Laparoscopic peritoneal lavage cytology and immunocytochemistry in pancreatic and periampullary carcinoma

MJ Midwinter¹, A Watson¹, V Wadehra² and RM Charnley²

¹HPB Surgery Unit, Freeman Hospital and ²Department of Cytology, Royal Victoria Infirmary, Newcastle upon Tyne, UK

Introduction

Exfoliation of cells from intra-abdominal tumours into the peritoneal cavity has been recognised for many years. In patients with ascites, the presence of malignant cells recognised cytologically has been utilised for diagnostic purposes. The recognition of tumour cells from a well-differentiated carcinoma is difficult cytologically. Reactive or degenerative mesothelial cells can make cytological interpretation with conventional stains difficult. Detection of the tumour-associated antigens carcinoembryonic antigen (CEA) and CA19-9 may improve detection.

Methods

At staging laparoscopy, 22 patients with pancreatic or peri-ampullary tumours had ascitic fluid aspirated or peritoneal lavage performed. Both conventional and immunocyto logically stained preparations were examined. Antibodies to CEA and CA19-9 and the epithelial marker BerEP4 were used. Lavage fluid from ten patients having operative treatment for benign pancreatic or biliary conditions was also examined.

Results

No malignant cells on conventional cytological criteria were recovered. Thirteen of the 22 patients with pancreatic or periampullary carcinoma had peritoneal cells that were positive for CEA and/or CA19-9. None was positive for BerEP4. No patients with resectable disease had cells that were positive for CEA or CA19-9 compared with 13 of 18 (72%) who had unresectable disease. One patient (10%) with benign disease (chronic pancreatitis) had cells recovered that were weakly positive for CEA but negative for CA19-9 and BerEP4.

Discussion

Recovery of cells from the peritoneal cavity of patients with pancreatic or periampullary carcinoma that are expressing the tumour-associated antigens CEA or CA19-9 does not indicate the presence of free tumour cells but is associated with advanced disease.

Keywords

pancreatic cancer, peritoneal cytology, immunocytochemistry, CEA, CA19-9, laparoscopy.
peritoneal washings from 7% [5] to 30% [6] of pancreatic cancer patients using conventional cytological stains. Warshaw and colleagues [6] suggested that previous percutaneous fine needle aspiration (FNA) for cytology increased the presence of free peritoneal tumour cells from 19% to 75%. This finding was not corroborated by Leach and associates [5], who found no increase in positive peritoneal cytology following FNA. Both groups found the presence of free peritoneal tumour cells to be a poor prognostic sign. The presence of reactive and degenerative mesothelial cells can hamper interpretation with conventional cytological stains.

It has been suggested that immunocytology may improve detection of scanty malignant cells in a peritoneal lavage specimen. Using conventional cytological stains plus immunocytological methods with antibodies to carcinoembryonic antigen (CEA) and the tumour-associated antigen CA19-9, Nomoto and co-workers [7] found some antibody-positive cells that were not recognised as malignant by conventional cytological criteria. These cells were not characterised further. In another immunocytological study, Juhl and colleagues [8] found some peritoneal cells positive for CA19-9 but negative to the epithelial membrane antigen C-54-0. None of these studies has examined a group of patients with benign pancreatic or biliary conditions.

The aim of this study was to examine cells recovered by laparoscopic peritoneal lavage in pancreatic and periampullary cancer patients and in those with benign pancreaticobiliary disease, using conventional cytological stains and immunocytochemical staining for the tumour-associated antigens CEA and CA19-9 and for the epithelial marker BerEP4.

Patients and methods

Consecutive patients presenting to the unit with pancreatic or periampullary adenocarcinoma were assessed by preoperative triple phase contrast-enhanced spiral computed tomography, gadolinium-enhanced magnetic resonance imaging and endoscopic ultrasound. Twenty-two patients had no evidence by these modalities of metastases or locally advanced disease involving the superior mesenteric vessels or portal vein and entered the study. Ten patients with benign pancreaticobiliary pathology also had peritoneal lavage performed. Lavage was performed at staging laparoscopy in the patients with carcinoma. None of the patients had had prior FNA. Pneumoperitoneum was induced and intra-abdominal pressure was set at 10 mmHg. An 11 mm trocar was used at the umbilicus and a 12 mm trocar was introduced under direct vision in the left upper quadrant of the abdomen. Any ascitic fluid was aspirated and sent as the specimen. If no ascitic fluid was found, peritoneal lavage was performed. Before any manipulation of the tissues, 50 ml 0.9% saline was introduced into the abdomen by a suction/irrigation cannula, irrigating over the greater omentum and around the right upper quadrant. The patient was gently agitated on the operating table for several minutes and the fluid was aspirated into two 20 ml universal containers. After peritoneal lavage a full laparoscopic assessment was performed, including evaluation with laparoscopic ultrasound. For patients with benign disease having a laparotomy, the irrigation was performed as soon as the peritoneal cavity was open and before any manipulation.

The specimens were processed immediately. The entire specimen was centrifuged at 1500 rpm for 5 min, then the supernatant decanted and the cell pellet re-suspended. Smear and cytospin preparations were made from the suspension.

Conventional cytological stains consisted of Papanicolau (PAP), May-Grunwald and Giesma (MGG), Periodic Acid Schiff (PAS) and Diastase Periodic Acid Schiff (DPAS). Slides for PAP staining were fixed in 95% ethyl alcohol immediately on preparation. Slides for MGG, PAS and DPAS were air-dried before fixation.

Immunocytology was performed using monoclonal mouse anti-human CEA and CA19-9 (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) and mouse anti-human BerEP4 (DAKO, Glostrup, Denmark). The BerEP4 antigen is expressed by normal and neoplastic epithelium (including pancreatic carcinoma) but not by mesothelium [9]. Antigen unmasking with microwave irradiation (700 W for 5 min, repeated once) was used for the CA19-9 slides. A biotin/avidin immunoperoxidase ( Vectastain, Vector Laboratories, Peterborough, UK) and diaminobenzadine with 0.01% hydrogen peroxide substrate (DAB) system was used to identify bound antibody. Positive control paraffin sections of colonic adenocarcinoma (known to express CEA, CA19-9 and BerEP4) were stained at the same time that immunocytochemical preparations were made from the lavage specimens. Counterstaining with haematoxylin was performed.
The conventionally stained cytological preparations were all examined by a single consultant cytopathologist (VW). The appropriate clinical information was available as is normal practice. Immunocytological interpretation was performed by two observers blinded to the results of the conventional cytology (MJM and AW). A positive immunocytological result was defined as any positive staining cell other than a polymorphonuclear leucocyte.

The findings of metastases were all confirmed histologically, and the vascular involvement was confirmed by surgical assessment at laparotomy.

Results

All peritoneal lavage specimens were BerEP4 negative. The control paraffin sections of colonic adenocarcinoma were positive to BerEP4, CEA and CA19-9.

Of the ten patients with benign pathology, three were undergoing procedures for chronic pancreatitis and seven for cholelithiasis. One of these lavage specimens was acellular. None showed abnormal cells on conventional cytology. One patient with chronic pancreatitis had cells that showed weak positivity for CEA. The remainder showed no positivity for CA19-9 or CEA.

Ascitic fluid was found in five of the 22 patients with pancreatic or periampullary carcinoma. No malignant cells were identified using conventional cytological stains from either ascitic fluid or peritoneal lavage in the pancreatic cancer patients. No cells were recovered that were positive for BerEP4. Thirteen patients had cells recovered from the peritoneum that were positive for CA19-9 or CEA (Table 1). Four patients showed positivity for CA19-9 alone, eight for CEA alone, and one was positive to both CA19-9 and CEA. Three of the five patients with ascitic fluid had immunocytologically positive cells (1 CEA, 1 CA19-9, 1 both antigens).

Four of the patients with pancreatic or periampullary cancer had a resection. All of these were considered curative with no evidence of microscopic residual disease at the resection margins. Three had peripancreatic lymph nodes involved but none had known lymph node metastases outside this area. The distribution of metastases or areas of locally advanced disease that precluded the patient from a resection is shown in Table 1. None of the four patients having tumour resection had cells on peritoneal lavage that were positive for either CA19-9 or CEA. Nine of 14 (64%) patients with metastatic disease and all patients with locally advanced disease and vascular infiltration had cells recovered from the peritoneum that were positive for CA19-9 or CEA. The finding of cells from peritoneal lavage that were positive for either CEA or CA 19-9 was significantly associated with unresectability (Fisher’s exact test, p<0.05).

In all cases in which CA19-9 or CEA was expressed, the antigens were found both around the cell membrane and within the cytoplasm (Figure 1).

Discussion

Improvement in the preoperative staging of pancreatic and periampullary carcinoma should mean fewer patients being subjected to a laparotomy without a realistic expectation of resection. Laparoscopy before laparotomy is the only reliable means of examining for the presence of peritoneal metastases. In the absence of peritoneal deposits, ascitic fluid or peritoneal lavage fluid can be examined. Detecting scanty malignant cells amongst reactive and degenerative mesothelial cells can be difficult. It has been suggested that immunocytology may improve the detection of malignant cells [3].

There are theoretical reasons why the antigens CEA and CA19-9 may be of interest. They are frequently expressed in the malignant cells at resection. Immunocytology has shown that positive results were associated with unresectability.

Table 1. Peritoneal immunocytology in pancreatic and periampullary cancer

<table>
<thead>
<tr>
<th>Clinical findings (No. of patients)</th>
<th>No. CA19-9 positive</th>
<th>No. CEA positive</th>
<th>Proportion of patients with positive immunocytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver metastases (8)</td>
<td>2</td>
<td>4</td>
<td>5/8</td>
</tr>
<tr>
<td>Peritoneal metastases (2)</td>
<td>1</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>LN metastases* (4)</td>
<td>0</td>
<td>3</td>
<td>3/4</td>
</tr>
<tr>
<td>Vascular infiltration (4)</td>
<td>2</td>
<td>2</td>
<td>4/4</td>
</tr>
<tr>
<td>Resected (4)</td>
<td>0</td>
<td>0</td>
<td>0/4</td>
</tr>
</tbody>
</table>

*Lymph node metastases outside field of surgical resection

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in pancreatic and periampullary carcinomas and have functions as adhesion molecules. Sialyl Lewis\textsuperscript{a} (sLe\textsuperscript{a}) is the immune determinant of the tumour-associated antigen CA 19-9 and is the ligand of E-selectin, a member of the selectin family of adhesion molecules. The selectin family of molecules have been implicated in the extravasation of neutrophils in acute inflammation [10]. Attachment of tumour cells from pancreatic cancer cell lines to endothelium has been associated with sLe\textsuperscript{a} in vitro [11]. It has been suggested that sLe\textsuperscript{a} is an important mediator of haematogenous metastasis in pancreatic cancer [12]. Carcinoembryonic antigen is a member of the immunoglobulin supergene family of adhesion molecules. Paradoxically, CEA seems to have both cell adhesion and cell contact inhibitory properties [13]. CEA has also been shown to act as an accessory adhesion molecule in colon epithelial cell-collagen interactions, and the staining pattern has been shown to be predictive of malignant potential in colorectal cancer [14]. The immunocytochemical staining pattern in the pancreas for each antigen changes from being localised to the luminal surface of the normal epithelium to the basolateral membrane and cytoplasmic expression in hyperplasia and over the entire cell surface, cytoplasm and stroma in neoplasia [15]. Similar changes in CEA localisation have been noted in colonic and gastric cancers [16]. The finding of sialyl-rich tumour-associated antigens in the stroma of gastric cancer has been associated with peritoneal metastases [17]. The presence of these molecules in the tumour stroma could saturate the receptors, leading to less cell–cell and cell–endothelium adhesion. When they reach an environment in which saturation of the adhesion molecules no longer pertains, cell–cell and cell–endothelium adhesion could take place. This process could then allow the formation of a metastatic deposit.

No malignant cells on conventional cytological staining were recovered from peritoneal lavage of patients with pancreatic or periampullary carcinoma in this study. This finding is at variance with the published rate of 7–30%. Taking the lower incidence, one would have expected two patients to have positive peritoneal cytology. The absence of malignant cells on conventional cytology could be due to detailed preoperative staging with spiral CT, MRI and endoscopic ultrasound before selecting patients for laparoscopy. These patients had potentially resectable disease from their staging investigations and would therefore tend to have less disease burden than in older series where the preoperative investigation was less exacting. In addition, no patients had prior FNA or biopsy. In the study of John and associates [3], they found positive peritoneal cytology on conventional staining in seven of 46 patients, three of whom had had prior percutaneous needle aspiration or biopsy. Laparoscopic ultrasound was used as part of our staging assessment, and it demonstrated lymph node and hepatic metastases (with histological confirmation) that were not detected by the other staging modalities. This fact explains the low resectability rate in the series despite the extensive preoperative imaging.

In this study, non-epithelial cells expressing the molecules CA19-9 (sLe\textsuperscript{a}) and CEA have been recovered from the peritoneal cavity of patients with pancreatic carcinoma. Cells recovered from peritoneal lavage in patients with benign disease did not express these antigens with the exception of one patient with chronic pancreatitis who had some weakly CEA positive cells. The presence of cells expressing these antigens in pancreatic and periampullary carcinoma patients was significantly associated with irresectable disease by virtue of local advancement or secondary spread. The absence of cells expressing CA19-9 or CEA was associated with resectable disease. The presence of these antigens may represent uptake of free CA19-9 and free CEA in the peritoneal cavity by mesothelial cells and reflect the disordered expression of these antigens in the primary tumour. Caution is therefore required in the interpretation of positively staining cells for CEA or CA19-9;
they do not necessarily represent free tumour cells as they are non-epithelial (BerEP4 negative). The usefulness of automated techniques examining cells on the basis of their immunoreactivity by, for example, flow cytometry is therefore limited. The relationship of immunopositivity of peritoneal cells and irresectability of the tumour may be an epiphenomenon related to the disordered expression of these antigens in the primary tumour and their adhesion properties as outlined above.

The presence of cells from peritoneal lavage expressing the tumour-associated antigens CA19-9 and CEA does not indicate the presence of free tumour cells but is associated with tumour irresectability.

References