Implications of Peritoneal Cytology for Pancreatic Cancer Management

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Objective: To assess the implications of positive cytology for malignant cells (positive results) from peritoneal washings in the management of patients with pancreatic cancer.

Design: Retrospective cohort study.

Setting: Referral practice in a university hospital.

Patients: A total of 32 consecutive pancreatic cancer patients with positive results from peritoneal washings during a 4-year period, 17 with visible biopsy-proven intra-abdominal metastases at the time of laparoscopy or laparotomy and 15 without visible metastases. A treatment-matched control group of 30 patients was randomly selected from a group of 105 patients with negative cytology for malignant cells (negative results) from peritoneal-fluid cytology.

Interventions: Eight of 17 patients with visible metastases underwent treatment with chemotherapy, radiation, or both; 13 of the 15 patients with no visible metastases underwent further treatment, including pancreatic resection in 2 patients and external beam radiation in 13 patients (3 with intraoperative radiation therapy).

Main Outcome Measures: Time to metastases and mortality.

Results: Median survival among patients with and without visible metastasis was 7.8 months and 8.6 months, respectively ($P= .95$), despite the fact that patients without visible metastases received more treatment. Patients without visible metastases at presentation were found to have metastatic disease as documented by computed tomographic scan or subsequent laparotomy at a median time of 2.9 months. The survival of treatment-matched patients with negative cytology was significantly longer (median, 13.5 months; $P=.04$).

Conclusions: Pancreatic cancer patients with peritoneal micrometastases have a dismal outcome even without macroscopic metastases. Since these patients do not benefit from local therapy, the finding of a positive result from peritoneal-fluid cytologic testing contraindicates further irradiation or surgery, except for specific complications.

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Despite advances in operative techniques, chemotherapy, and radiation medicine, pancreatic cancer remains the gastrointestinal malignancy with the worst prognosis.1 Surgery is potentially curative for select patients with local disease, and radiation therapy may significantly prolong survival in those with localized, but unresectable, tumors.2-3 However, most of these patients subsequently develop metastatic disease. Identifying optimal candidates for aggressive treatment remains difficult.

Peritoneal washings from patients with pancreatic cancer demonstrate malignant cells in 8% to 30% of patients.4-9 While most of these malignant cells are seen in conjunction with macroscopic metastatic spread, as many as one third of the instances of micrometastasis occur in the absence of any visible intra-abdominal metastases.8 Their potential for implantation and growth and, therefore, their significance is not established. This study investigates the implications of peritoneal micrometastases by comparing the
PATIENTS AND METHODS

PATIENTS

Medical records of all patients with pancreatic cancer receiving peritoneal washings between September 15, 1991, and January 1, 1997, at the Massachusetts General Hospital, Boston, were reviewed. Out of 137 consecutive patients, 32 (23%) had positive results and biopsy-proven pancreatic cancer. These patients were being evaluated for cancer stage, initially presumed to be localized. Their ages ranged from 46 to 88 years, with a mean age of 63 years. The patients were subdivided into 2 groups: those who had visible, intra-abdominal metastases at the time of laparoscopy or laparotomy (17 patients) and those who did not have visible metastases (15 patients). Eight of the 17 patients with visible metastases underwent treatment with chemotherapy, radiation, or both, while the other 9 patients received no cancer-specific therapy. A total of 13 of the 15 patients with no visible metastases underwent further treatment, including pancreatic resection in 2 cases and external beam radiation in 13 cases (3 with intraoperative radiation therapy boost). An additional control group of 30 patients was randomly selected from a series of 105 patients with negative cytology (negative results) from peritoneal washings and pancreatic cancer treated in the same time period. To minimize a potential confounding effect of treatment, including pancreatic resection in 2 cases and external beam radiation in 13 cases (3 with intraoperative radiation therapy boost). An additional control group of 30 patients was randomly selected from a series of 105 patients with negative cytology (negative results) from peritoneal washings and pancreatic cancer treated in the same time period. To minimize a potential confounding effect of aggressive treatment when comparing patients with negative results with those with positive results and no visible metastases, patients were proportionally matched according to intraoperative radiation therapy and pancreatic resection. Survival follow-up information was available for 100% of cases.

CYTOLOGIC EXAMINATION

All peritoneal fluid washings were evaluated for malignant cells using standard cytomorphologic criteria (Figure 1). Cytomorphologic examination showed malignant cells in 27 patients and atypical/highly suspicious cells in 1 patient. Immunocytochemistry was also performed in a later phase of the study, using antibodies against the antigens cytokeratin (a positive control), CA 19-9, B72.3, carcinoembryonic antigen (CEA), and Leu-M1, according to standard techniques for frozen tissue. Fluid specimens containing cells that reacted with at least 1 antibody in addition to cytokeratin were also considered as having positive results for this study (Figure 2). Immunocytochemical staining identified 4 additional patients as having malignant cells in the peritoneal washings, 3 of these patients' results had been initially interpreted as negative for malignant cells and the other patient's results were considered suspicious for malignant cells using standard cytomorphologic criteria.

STATISTICAL ANALYSIS

Statistical analysis was performed by the Kaplan-Meier method, and survival curves were compared with the log-rank test. \( P \) values less than .05 were considered significant. All statistical analysis was performed using Stata 5.0 software.

RESULTS

Among patients with positive results, no difference in survival was observed between those with or without visible metastases (median survival time, 7.8 and 8.6 months, respectively; \( P = .95 \)) (Table 2). This survival time was notably shorter than that of patients with negative results (median survival time, 13.5 months; \( P = .04 \)) (Figure 3). At 1 year, only 34% of patients with positive cytology were alive, compared with 53% in the negative-cytology group. Among patients with positive cytology without visible metastases at presentation, metastatic disease was found by computed tomographic (CT) scan or subsequent laparotomy at a median interval of 2.9 months.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Cytologic Findings</th>
<th>No.</th>
<th>Mean Age, y</th>
<th>Age Range, y</th>
<th>Male, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cytology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible metastases</td>
<td>17</td>
<td>62</td>
<td>46-88</td>
<td>53</td>
</tr>
<tr>
<td>No visible metastases</td>
<td>15</td>
<td>64</td>
<td>53-72</td>
<td>73</td>
</tr>
<tr>
<td>Negative cytology</td>
<td>30</td>
<td>63</td>
<td>39-79</td>
<td>53</td>
</tr>
</tbody>
</table>

For patients with known metastatic pancreatic cancer, there is no treatment proven to prolong survival for more than a brief time, and surgical bypass and radiation are only offered for palliation of symptoms, usually when this cannot be achieved by other means. The data from this study show that patients with positive results from peritoneal-fluid cytology have the same brief duration of remaining life whether they have grossly visible metastatic disease and irrespective of further aggressive local treatment with resection or radiation. The observation is further underscored by the significantly better survival of the similarly treated, matched control group with negative results from peritoneal-fluid cytology. It is clear from our findings that patients with peritoneal micrometastases did not benefit from therapies aimed at local control.

Exfoliation of free malignant cells is a well-described feature of human carcinomas. Malignant outcomes of patients with positive cytology for malignant cells (positive results) from peritoneal washings who have no other detectable metastases with patients who already developed grossly visible metastatic lesions.
transformation of cells is understood to alter expression of surface adhesion molecules, releasing free cells into the peritoneum,\textsuperscript{15,16} and it is believed that microscopic occult peritoneal metastases precede the appearance of malignant cells in the abdominal fluid.\textsuperscript{17} In gastric cancer, there is a clear association of positive results from peritoneal-fluid cytology with serosal invasion and lymph node infiltration.\textsuperscript{18,19} Moreover, in gynecological malignancy, peritoneal cytology is established as a routine component of accurate cancer staging.\textsuperscript{20} Because the finding of free malignant cells in the peritoneal cavity has implications for the natural history that is identical with established metastases, we suggest that a positive test result from peritoneal-fluid cytology with serosal invasion and lymph node infiltration demonstrates single cells with strong cytoplasmic, granular staining (thin arrow). The pale nuclei of nonmalignant cells can be recognized in the background (thick arrow) (×640).

We and others have shown that laparoscopy can detect metastases not shown by high-quality spiral or thin-section CT in 22% to 73% of patients with pancreatic cancer and is thus a valuable adjunct in preoperative clinical staging.\textsuperscript{8,26-30} Peritoneal washings, obtained during laparoscopic evaluation, will uncover an additional 6% of patients with micrometastatic spread,\textsuperscript{8} and immunocytochemical techniques will nearly double the yield.\textsuperscript{31} These patients can be spared the futility, expense, and potential morbidity of treatments impossibly aimed at local control and, instead, be offered simple palliative care or participation in trials using systemic therapy. We found that the increased use of laparoscopy combined with cytology consequently led to a significant cost savings (M.A.M.; A.L.W.; Muhammad M. Mamdani, PharmD; Robert F. Seger, MBA; C.F.C., unpublished data, 1998).

**Figure 1.** Top, Peritoneal wash with negative results for malignant cells showing typical flat sheet of mesothelial cells with intracytoplasmic spaces (“windows”) (arrow) and inflammatory cells (Papanicolaou, ×500). Bottom, Peritoneal wash with positive results for malignancy showing a cluster of adenocarcinoma cells with nuclear overlapping and cytomorphic features of malignancy, including an increased nuclear to cytoplasmic ratio and nuclear membrane irregularities (arrow) (Papanicolaou, ×640).

**Figure 2.** Peritoneal washing originally evaluated as negative for malignant cells. Immunocytochemical staining for carcinoembryonic antigen demonstrated single cells with strong cytoplasmic, granular staining (thin arrow). The pale nuclei of nonmalignant cells can be recognized in the background (thick arrow) (×640).

**Figure 3.** Survival time of patients with pancreatic cancer, comparing patients (n=15) with positive test results for malignant cells (positive results) from peritoneal-fluid cytology and no metastases, patients (n=17) with positive results and visible metastases, and patients (n=30) with negative test results for malignant cells from peritoneal-fluid cytology and no visible metastases.

**Table 2. Survival of Patients With Pancreatic Cancer**

<table>
<thead>
<tr>
<th>Cytologic Findings</th>
<th>Mean Survival, mo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No visible metastases</td>
<td>7.8</td>
<td>Reference</td>
</tr>
<tr>
<td>Visible metastases</td>
<td>8.6</td>
<td>.95</td>
</tr>
<tr>
<td>Negative*</td>
<td>13.3</td>
<td>.006</td>
</tr>
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</table>

*Treatment-matched control group.
To our knowledge, there are no reported cases of pancreatic cancer patients with positive cytology surviving 3 years. Bonenkamp et al., in a study of 38 patients with intraperitoneal cancer cells from gastric carcinoma, observed similarly that none of the 18 patients with unresectable gastric cancer lived beyond 1 year, and only 10% of patients who underwent surgery for resection of their cancer were alive at 2 years. In the present study, only 34% of patients with positive cytology were alive at 1 year, compared with 53% of the group with negative cytology. The overwhelming implication is that the stage of peritoneal micrometastases lags only slightly behind gross metastatic disease, and, therefore, peritoneal washings should probably be an integral component in the management of pancreatic cancer. Even if laparoscopy is not used in the staging algorithm, peritoneal washings obtained at the time of exploration or resection may prove to be a predictor of survival as strong or stronger than other factors that have been described. 

While the ultimate goal is to improve survival of all patients with pancreatic cancer, the proclivity of this tumor to disseminate early and widely means that this goal cannot be achieved until better systemic therapies are found. For now, care is optimized by appropriately tailoring treatment to the stage of the disease. Therapies, such as surgical resection and irradiation, that confer only local benefits, are best applied to patients with truly localized cancer. The present study supports the contention that routine use of peritoneal cytology can add significantly to the accuracy of staging and strategic planning of therapy.


References:


Discussion:

Harold J. Wanebo, MD, Providence, RI: Dr. Makary, and his colleagues, have brought to our attention an area they have focused on for many years and still has some controversy. They have confirmed the significant adverse impact of a positive peritoneal cytology on outcome in patients with cancer of the pancreas. They demonstrated that the median survival in patients with occult peritoneal metastases is essentially equivalent to that which accompanies macroscopic disease and is about 8 months which is much less than a historic control which would be around 13 months survival. They have demonstrated the tremendous negative impact on short-term survival in these patients in which after therapy was initiated was only a median of about 3 months in about 11 out of 15 patients. They conclude that the presence of positive peritoneal cytology would contraindicate further treatment, at least surgery and perhaps radiation for those patients. They have raised many questions. First of all, in the past the authors have pointed out that there seems to be a correlation between the performance of a diag-
nostic fine-needle aspiration cytology of the pancreas preoperatively with positive test results for malignant cells (positive results) from peritoneal-fluid cytology and a correlated negative outcome. My question here is, do you still believe this or are these rather unrelated happenstance findings, that is, a fine-needle biopsy has nothing to do with the positive cytology?

The second question relates to their group of the patients with occult metastases. As I looked at their data, only 2 of these 15 patients had resection. Does this mean these patients had locally advanced disease and the cytologic results were just a surrogate marker of what appeared to be obvious locally advanced disease even though there were no metastases? In that case, then it is a marker for ______ disease. They are correct that it should be considered an important factor in classifying these patients as having M-1 disease.

Third, in the patient who truly has resectable disease and has positive cytology, would you deny a resection in that patient? Do you think that there is a role of neoadjuvant therapy in patients with positive cytology who might have an otherwise favorable local disease that might be amenable to resection?

Last, one of the problems that comes up is in diagnosing positive cytology: Do you recommend the laparoscopic approach in all of these patients, which I think is certainly adaptable in many cases, or can this be achieved with image-directed cytology, saving those patients unnecessary laparoscopy?

Richard Swanson, MD, Worcester, Mass: I have 2 questions. First, do you have any long-term survivors in that group of 15 patients without visible metastases? You talked about the median survival of 8 to 9 months. Second, in the negative cytology group, median survival time is 13 months. What median survival time do you consider necessary before you think treatment is beneficial? I believe you think 8 to 9 months is too short, but evidently 13 months is long enough.

Thomas Colacchio, MD, Lebanon, NH: Two other questions. The first has to do with your method of diagnosis. Were there any false-positive results using the immunocytochemical technique for diagnosis? And the second is, is there any relationship between standard staging and the probability that someone will have a positive cytology?

Dr Makary: I will respond first to Dr Wanebo’s comments. There does appear to be some potential harm from fine-needle–aspiration biopsy of pancreatic cancer demonstrated. Our first report noted that a positive cytology was found in 6 of 8 patients who had received fine-needle aspiration, significantly more than the comparative group of patients who had not. We continue to suggest that fine-needle aspiration should be considered with caution and perhaps avoided if not necessary to deciding on a course of treatment.

In regard to the question of whether resection should be denied if the cytology is positive, I will refer that question to Dr Warshaw. It seems logical, however, that systemic therapy is most appropriate because these patients are properly categorized as having metastatic disease.

The finding of positive peritoneal cytology in pancreatic cancer was first described at the Mayo Clinic, Rochester, Minn, in 1986. They reported that 23% of their patients had a positive cytology. Our experience at the Massachusetts General Hospital is similar, ranging between 17% and 30% in different time periods.

There are no long-term survivors in this group. Two patients were still alive at the end of the study period, but both had either positive margins or lymphatic metastases.

There were no false-positive results. Immunocytochemistry appears thus far to be 100% specific, but the sensitivity of our technique is surely less than that. The problem is that there is no alternative benchmark.

At this point I would like to turn to Dr Warshaw to comment on his experience.

Dr Warshaw: Let me first be a little bit out of order and express my pride in the presentation you just heard. Martin is a fourth-year medical student, and I think you’ll agree his is really very sophisticated work.

Dr Wanebo, as you know, we did report a relationship between percutaneous needle aspiration and subsequent positive peritoneal cytology. We continue to be concerned about that. In our latest series of 200 patients, the correlation had a P value of .07. The reduced prevalence may have been influenced by our current practice to radiate prior to biopsy to reduce the chance of dissemination. Dissemination, whatever the cause, is probably an adverse circumstance.

In answer to both Dr Colacchio and Dr Wanebo, there does appear to be a relationship between positive cytology and other aspects of locally advanced disease. In other words, a smaller tumor, perhaps 2 cm or less, is very unlikely to have a positive cytology. Tumors that have other evidence of locally advanced disease, such as retroperitoneal extension to the superior mesenteric vein shown by CT, are more likely to have positive cytology. That factor is reflected in the selection of the patients who undergo laparoscopy, as was noted by Dr Swanson. Most of the patients with positive peritoneal cytology are not resected because they have locally advanced disease. We have not seen a benefit to neoadjuvant therapy in this setting, but we have not studied it in a systematic way. The role of neoadjuvant therapy in downstaging pancreatic cancer is still very uncertain.

More than 17% of our resected patients become long-term survivors, but that is obviously a very different group of patients than those included in this study.