

EXPRESSION OF CK-19 AND CEA MRNA IN PERIPHERAL BLOOD OF GASTRIC CANCER PATIENTS

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Aim: To investigate the clinical and pathological relevance of detection of circulating tumor cells (CTC) in the peripheral blood of gastric carcinoma patients before operation. **Patients and Methods:** Fifty patients with gastric adenocarcinoma were analysed prospectively. Patients were divided into two groups according to the extent of the tumor. Group I (unresectable) consisted of 22, and group II (resectable) consisted of 28 patients. Peripheral blood samples were collected pre-operatively from all 50 patients as well as from ten healthy controls and analyzed for carcinoembryonic antigen (CEA) and cytokeratin-19 (CK-19) messenger ribonucleic acids (mRNAs). Tumor localisation, stage, presence of signet cell formation, nodal metastases, serousal and lympho-vascular invasion were recorded for all patients. **Results:** Expression of CK-19 was detected in 24 (48%), and CEA in 10 (20%) cases. Nine patients (40%) in group I and 15 (53.6%) in group II were positive for CK-19 expression. CEA expression was more frequent among group I patients (6 vs. 4 cases). There was no significant difference between the groups in the expression of CK-19 and CEA mRNA, tumor localisation, presence of signet formation, and presence and extent of nodal metastases. Patients with major vascular invasion (MVI) expressed significantly higher levels of CTC mRNA compared to those without MVI ($p = 0.023$ for CEA, and $p = 0.009$ for CK-19). The median 1 and 2-year survival was 9.5 and 10.5 months for group I, and 20 and 28.5 months for group II, respectively ($p = 0.001$). The mean survival was 6.7 months for patients with MVI, and 30.2 months for those without MVI ($p = 0.0001$). **Conclusions:** High levels of CTCs were observed in patients with MVI invasion, rather than other causes of unresectability. It can be suggested that expression of both CEA and CK-19 in the peripheral blood of gastric cancer patients are strong predictors of MVI and significantly worse survival rates.

Key Words: gastric carcinoma, micrometastases, mRNA, cytokeratin-19, carcinoembryonic antigen, vascular invasion.

Gastric cancer is the eighth most common malignancy in the United States, and has an annual incidence of 1 per 10 000 people. The incidence in Japan is approximately 78 per 10 000 [1], and it is one of the commonest types of gastrointestinal tumor. Survival of patients with gastric cancer is poor, with an overall 5-year survival rate of 15%. The survival rate declines linearly with advanced stage. While patients with early gastric carcinoma have a 90% 5-year survival rate, only 10% of patients with advanced gastric cancer survive for 5 years [2].

From 60–65% of gastric cancer patients staged according to conventional methods have local disease.

However, 50–60% of these patients experience recurrence and die from disseminated disease. Despite advances in surgical techniques, these ratios have remained stable over the last 50 years. In general, it is accepted that a number of gastric carcinoma cases have disseminated disease that cannot be diagnosed by conventional screening methods. The diagnosis of the patients with advanced disease is usually established during surgical exploration. It emphasizes the importance of the identification of useful diagnostic and prognostic markers for gastric cancers.

Disseminated cells from primary solid tumors are considered the cause of metastases formation and relapse of the disease. Consequently, its detection is of great importance for staging, prognosis and decisions about therapy. Transcripts of the tumor-associated marker carcinoembryonic antigen (CEA) and the epithelial tumor marker, cytokeratin (CK)-19 have been used successfully to detect disseminated cancer cells. We have therefore aimed to investigate the combined use of CEA and CK19 to detect micrometastases in advanced gastric carcinoma patients before operation. We have detected circulating cancer cells in patients with gastric cancer using reverse transcriptase-polymerase chain reaction (RT-PCR) for CK-19 and CEA

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Abbreviations used: CEA – carcinoembryonic antigen; CTC – circulating tumor cell; CK-19 – cytokeratin-19; cDNA – complementary deoxyribonucleic acid; CT – computed tomography; DNA – deoxyribonucleic acid; EUS – endoscopic ultrasound; GAPDH – glyceraldehyde 3-phosphate dehydrogenase; H&E – hematoxylin and eosin; IHC – immunohistochemistry; LN – lymph node; LNM – lymph node metastases; LVI – lymphovascular invasion; mRNA – messenger ribonucleic acid; MVI – major vascular invasion; N – lymph node; PET – positron emission tomography; RT-PCR – reverse transcriptase polymerase chain reaction; T – tumor size; US – ultrasound.

transcripts and have explored their possible correlations with prognostic parameters.

PATIENTS AND METHODS

Patients. We analysed prospectively 50 patients with gastric adenocarcinoma treated at Ankara Oncology Hospital between 2002 and 2005. Abdominal ultrasound (US) and chest-X-ray were done for each patient. The Ethical Committee of Ankara Oncology Hospital gave approval for this study, and written informed consent was obtained from all patients. Survival periods were obtained from hospital records or by phone contact. In patients unavailable for phone contact, the last contact was recorded as the survival period. Peripheral blood was obtained pre-operatively from 50 patients, as well as from ten healthy controls.

The patients were divided into two groups: those with unresectable tumors (group I) and those with resectable tumours (group II). There were 22 (44%) patients in group I and 28 (56%) patients in group II. Age, gender, tumor localisation, stage and presence of signet cell formation were recorded for all patients. The patients who underwent surgical resection (group II), lymph node (LN) status, serousal and lymphovascular invasion were recorded if present.

Semi-quantitative expression of micrometastases by polymerase chain reaction (PCR) was as follows: 1) no expression; 2) baseline (minimal) expression; 3) 1+ expression; 4) 2+ expression; 5) 3+ expression; 6) 4+ expression.

RNA preparation and RT-PCR. Total RNA was isolated from 300 µl of whole blood using the QIAamp RNA Blood Mini Kit (QIAGEN) according to the manufacturer's instructions. Reverse transcription was performed using 100 ng to 1 µg of RNA, 50 pmol of oligo(dT)₁₈ primer, 200 U of OMNISCRIPT reverse transcriptase (QIAGEN, USA), 40 units of rRNasin RNase inhibitor (Promega), and the deoxynucleotide triphosphates (final concentration 500 µmol/L) in a total volume of 20 µL. Samples were incubated at 37 °C for 1 h and then heated at 95 °C for 3 min. For PCR, 1 µL of the reverse transcription sample was used. The integrity of RNA specimens was verified by performing RT-PCR with the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Nested PCR was conducted by addition of 1 µL of complementary deoxyribonucleic acid (cDNA) to 20 µl of reaction mixture containing 10 mM Tris–hydrochloric acid (pH 9.0), 50 mM potassium chloride, 2.5 mM magnesium chloride, 250 nM deoxynucleotid triphosphate 10 pmol of each outer primer, and 2.5 units of Taq DNA polymerase (QIAGEN, USA). The reaction mixtures were subjected to 35 cycles of amplification in a programmable thermal cycler (Perking-Elmer Cetus, USA) using the following sequence after a denaturation step at 94 °C for 5 min: 94 °C for 30 sec, 56 °C for 30 sec and 72 °C for 1 min, and a final extension step at 72 °C for 10 min. A sample of 2 µl of 20-fold diluted first amplification product was further amplified using an inner

pair of primers. The inner and outer pairs of primers are given in Table 1.

Table 1. The inner and outer pairs of both CEA and CK-19 primers

| Primer name | Sequence | TM | Product size |
|--------------|-------------------------|----|--------------|
| CEA outer F | GGACCTATGCCTGTTTGTCT | 58 | 250 |
| CEA outer R | AGCAATTTTAGACTGTAGCTGTG | 57 | |
| CEA inner F | GCATCTGGAACCTCTCCTGGT | 61 | 154 |
| CEA inner R | TGCAAATGCTTTAAGGAAGAAGC | 62 | |
| CK19 outer F | GTTTGAGACGGAACAGGCTCT | 58 | 425 |
| CK19 outer R | CAGCTCAATCTCAAGACCCTG | 57 | |
| CK19 inner F | GCAGATCGAAGGCCTGAA | 61 | 209 |
| CK19 inner R | TGAACCAAGGCTTCAGCATC | 62 | |

PCR primer sequences for each gene were designed to span at least one intron region to avoid amplification of genomic DNA. According to this design, the PCR products of 154 and 209 base-pairs were amplified from CEA and CK19 cDNAs with nested PCR, respectively. The total RNA extracted from col15 and SW620 cells was used for optimization of the PCR and as a positive control. Blood cells from healthy volunteers were used as negative controls for every PCR reaction. Each sample was subjected to electrophoresis in 1.5% agarose gels and stained with ethidium bromide. Samples from each patient were considered to have a positive score if any fragment showed a band of the expected size as positive control both for CEA and CK-19. Some PCR amplification products were sequenced to confirm the specificity of CK19 and CEA cDNA amplification products. The samples were judged to be positive (from + to +++) or negative on the basis of the intensity value for the PCR product on the gel. Quantification of the expressions was based on the hypothesis that advanced stage tumors might present with greater amounts of micrometastatic tumor cells. Comparison between the groups was performed using this scale.

Statistical analysis. The χ^2 test or Fisher's exact test was used for categorical variables. Comparison according to age between groups was done using Student's *t*-test. The 1 year and 2 year survival rates were evaluated by the Kaplan-Meier method. The significance of differences between the groups was calculated by the log-rank test. All statistical analyses were performed using the programme package number Crucher statistical system 2004 (Kaysville, UT, USA), and a *p* value < 0.05 was considered as significant.

RESULTS

Characteristics of patients and surgical procedures. A total of 50 patients with gastric cancer were included in our study; 22 (44%) females and 28 (56%) males, with a mean age of 60.9 years (range 42–84). The tumors were localized in the cardia in 16 (32%), in the corpus in 11 (22%), and in the antrum in 22 (44%) patients. One patient (2%) presented with diffuse gastric cancer. Signet cell formation was detected in 18 (36%) patients.

Eleven patients in group II underwent subtotal gastrectomy, and total gastrectomy was applied to the remaining 17 patients. Total gastrectomy combined with partial colon resection was applied to one patient, and three patients underwent total gastrectomy com-

bined with splenectomy. Fourteen patients underwent D2, and 5 patients underwent D3 lymph node dissection. All palpable lymph nodes were removed in the remaining 9 cases.

In group I, we detected peritonitis carcinomatosa in 12 patients, pancreatic invasion in 14, invasion of celiac truncus in 7, hepatic pedicle invasion in 6, Cruikshank tumor in 2 and liver metastases in 2 patients. The reasons for unresectability consisted of pancreatic invasion, celiac truncus and or hepatic pedicle invasion. Treatment options for unresectable tumors were gastroenterostomy in 10, jejunostomy in 4, and gastrostomy in 3 patients. Explorative laparotomy and biopsy were applied to 5 patients. All patients received chemotherapy consisted of 5-fluorouracil and leucovorin at four weeks for 6 cycles.

Detection of CK-19 and CEA expression in peripheral blood. No amplified product was obtained after RT-PCR when evaluating CK19 and CEA expression in the 10 healthy controls. CK-19 expression was detected in 24 gastric cancer patients (48%). Expression was not observed in 26 cases (52%). In patients with positive expression, 11 (22%) had baseline activity, 3 (6%) had 1+ expression, 3 (6%) had 2+ expression, 4 (8%) had 3+ expression, and the remaining 3 (6%) had 4+ expression. CEA expression was detected in 10 patients (20%), and was not observed in 40 cases (80%). In patients with positive expression, 2 (4%) had 2+ expression, 2 (4%) had 3+ expression, and the remaining 6 (12%) had 4+ expression (Table 2). Semi-quantitative expression of micrometastases by PCR are given in Table 3.

Table 2. CK-19 and CEA expressions among groups

| Groups | Cytokeratin-19 | | Total | Carcinoembryonic antigen | | Total |
|----------|----------------|----------|----------|--------------------------|----------|----------|
| | Negative | Positive | | Negative | Positive | |
| Group I | 13(59%) | 9(41%) | 22(44%) | 16(79%) | 6(21%) | 22(44%) |
| Group II | 13(40%) | 15(60%) | 28(56%) | 24(90%) | 4(10%) | 28(56%) |
| Total | 26(52%) | 24(48%) | 50(100%) | 40(80%) | 10(20%) | 50(100%) |

Table 3. Semi-quantitative expression of CK-19 mRNA (in 24 patients) and CEA mRNA (in 10 patients) micrometastases by RT-PCR in patients with gastric cancer

| Expression | CK-19 | CEA |
|---------------|----------|----------|
| Baseline | 11(22%)* | |
| 1+ expression | 3(6%) | |
| 2+ expression | 3(6%) | 2(4%) |
| 3+ expression | 4(8%) | 2(4%) |
| 4+ expression | 3(6%) | 6(12%) |
| Total | 24(100%) | 10(100%) |

Note: * number of the patients.

There was no significant difference for either of the markers studied ($\chi^2 = 4.097, p = 0.536$ for CK-19 and $\chi^2 = 0.791, p = 0.374$ for CEA). Expressions of CEA and CK-19 without quantification did not also reveal a significant difference ($\chi^2 = 2.052, p = 0.562$). The difference in expressions of CK-19 and CEA mRNA according to age, gender, tumor location, and presence of signet cell formation were not significant ($p = 0.631; \chi^2 = 0.034, p = 0.854; \chi^2 = 2.037, p = 0.565; \chi^2 = 2.063, p = 0.151$, respectively). The degree of expression did not differ significantly between the groups ($\chi^2 = 5.396, p = 0.145$).

Serousal invasion was observed in 25 of 28 patients who underwent surgical resection. Lymphovascular invasion was detected in 18 (36%) individuals. Lymphatic metastases were detected in 21 (42%) of the 28 (56%) patients in group II. In the remaining 7 patients, dissected lymph nodes (LNs) were free of metastases.

CK-19 was positive in 4 of 7 patients without lymphatic involvement. However, this difference was not significant ($p = 0.28$). CEA was negative in all patients without lymphatic involvement. In cases with lymphatic invasion CEA expression was not different from those without LN metastasis ($\chi^2 = 5.483, p = 0.140$). We compared the difference between groups without quantifying the number of metastatic lymph nodes, and the difference was also not significant ($\chi^2 = 2.775, p = 0.521$ for CK-19 and $\chi^2 = 7.885, p = 0.098$ for CEA). Consideration of parameters in group II revealed no significant difference according to serousal invasion ($p = 0.556$ for CK-19 and $p = 0.618$ for CEA) and lymphovascular invasion ($p = 0.247$ for CK-19 and $p = 0.335$ for CEA).

Median 1 year survival in group I and group II was 9.5 months (range 2–24) 20 months (range 11–29 months) respectively ($p = 0.001$). Median 2 years survival in group I was 10.5 months (range 2–24 months), and was 28.5 months (range 11–29) in group 2 ($p = 0.001$). MVI was detected in 7 cases. CK-19 was positive in 6 of these seven patients (85.7%) and CEA was positive in 5 patients (71.4%) with celiac truncus involvement, and both were positive in 4 (57.1%) of them. We detected both CK-19 and CEA positivity in 5 patients, and in 4 of them (80%) celiac truncus was invaded by the tumor. Furthermore, patients with celiac truncus invasion expressed higher levels of micrometastases: for CEA; two patients had +++ expression, and three cases had ++++ expression. For CK-19; two patients had ++, two had +++, and the remaining two had ++++ micrometastatic expression. Patients with MVI expressed significantly higher levels of micrometastases compared to those without MVI ($\chi^2 = 12.311, p = 0.023$ for CEA, $\chi^2 = 9.844, p = 0.009$ for CK-19). All the patients with MVI have died within one year. We have evaluated the survival of patients according to expression patterns of CEA and CK-19. Mean survival of patients with high levels of CEA and CK-19 were 11.1 months and 11.9 months, respectively. Those of the remaining cases with minimal or no expression for CEA and CK-19 were 30.1 months and 29.4 months, respectively. The difference for both was significant ($p < 0.001$ for CEA and $p = 0.002$ for CK-19). Mean survival periods of patients due to the expression patterns were given in Table 4, survival curves are depicted in Fig. 1, a, b.

Table 4. Mean survival of patients according to expression patterns of CEA and CK-19

| | | Time (months) (mean ± SD) | | Log rank (χ^2) | p value |
|-------|---------------------|---------------------------|--------------|-----------------------|---------|
| | | Death | Alive | | |
| CEA | Absent or low level | 9.75 ± 2.18 | 27.52 ± 5.70 | 14.851 | <0.001 |
| | High level | 5.80 ± 3.96 | 20.50 ± 9.19 | | |
| CK-19 | Absent or low level | 10.27 ± 1.27 | 27.48 ± 5.69 | 9.807 | 0.002 |
| | High level | 5.50 ± 3.61 | 21.00 ± 9.90 | | |

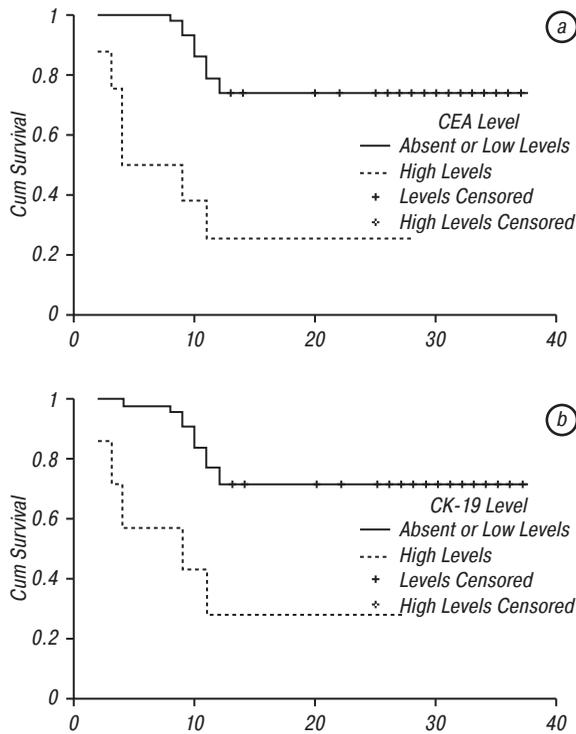


Fig. 1. One year survival curves of patients according to the expression levels of CEA (a) and CK-19 (b); χ^2 (log-rank) = 14.851, $p < 0.001$ for CEA and χ^2 (log-rank) = 9.807, $p = 0.002$ for CK-19

The sensitivity and specificity of CK-19 in detecting MVI was 26.08 and 96.15%, respectively. For CEA the sensitivity and specificity was 50 and 95%, respectively. When we consider only high titres of markers, sensitivity increased to 85.7% and 62.5% for CK-19 and CEA, respectively. In combined usage of CEA and CK-19 for detecting MVI, sensitivity was 80%. Considering combined usage of high titres in detecting MVI, sensitivity was 100%.

Median survival of patients with MVI was 6.7 months (95% CI: 3.554–9.875 months), and those of the remaining was 30.2 months (95% CI: 26.672–33.653 months). Patients with MVI had a significantly lower survival rates (χ^2 (log-rank) = 23.324, $p = 0.0001$), (Fig. 2).

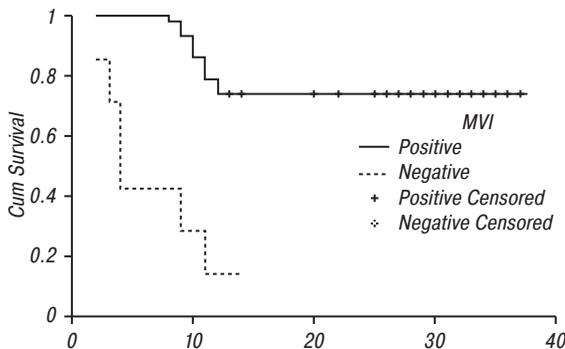


Fig. 2. One year survival curves of patients according to the presence of major vascular invasion. (χ^2 (log-rank) = 23.324, $p = 0.0001$)

DISCUSSION

Malignant process initiates from neoplastic proliferation of a cell leading to local enlargement and as the

progression advances, lymphatic and hematogenous metastases occur [3]. There is still a dilemma concerning the significance of detecting circulating tumor cells (CTC), since only a portion of CTC have the ability to perform adhesions and invasion. This raises the question of whether there is a correlation between CTC and tumor stage, and what portion of patients with CTC will develop distant metastases. Detection of distant metastases plays a vital role in accurate staging and treatment of malignant tumor. For this reason, the goal of a screening method should be to identify individuals who are at high risk of developing distant metastases. It is common to observe cases with micrometastases in which conventional screening methods failed to demonstrate the course [4]. From this point of view, we investigated the role of PCR in detecting patients with locally advanced disease, and the correlation of prognostic parameters with PCR expression.

Detection of CTC by conventional haematological methods is both difficult and inaccurate [5, 6]. Using improved methods like immunohistochemistry (IHC) and PCR, not only the metastatic focus but only one metastatic cell can be identified [7–9]. Due to its great sensitivity, PCR can detect trace amounts (1 per 10^6) of cells in peripheral blood, lymph nodes, cerebrospinal fluid, and bone marrow [10]. Furthermore, micrometastases undetected by IHC can be detected by PCR [11–13]. We applied nested RT-PCR to determine the CEA and CK19 mRNA molecules in combination, in the peripheral blood of gastric cancer patients. CEA is commonly used as a tumor marker and CK-19 is expected to display some specificity for epithelial cells.

Some markers like CEA, CK-19, CK-20 and α -fetoprotein have been used to detect CTC and micrometastases. In different series micrometastasis and CTC were detected in 20 to 70 percent of patients with gastric cancer, and stated as favourable markers of distant spread [10, 14, 15]. Conflicting results have been reported concerning the use of CK19 mRNA in the peripheral blood of patients with solid tumor due to the presence of pseudo genes and DNA contamination. To avoid these problems, RNA samples were incubated with DNase before cDNA synthesis, and primers were designed on the exon boundaries for both genes. Our results suggest that this method seems to be highly specific for micrometastatic cell detection because we have not detected any amplification product for both genes in healthy donor samples. But expression of both genes was observed in gastric cancer patients (CEA in 20% and CK-19 in 48% of the cases). Our results emphasize the heterogeneity of gene expression in tumors and justify the use of more than one mRNA marker for tumor cell detection.

Unlike some previous studies with different tumor types, we were not able to find any correlation between CK-19 and CEA mRNA expression with prognostic parameters. The positivity for PCR s in our samples from peripheral blood was lower than those of bone marrow samples and lymph nodes, when compared with published data. A possible explanation of this

controversy is the phenomenon described, particularly in patients with breast [16] and gastric [17] cancers, caused by the peculiar adhesive interaction between metastatic epithelial cells and stromal cells. In the report by Bonavina and co-workers [17], the role of micrometastatic expression by PCR in patients with non-metastatic tumors of the esophagus or gastric cardia was evaluated. The authors found PCR positive expression in peripheral blood in 3 of 18 (16.6%) patients, but they could not demonstrate any correlation with tumor type or lymph node status. Micrometastatic expressions in bone marrow were detected in 14 of 18 patients (77.7%), and it was concluded that this ratio agrees with data in the literature [17]. Decreased ratios of peripheral micrometastatic expression might result from the degradation caused by circulating lymphocytes, and disseminated tumor cells in the circulation detected by RT-PCR might represent the subgroup that are able to preserve cell-surface antigens from lymphocytes. Comparison of survival curves of patients with different micrometastatic focuses might lead to a better understanding of the micrometastatic process.

Kodera *et al.* [18] have investigated the role of PCR in detecting peritoneal disseminated tumor cells, and found that positive PCR results were predictive of survival even more strongly than LN metastasis (LNM) in patients who underwent curative surgical procedure for gastric carcinoma. Their results suggested that LN involvement correlated with peritoneal micrometastases. LN micrometastases in gastric cancer patients have been investigated. Even if it does not parallel with survival, CK-20 immunostaining has been detected in 20.7% of node negative gastric cancer patients [19], although the micrometastatic expression pattern affected the prognosis. Moreover, detection of multiple individual isolated tumor cells had the worst prognosis [19]. Single cells and small clusters of LNM are not uncommon in gastric cancer. Especially in diffuse type gastric cancer, the neoplastic cells lose their intercellular adhesion molecules, and unpredictable lymphatic spread can occur. Therefore, micrometastatic involvement of LN in gastric cancer is not well defined. Wu *et al.* [20] detected LNM in 10.8% of node negative patients assessed by routine hematoxylin and eosin (H&E) staining. They concluded that detection of cytokeratin by RT-PCR was useful for the detection of LNM, and that it correlated with diffuse histological subtype and depth of tumor invasion. If we could detect a correlation between conventional prognostic parameters and micrometastases, we might be able to detect patients with locally advanced tumors. In other words, if there had been a correlation, patients with early gastric carcinoma who do not have micrometastatic expression might undergo non-invasive surgical resections. Otherwise, adjuvant chemotherapy and antiangiogenic treatment may be necessary for patients with micrometastases.

The major important point of our study is the high percentage of micrometastases in patients with celiac

invasion. In these patients, the levels of micrometastatic expression were much higher than others. To the best of our knowledge, this is the first report showing that high levels of micrometastatic expression suggested by the expression of both CK-19 and CEA might be a clue to predicting MVI in gastric cancer patients.

The imaging modalities used commonly in clinical staging of gastric cancer are gastroscopy, abdominal US, endoscopic US (EUS), computed tomography (CT) and occasionally positron emission tomography-CT (CT-PET). The combination of these methods is crucial in making the right diagnosis as well as the stage and follow-up after multimodal treatment. EUS and CT are especially beneficial for identifying invasion of gastric tumor within and beyond the gastric wall to adjacent structures. There are a number of reports indicating that EUS and CT have the ability to detect the tumor size and involvement of lymph nodes up to 80 and 60%, respectively [21–23]. However, disappointing reports of CT on pre-operative staging for gastric cancer with low accuracy in tumor size (T) and node (N) staging exist in the literature [24, 25]. Magnetic resonance imaging (MRI) yielded discouraging results in gastric tumor staging as compared to CT or EUS [26]. Even with modern multislice CT, it may not be possible to evaluate infiltration of the vascular structures with any certainty. There are some nonspecific signs, like the degree of deformity in the circumference of the vascular structure or more than 90° of contact between the tumor and the vessel wall [27]. From this point of view our study permitted us to conclude that appropriate vascular invasion might be predicted by the combined detection of CEA and CK-19 mRNA in peripheral blood of gastric carcinoma patients.

We did not observe a significant correlation between the presence of circulating tumor cells and stage, LNM and serosal invasion. We had only one case of early gastric carcinoma, all the remaining patients had at least subserosal invasion. In the present series early gastric cancer is infrequent. If we had more patients with early gastric carcinoma, we might be able to exclude the significance of the expression of micrometastases within this subgroup, and we believe that our results might then be different.

In the future, staging systems will have to provide specific information on the biological properties of residual cancer cells in order to provide more exact prognostic estimates and guide patients to an individually tailored multimodal treatment. However, it should not be forgotten that any cancer cell detected in peripheral blood may not be able to metastasize, and the significance of micrometastatic cell detection remains to be proven by multicentre prospective randomized trials with long-term follow-up.

In conclusion, pre-operative combined nested RT-PCR for CK-19 and CEA (if both are positive) might predict MVI and thereby avoid the morbidity of invasive interventions like angiography. We believe that this procedure is minimally invasive and cost-effective,

and could be the method of choice for pre-operative evaluation of local invasion in gastric cancer.

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