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Original article

Distribution of free cancer cells in the abdominal cavity suggests limitations of bursectomy as an essential component of radical surgery for gastric carcinoma

Yoshitaka Yamamura[1], Seiji Ito[1], Yoshinari Mochizuki[1], Hayao Nakanishi[2], Masae Tatematsu[2], and Yasuhiro Kodera[3]

[1]Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan
[2]Laboratory of Pathology, Aichi Cancer Center Research Institute, Nagoya, Japan
[3]Second Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan

Abstract

Background. **Bursectomy, which has been performed so as to resect peritoneal deposits disseminated within the omental bursa, is considered as an essential component of radical surgery for gastric carcinoma in Japan. Bursectomy has also been described in the Japanese Treatment Guidelines for Gastric Carcinoma as a mandatory procedure for the treatment of serosa-positive cancer. However, no evidence to support the prognostic significance of this procedure has been reported to date.**

Methods. **Cytologic examination and real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of the peritoneal washes obtained from the Douglas pouch, left subphrenic cavity, and inside the omental bursa were performed for 136 patients who underwent potentially curative surgery for gastric carcinoma.**

Results. **Carcinoembryonic antigen (CEA) or cytokeratin (CK) 20 mRNA was detected in one or more samples from the three different sites of peritoneal washes in 43 of the 136 patients. In 14 patients, the mRNAs were detected in samples obtained from the bursa omentalis (10.3% of all patients and 32.6% of patients with positive RT-PCR results). In 12 of these 14 patients, the mRNAs were also detected in samples taken from either or both of the remaining two sites. Only in the 2 other patients was the sample only from inside the omental bursa positive for CEA.**

Conclusion. **It is unlikely that viable cancer cells disseminated into the bursa remain restricted to this cavity without migrating into the free abdominal cavity. Routine bursectomy may not be an essential procedure for resecting gastric cancer, from the viewpoint of eliminating microscopic peritoneal deposits within the omental bursa.**

Key words Bursectomy · Real-time RT-PCR · Cytology · Peritoneal metastasis

Offprint requests to: Y. Yamamura
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Introduction

Bursectomy, a procedure to dissect the peritoneal lining covering the anterior plane of the transverse mesocolon together with the omentum, has been described in the *Japanese Gastric Cancer Treatment Guidelines* [1] as being mandatory for radical gastrectomy to resect disease of stage T3 or higher. The purpose of this procedure is to remove cancer cells and micrometastases disseminated within the bursa omentalis and, arguably, to dissect lymphatic disease. However, nowadays, in general, routine application of this time-consuming procedure is often not done. Moreover, the theoretical background of bursectomy may seem rather unsound from the anatomical point of view. The bursa omentalis is actually not a closed cavity, and is connected to the free abdominal cavity through the foramen of Winslow. Consequently, free cancer cells that have been primarily shed into the bursa can, in theory, migrate into the whole abdominal cavity after a certain amount of time.

Cytologic examination of peritoneal washes has been performed to detect free cancer cells, but the results have often turned out to be false-negative when the number of cells present in the abdominal cavity was small. We [2–5] extensively explored the application of a reverse transcriptase-polymerase chain reaction (RT-PCR) technique with carcinoembryonic antigen (CEA) mRNA as a target to more sensitively detect free cancer cells in peritoneal washes. Subsequently, we found, through the analysis of findings in 284 patients who completed 5 years of follow-up, that with our method, the sensitivity, specificity, positive predictive value, and negative predictive value for predicting peritoneal carcinomatosis within 5 years of surgery was 88.5%, 81.6%, 64.5%, and 94.9%, respectively [6]. In addition, our method and the cutoff value of the assay system have been validated in a new series of samples [7]. A similar method has been reported to be a reliable predictor of

peritoneal recurrence by other investigators [8,9], while the use of cytokeratin 20 (CK20), a marker with greater specificity and inferior sensitivity [10], was found to be potentially useful to augment the diagnostic accuracy of CEA RT-PCR [11].

Resection of micrometastases trapped within the bursa would, no doubt, be meaningless when free cancer cells have already been scattered into the free abdominal cavity. However, if disseminated cancer cells are shown to remain trapped within the bursa for a certain length of time, bursectomy may have a prognostic value in a limited subset of patients. To test this hypothesis, we measured CEA and CK20 mRNA levels in the omental bursa and other abdominal cavities, using the validated RT-PCR technique.

Patients, materials, and methods

Patients

One hundred and thirty-six patients operated on between July 2002 and August 2005 and who fulfilled the following criteria were entered in the study. Patients had to have a histological diagnosis of adenocarcinoma of the stomach and to have been diagnosed preoperatively to have T2-T4 stage disease according to the *Japanese Classification of Gastric Carcinoma* [12]. They had to have been intraope diagnosed to have no gross peritoneal deposits, and to be negative for cytologic examination of peritoneal washes. This is because intraoperative findings of visible peritoneal deposits and being positive for the cytologic examination reportedly are equally suggestive of a dismal prognosis and are both considered sufficient to classify a patient as stage IV in the current version of the *Japanese Classification of Gastric Carcinoma* [12]. Hence, formal D2 dissection with bursectomy would not be considered a standard procedure for these patients. In addition, peritoneal washing samples had to be collected from the Douglas pouch, left subphrenic cavity, and within the bursa omentalis. Thus, patients with extensive intraperitoneal adhesions due to prior surgery were excluded. Patients were also excluded if they were positive for type B or C hepatitis. Informed consent was obtained from all patients who participated in the study.

Sample collection

The subphrenic cavity was rinsed with 100 ml saline immediately after laparotomy and the washing sample was aspirated. The same procedure was repeated in the Douglas pouch. Then, the omentum was dissected at the mid-transverse colon and 100 ml saline was introduced into the omental bursa, stirred, and collected. New syringes and tubes were used for each sample collection procedure to prevent contamination of each washing sample by other samples. All samples were divided into halves and one half was sent for conventional cytologic examination with Papanicolaou staining while the other half was submitted to RT-PCR. Samples for RT-PCR were centrifuged at 1800 rpm for 5 min to collect intact cells, rinsed with phosphate buffered saline, dissolved in ISOGEN RNA extraction buffer (Nippon Gene, Tokyo, Japan), and stored at –80 °C until use.

Real-time RT-PCR

Real-time RT-PCR detection of CEA, CK20, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in the peritoneal washing samples was performed as described elsewhere [4]. In brief, total RNA was extracted using a guanidinium isothiocyanate-phenol-chloroform method. The extracted total RNA was converted to first-strand cDNA and was immediately used for PCR amplification with a LightCycler (Roche Diagnostics, Mannheim, Germany). Real-time RT-PCR was performed by a single-step method (50 cycles), using hybridization probes. The design of the primers and probes used in the study, together with the conditions for amplification, have been described previously [4,11]. All the primers and probes were synthesized and purified by reverse-phase high-performance liquid chromatography by Nihon Gene Research Laboratories (Sendai, Japan). Real-time PCR monitoring was achieved by measuring the fluorescent signal at the end of the annealing phase for each cycle. GAPDH was quantified only to ensure that mRNA was successfully extracted. A cutoff value of 0.1 for CEA mRNA (equivalent to one-tenth of CEA mRNA contained in a single COLM-2 cell) was established by the aid of a receiver operating a characteristics curve, which was constructed using data for CEA mRNA values and the presence or absence of free intraabdominal cancer cells (the presence of cancer cells in patients in this analysis was defined as their either having peritoneal metastasis at surgery or suffering from relapse as peritoneal carcinomatosis within 2 years of surgery), as reported previously [5]. Because CK20 is more specific and less sensitive than CEA, CK20 mRNA exceeding 0 was considered as positive [11]. Patients were considered as being positive for intraperitoneal cancer cells if either or both of the two markers exceeded the cutoff values.

Results

Either or both of the target mRNAs were detected in one or more samples from the three different cavities

Table 1. Results of lavage cytology by RT-PCR method

	Results of lavage cytology							Number of positive cases (%)
Douglas pouch	+	+	+	−	+	−	−	34 (79.1)
Left subphrenic cavity	+	+	−	+	−	+	−	17 (39.5)
Bursa-omentalis cavity	+	−	+	+	−	−	+	14 (32.6)
Number of cases	6	4	6	0	18	7	2	43 (100)

in 43 of the 136 patients (31.6%). Of these 43 patients, the mRNA was detected in all three samples in 6 patients, in two samples in 10 patients, and in one sample in 27 patients (Table 1). CEA was detected in 41 of the 43 patients, whereas the samples in the other 2 patients were diagnosed as positive through detection only of CK20 mRNA. The mRNAs were detected most frequently in the Douglas pouch (in 34 patients [25.0% of all patients and 79.1% of patients who had positive RT-PCR results]), followed by the left subphrenic cavity (17 patients [12.5% of all patients and 39.5% of patients who had positive RT-PCR results]). In contrast, the mRNAs were detected in only 14 patients in samples obtained from the omental bursa (10.3% of all patients and 32.6% of all patients with positive RT-PCR results). In 12 of these 14 patients, the mRNAs were also detected in samples from either or both of the other two cavities; it was only in the 2 remaining patients that the samples only from the omental bursa were diagnosed as positive for free cancer cells. These samples were positive for CEA, the more specific of the two markers, and not for CK20 [6].

Discussion

Free cancer cells in the abdominal cavity are reported to adhere primarily to the milky spots that are found abundantly in the omentum [13]. Consequently, peritoneal deposits tend to be harbored within the omentum before they are found scattered throughout the abdominal cavity [14]. For this reason, omentectomy is performed for the surgery of tumors with a high propensity towards peritoneal metastases, such as gastric and ovarian cancers. Bursectomy, a time-consuming procedure to meticulously remove the peritoneal lining that covers the anterior plane of the transverse mesocolon and the pancreas, has also been incorporated in radical surgery for gastric carcinoma. In addition, the retroperitoneal lining that covers the second-tier lymph nodes along the hepatic and splenic arteries has to be removed in order to perform a D2 lymphadenectomy, and only through a combination of all three procedures could peritoneal metastasis confined to the omental bursa be removed. In other words, bursectomy constitutes only a part of the whole process of removing micrometastasis within the omental bursa. Bursectomy, in combination with other procedures, is also considered to have some value in clearing lymphatic disease. It could be argued, however, that the complete removal of the peritoneal lining over the anterior plane of the transverse colon, per se, would not result in the harvesting of a large number of additional lymph nodes. Removal of the peritoneal lining covering the right half of the transverse mesocolon does indeed facilitate the retrieval of lymph node stations numbers 6 and 14, but this procedure would not be referred to as a formal bursectomy. We therefore concentrated on examining the reasoning for the procedure only in relation to the clearance of peritoneal disease. However, the removal of microscopic cancer progression through lymphatic pathways may be an additional benefit conferred by formal bursectomy. Furthermore, the spillage of cancer cells that have already adhered to the pancreatic capsula or mesocolon and which can be removed only by omento-bursectomy may not be detected through the evaluation of washing samples. These issues have not been fully addressed here and may be considered a weakness in the present study.

Serum CEA is not a sensitive tumor marker for gastric carcinoma [15], and the use of this CEA as a target for RT-PCR to detect gastric cancer cells could be questioned. However, it has been documented that RT-PCR detects CEA mRNA in most gastric cancer cell lines and tissues, despite the fact that the expression may not be as readily detectable using less sensitive assays such as Northern blotting and immunostaining [2,16]. Moreover, CEA RT-PCR analysis of peritoneal washings has been validated by the present authors as a useful predictor of peritoneal carcinomatosis [7]. Admittedly, a positive predictive value of 64.5%, meaning the detection of CEA mRNA, does not predict the later development of peritoneal carcinomatosis in a third of cases, and this may seem insufficient, given that the research concept of the present study is based on the principle that clinical outcomes should be highly correlated with the parameter evaluated. Possible false-positive results have indeed obscured some of the results, as discussed below.

In this study, however, the most fundamental quality required for the method was a high negative predictive value. With a negative predictive value of 94.9%, the absence of CEA mRNA in a sample will convincingly deny the presence of viable cancer cells [6]. Despite various weaknesses, therefore, the CEA RT-PCR method may represent one of a limited number of options that are suitable for this study.

It became evident through this study that free cancer cells are rarely found confined to the bursa omentalis and they remain undetectable in other parts of the abdominal cavity. The primary cancer in the two patients who had CEA mRNA detected only in the bursa omentalis had penetrated the anterior wall of the stomach, meaning that the cancer cells, if exfoliated from the serosa, would more likely have spread into the free abdominal cavity rather than into the bursa omentalis. Neither of these patients was diagnosed to have peritoneal carcinomatosis during the course of postoperative follow-up however, and the CEA mRNA in these patients could therefore be interpreted as false-positive. If this speculation is correct, we can conclude that none of the 136 patients had free cancer cells confined to the omental bursa. This finding, although not as yet associated with any survival data, could begin to challenge the principle — in classic Japanese-style, radical gastrectomy — whereby bursectomy is considered a standard procedure for resectable advanced gastric cancer. There are some other data that cast doubt on the benefit of bursectomy. Theoretically, the population most likely to benefit from bursectomy has penetration of the serosa on the posterior gastric wall, with no evidence of peritoneal deposits or floating cancer cells in the free abdominal cavity. A retrospective analysis of this particular population failed to reveal a survival benefit of bursectomy [17]. In addition to the standard bursectomy procedure, a more thorough resection of all components of the retroperitoneum could be accomplished by extensive surgery such as left upper abdominal evisceration, with or without Appleby's procedure [18,19]. However, even these procedures have been reported not to reduce the incidence of peritoneal metastasis.

To conclude, the finding in the present study that CEA mRNA was detected only in the bursa in only 2 of 136 patients, while being undetectable in the free abdominal cavity, suggests that cancer cells shed from the primary tumor and disseminated into the bursa are either eliminated or migrate swiftly into the free abdominal cavity. These cells are unlikely to be optimal targets for surgical removal, and the emergence of more effective locoregional therapy [20,21] is urgently needed to improve the survival of patients with stage T3 or more gastric cancer. A randomized trial is ultimately needed to reach a definite conclusion regarding the prognostic value of bursectomy. Given that a depressingly small proportion of patients with gastric carcinoma currently participate in clinical trials in Japan, however, investigators are confronted with the issue of whether the precious patient resources should be expended to explore a procedure that now seems rather unlikely to have a profound impact on patients' wellbeing.

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