcinomatosis.

Survival curves showing the result of the multimarker analysis are shown in Fig. 4. In this analysis, being positive for either CEA or CK20 mRNA was regarded as being positive for free cancer cells in the abdominal cavity. Among the patients who were negative for CK20 mRNA, 8 patients were positive for the multimarker analysis and, among those negative for CEA, 11 patients were positive for the multimarker analysis.

Value of cytology and RT-PCR as independent prognostic factors

Both the CK20/GAPDH ratio and the CEA/GAPDH ratio were highly significant as prognostic factors by univariate analysis. In a multivariate analysis involving 156 patients who had no concomitant peritoneal deposits and were negative for the conventional cytology examination, the prognostic significance of CEA RT-PCR, CK20 RT-PCR, and the multimarker analysis was evaluated separately in three independent multivariate analyses, with nodal metastasis and serosal invasion as other covariates. The multimarker analysis was the only PCR-related covariate that was identified as a significant independent prognostic factor (Table 1).

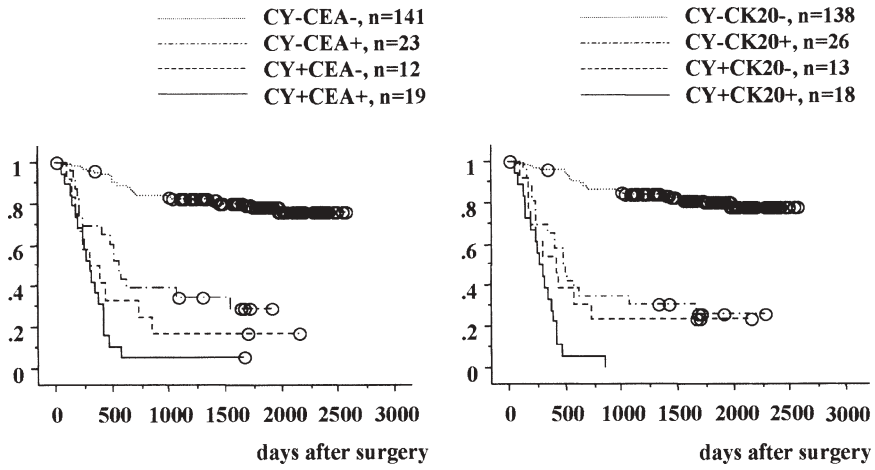

Fig. 3. Survival curves of all 195 patients stratified according to the results of conventional cytologic examination and reverse transcriptase-polymerase chain reaction (RT-PCR) for CEA and CK20

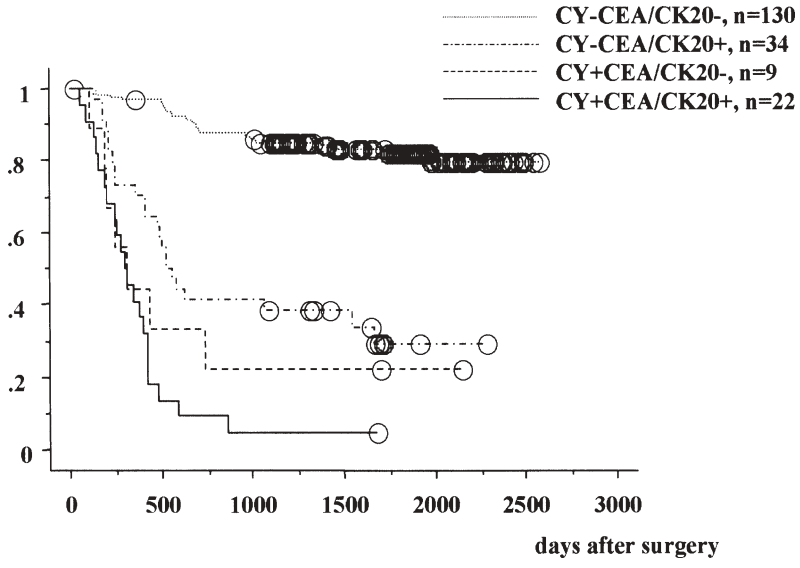

Fig. 4. Survival curves of all 195 patients stratified according to the results of the conventional cytologic examination and the multimarker assay, in which being positive for either CEA or CK20 was considered as being positive for free cancer cells in the abdominal cavity

Discussion

In countries where an extended lymphadenectomy is a standard procedure, the most common type of gastric cancer recurrence is peritoneal carcinomatosis [1,2], local recurrences being relatively uncommon. In the United States, adjuvant chemoradiation as a new standard of care [21] could also reduce the incidence of local recurrences. Thus, the detection of intraperitoneal cancer cells by the cytologic examination of peritoneal washes may become increasingly important as a prognostic factor worldwide. In general, CEA mRNA expression is strong in cancer tissues of gastric carcinoma of the differentiated (intestinal) type, but relatively weak or undetectable in poorly differentiated gastric carcinoma [22]. The use of other, or multiple, markers could improve sensitivity, and CK20 had been selected in a previous study for the detection of nodal micrometastases [18]. More recently, an extensive gene-screening strategy, using microarrays, identified CK20 as one of five potential mRNAs that could be used as markers of free cancer cells in peritoneal washing samples [19]. These findings prompted us to explore the potential of CK20 RT-PCR. A multimarker assay, employing both CEA and CK20 RT-PCR, has already been reported as an option for the prediction of peritoneal recurrence [23]. In that report, however, the predictive value of CK20 alone had not been presented.

One weakness of the present study concerns the determination of the cutoff value. Limited access to peritoneal washes from patients with benign disease at a cancer center hospital precluded us from testing a large number of samples to serve as controls. In the present study, therefore, only five samples collected at surgery for benign disease were tested, and neither CEA nor

Table 1. Results of multivariate analyses of data for 156 gastric carcinoma patients without concomitant peritoneal metastasis

Covariate	Hazard ratio	95% Confidence interval	*P* value
Lymph node metastasis			
Negative	1		
Positive	4.88	2.01–11.8	0.0005
Serosal invasion			
Negative	1		
Positive	2.08	1.00–4.34	0.0506
CEA mRNA			
Negative	1		
Positive	2.06	0.92–4.62	0.0807
Lymph node metastasis			
Negative	1		
Positive	4.61	1.90–11.2	0.0008
CK20 mRNA			
Negative	1		
Positive	2.11	0.97–4.57	0.0588
Serosal invasion			
Negative	1		
Positive	2.02	0.96–4.57	0.0627
Lymph node metastasis			
Negative	1		
Positive	4.61	1.89–11.2	0.0008
Multimarker analysis			
Negative	1		
Positive	2.09	1.01–4.33	0.0477
Serosal invasion			
Negative	1		
Positive	1.97	0.94–4.14	0.0736

CK20 was detectable in any of the samples. Sugita et al. [23] examined 25 peritoneal lavage samples from patients with benign disease and found that only 1 sample contained a detectable amount of CK20, while CEA was not detectable in any of the samples. On the other hand, Oyama et al. [24] detected CEA mRNA in 10 of 20 peritoneal lavage samples that they collected. They subsequently determined their cutoff level as the mean plus two SDs of these 20 samples. Although the determination of the cutoff value should preferably be performed by measuring a large number of control samples, investigators should be aware that, unlike peripheral blood, peritoneal washes cannot be obtained from healthy volunteers. Patients who undergo surgery for benign disease often suffer from inflammatory disease, and the profile of the mRNA in the peritoneal washes from these patients may not necessarily represent that of healthy controls.

The problem consistently encountered when employing an RT-PCR technique concerns specificity. After numerous reports describing the successful application of this technique for the detection of micrometastases, a criticism arose in the literature, pointing to unexpectedly high rates of false-positives with CK20 [25]. The false-positive results were attributed to the illegitimate expression of target mRNA in granulocytes. This becomes a considerable problem when the samples to be evaluated are of peripheral blood or bone-marrow aspirates, but perhaps there is less of a problem when we are dealing with peritoneal washes. In the present study, the specificity achieved by CK20 RT-PCR was 91%, and that of CEA RT-PCR was 90%. These values approached that for the conventional cytology examination (95%). Rather disappointingly, however, the maximal sensitivity obtained by CK20 RT-PCR was no more than 64%, even when the lowest possible cutoff value was employed. One way of utilizing the data would be to combine the data with data obtained from CEA RT-PCR, as recommended by Sugita et al. [23], so that a sample would be diagnosed as positive when either of the two mRNAs exceeded the cutoff value. The sensitivity of the multimarker analysis reached 73%, although the specificity declined, to 86%, and multivariate analysis eventually identified the multimarker analysis as the only significant independent prognostic factor among the various data obtained with the RT-PCR technique.

Finally, the present method of detection (i.e., real-time RT-PCR) remains time-consuming, in that it takes at least 2 h from the collection of samples to obtain

results. The result could still be used to decide whether or not to place an indwelling catheter for intraperitoneal chemotherapy following debulking surgery or whether to add more aggressive options, such as intraperitoneal chemohyperthermia [26]. This technique can certainly be used to select candidates for postoperative adjuvant chemotherapy. On the other hand, this technique can, no doubt, be applied to evaluate samples obtained at staging laparoscopy [27].

To summarize, the result of diagnosis shown by multimarker analysis was marginally more significant as a prognostic determinant compared with the RT-PCR-mediated detection of CEA or CK20 alone. CK20 RT-PCR demonstrated a relatively high specificity, but limited sensitivity, which may hinder its use alone for the detection of disseminated cancer cells in the abdominal cavity.

References

1. Yoo CH, Noh SH, Shin DW, Choi SH, Min JS. Recurrence following curative resection for gastric carcinoma. Br J Surg 2000;87:236–42.
2. Maehara Y, Hasuda S, Koga T, Tokunaga E, Kakeji Y, Sugimachi K. Postoperative outcome and sites of recurrence in patients following curative resection of gastric cancer. Br J Surg 2000;87: 353–7.
3. Koga S, Kaibara N, Iitsuka Y, Kudo H, Kimura A, Hiraoka H. Prognostic significance of intraperitoneal free cancer cells in gastric cancer patients. J Cancer Res Clin Oncol 1984;108:236–8.
4. Boku T, Nakane Y, Minoura T, Takeda H, Yamamura M, Hioki K, et al. Prognostic significance of serosal invasion and free intraperitoneal cancer cells in gastric cancer. Br J Surg 1990;77:436–9.
5. Bonenkamp JJ, Songun I, Hermans J, van de Velde CJ. Prognostic value of positive cytology findings from abdominal washings in patients with gastric cancer. Br J Surg 1996;83:672–4.
6. Kodera Y, Yamamura Y, Shimizu Y, Torii A, Hirai T, Yasui K, et al. Peritoneal washing cytology: prognostic value of positive findings in patients with gastric carcinoma undergoing a potentially curative resection. J Surg Oncol 1999;72:60–5.
7. 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;79:520–4.
8. Bando E, Yonemura Y, Takeshita Y, Taniguchi K, Yasui T, Yoshimitsu Y, et al. Intraoperative lavage for cytological examination in 1297 patients with gastric carcinoma. Am J Surg 1999; 178:256–62.
9. Abe S, Yoshimura H, Tabata H, Tachibana M, Monden N, Nakamura T, et al. Curative resection of gastric cancer: limitation of peritoneal lavage cytology in predicting the outcome. J Surg Oncol 1995;59:226–9.
10. Benevolo M, Mottolese M, Cosimelli M, Tedesco M, Giannarelli D, Vasselli S, et al. Diagnostic and prognostic value of peritoneal immunocytology in gastric cancer. J Clin Oncol 1998;16:3406–11.
11. Juhl H, Stritzel M, Wroblewski A, Henne-Bruns D, Kremer B, Schmiegel W, et al. 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;57:330–5.
12. Nakanishi H, Kodera Y, Yamamura Y, Ito S, Kato T, Ezaki T, et al. Rapid quantitative detection of carcinoembryonic antigen-expressing free tumor cells in the peritoneal cavity of gastric-cancer patients with real-time RT-PCR on the LightCycler. Int J Cancer 2000;89:411–7.
13. Kodera Y, Nakanishi H, Ito S, Yamamura Y, Kanemitsu Y, Shimizu Y, et al. 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.
14. Fujimura T, Ohta T, Kitagawa H, Fushida S, Nishimura DI, Yonemura Y, et al. Trypsinogen expression and early detection for peritoneal dissemination in gastric cancer. J Surg Oncol 1998; 69:71–5.
15. Yonemura Y, Fujimura T, Nimomiya U, Kim BS, Bandou E, Sawa T, et al. Prediction of peritoneal micrometastasis by peritoneal lavaged cytology and reverse transcriptase-polymerase chain reaction for matrix metalloproteinase-7 mRNA. Clin Cancer Res 2001;7:1647–53.
16. Moll R, Zimbelmann R, Goldschmidt MD, Keith M, Laufer J, Kasper M, et al. The human gene encoding cytokeratin 20 and its expression during fetal development and in gastrointestinal carcinomas. Differentiation 1993;53:75–93.
17. Burchill SA, Bradbury MF, Pittman L, Southgate J, Smith B, Selby P. Detection of epithelial cancer cells in peripheral blood by reverse transcriptase-polymerase chain reaction. Br J Cancer 1995;71:278–81.
18. Kubota K, Nakanishi H, Hiki N, Shimizu N, Tsuji E, Yamaguchi H, et al. Quantitative detection of micrometastases in the lymph nodes of gastric cancer patients with real-time RT-PCR: a comparative study with immunohistochemistry. Int J Cancer 2003;105: 136–43.
19. Mori K, Aoyagi K, Ueda T, Danjoh I, Tsubosa Y, Yanagihara K, et al. Highly specific marker genes for detecting minimal gastric cancer cells in cytology negative peritoneal washings. Biochem Biophys Res Commun 2004;313:931–7.
20. Sobin LH, Wittekind C. TNM classification of malignant tumors, 5th ed. New York: John Wiley & Sons; 1997.
21. Macdonald JS, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN, et al. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 2001;345:725–30.
22. Kodera Y, Isobe K, Yamauchi M, Satta T, Hasegawa T, Oikawa S, et al. Expression of carcinoembryonic antigen (CEA) and nonspecific crossreacting antigen (NCA) in gastrointestinal cancer; the correlation with degree of differentiation. Br J Cancer 1993;90:130–6.
23. Sugita Y, Fujiwara Y, Taniguchi H, Mori T, Ishii T, Niwa H, et al. Quantitative molecular diagnosis of peritoneal lavage fluid for prediction of peritoneal recurrence in gastric cancer. Int J Oncol 2003;23:1419–23.
24. Oyama K, Terashima M, Takagane A, Maesawa C. Prognostic significance of peritoneal minimal residual disease in gastric cancer detected by reverse transcription-polymerase chain reaction. Br J Surg 2004;19:435–43.
25. Jung R, Petersen K, Kruger W, Wolf M, Wagener C, Zander A, et al. Detection of micrometastasis by cytokeratin 20 RT-PCR is limited due to stable background transcription in granulocytes. Br J Cancer 1999;81:870–3.
26. Glehen O, Mohamed F, Gilly FN. Peritoneal carcinomatosis from digestive tract cancer: new management by cytoreductive surgery and intraperitoneal chemohyperthermia. Lancet Oncol 2004;5: 219–28.
27. Mori T, Fujiwara Y, Sugita Y, Azama T, Ishii T, Taniguchi K, et al. Application of molecular diagnosis for detection of peritoneal micrometastasis and evaluation of preoperative chemotherapy in advanced gastric carcinoma. Ann Surg Oncol 2003;11: 14–20.