Genes and surgery in pancreatic cancer
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Usefulness of conventional cytologic and molecular analyses for detection of peritoneal spread of periampullary cancer

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Background
Standard staging procedures of periampullary tumors do not identify all patients with an adverse outcome despite apparently successful resection. The purpose of this study was to determine whether cytologic examination and K-ras mutation analysis of intraoperative abdominal washings obtained from patients with periampullary carcinoma could identify a subset of patients with a worse prognosis.

Methods
Between May 1998 and May 1999 136 patients with a periampullary tumor underwent exploration with intent to undergo surgical resection. Abdominal washings were obtained prior to (n=134) and following (n=83) surgical resection. The washing fluid was evaluated by conventional cytology and for mutations in the K-ras oncogene mutation analysis using a PCR and ASO hybridization based methodology. The results were correlated with the final diagnoses and survival of the patients.

Results
Of the 136 patients, 94 underwent a pancreaticoduodenectomy and 42 a palliative surgical procedure. In total, eight of the 134 (6%) pre-manipulation washings were positive for malignancy by cytology, 13 (10%) were atypical, and 111 (84%) were negative. Four of 134 (3%) pre-manipulation washings harbored K-ras gene mutations and 2 of these 4 were positive by cytology. Two (2%) of the 83 post-resection washings were positive for malignancy by cytology, 8 (10%) were atypical, and 71 (88%) were negative. Two (2%) of the 83 post-resection washings harbored K-ras gene mutations, both negative for malignancy by cytology. While patients with a positive cytology had a significantly shorter survival than patients with a negative cytology, no survival differences based on cytology were found within the resected and unresected subgroups. Patients with a positive cytologic abdominal washing were significantly more often found to be unresectable than patients with an atypical or a negative abdominal cytologic washing.

Conclusions
Positive peritoneal cytology in intraoperative abdominal washings from patients who undergo explorative laparotomy for periampullary cancer is rare. The overall value of positive peritoneal cytology was low in this relatively small series. However when it is present it does convey a bad prognosis and often indicates advanced disease characterized by unresectability. K-ras gene mutation analysis was of no additional value in this regard.
INTRODUCTION

Periampullary cancer comprises pancreatic cancer, distal common bile duct cancer and ampullary cancer, all for which resection is the only curative option. Pancreatic cancer ranks fifth in cancer-related mortality in men and women. The treatment of invasive pancreatic cancer has progressed substantially in the last three decades, however the overall national 5-year survival rate has remained low, being 3% in 1974 and rising only to 4% in 1995. Survival for pancreatic cancer depends on stage and extent of disease. Analysis of a series of 616 patients undergoing resection for pancreatic cancer at The Johns Hopkins Hospital revealed an overall 5-year survival of 17%. Yet, a subset of these patients with localized small tumors and negative lymph nodes were found to have a 5-year survival rate of 40% following resection.

Given the critical role of accurate staging in determining prognosis, and the finding that standard staging procedures do not identify all patients who ultimately succumb despite apparently successful resection, there is a need to identify factors that predict adverse outcome in resected patients. Such data would identify those patients who might benefit from more aggressive adjunctive therapy, or potentially should not be resected. Important prognostic features for patients with pancreatic adenocarcinoma include tumor size, margin and node positivity, degree of differentiation and DNA ploidy. Several studies have found that positive peritoneal cytology obtained during preoperative laparoscopy was associated both with metastatic disease at the time of resection and a worse prognosis if resected. In one of the studies conducted at the Massachusetts General Hospital the survival of 15 patients with positive peritoneal cytology and no visible metastases was compared to a treatment-matched control group. They found a markedly decreased survival in the presence of positive cytology and conclude that a positive cytology, even without visible metastases, contraindicates further surgery or irradiation.

The goal of this study was to evaluate the results of cytologic analysis in abdominal washings and to extend the work of the other groups by determining whether molecular analysis for activating point mutations in the K-ras gene would increase the sensitivity of cytologic examination of peripancreatic washes obtained from patients with periampullary cancer. Activating point mutations in codon 12 of the K-ras oncogene have been identified in 80-90% of pancreatic carcinomas making K-ras gene mutations a potentially sensitive marker for cells shed from pancreatic cancer. The identification of neoplastic cells in abdominal washing fluid by cytology or the presence of mutant DNA could indicate more extensive disease than that detected by conventional means.
MATERIALS AND METHODS

Patients
Between May 1998 and May 1999 136 patients underwent exploratory laparotomy for possible pancreaticoduodenectomy for a periampullary tumor. One hundred and thirty-four patients participated in this study after giving appropriate informed consent as approved by the Johns Hopkins Joint Committee for Clinical Investigation. Preoperative evaluation in patients with suspected periampullary adenocarcinoma consisted of abdominal computed tomography scanning, cholangiography, and magnetic resonance imaging as clinically indicated. Diagnostic laparoscopy was not used in any of the patients.

Specimen collection
Immediately upon entering the peritoneum at laparotomy 200 mL of normal saline was introduced into the peripancreatic area and gently agitated. After ten minutes dwell time the entire volume was collected from the subphrenic area prior to tumor manipulation. Subsequently, any suspicious sites of liver metastases or peritoneal implants were biopsied and frozen section diagnosis was obtained. If after complete evaluation the tumor was found to be resectable, pancreaticoduodenectomy was completed. After resection, the peritoneal washing procedure was repeated.

Cytologic evaluation
The collected specimens were submitted for cytologic evaluation, 200 cc was spun down for 10 minutes, and four cytospin slides were prepared. Two were stained using the conventional Giemsa and Papanicolaou methods, and were evaluated by a staff pathologist from the Johns Hopkins Medical Institutions (RHH) and a staff pathologist from the Academic Medical Center Amsterdam (GJAO). The cytologic criteria utilized to declare a specimen positive, negative or atypical, respectively, were as follows. In a "positive" or malignant sample the cells are disordered and crowded with loss of polarity and cell cohesion with sometimes acinar groups. The nuclei are conspicuously enlarged and show anisokaryosis, chromatin clumping and an irregular nuclear membrane (figure 1). In a "negative" or benign sample the cells are typically grouped in monolayers or palisading strips with a low nucleus cell ratio, demonstrating no atypia. The nuclei have a fine chromatin pattern and smooth nuclear membranes (figure 2). In an "atypical", indefinite or suspicious sample the cells show some of the malignant features mentioned above, but only in a few single cells or insufficiently outspoken (figure 3). The other two slides were airdried for K-ras gene analysis on material equivalent to that examined morphologically. The results of the abdominal washings were not used to plan further treatment.
Figure 1
(A) Periampullary wash positive for adenocarcinoma with crowded tissue fragments and marked pleomorphism
(B) Corresponding pathology of the resection specimen showing pancreatic adenocarcinoma (25, page 258)

Figure 2
(A) Periampullary wash negative for adenocarcinoma with a low nuclear : cytoplasmic ratio, smooth nuclear borders, and lack of tissue fragments
(B) Corresponding pathology of the resection specimen showing pancreatic adenocarcinoma (25, page 258)

Figure 3
(A) Periampullary wash atypical for adenocarcinoma with crowded tissue fragments and irregular nuclear borders
(B) Corresponding pathology of the resection specimen showing pancreatic adenocarcinoma (25, page 258)
Clinical data
Tumor type and size, margin and lymph node positivity were determined by standard pathologic analysis. Follow-up information including survival, recurrence, and death was collected from the prospectively collected pancreaticoduodenectomy database from the Department of Surgery at the Johns Hopkins Medical Institutions.

K-ras gene mutation analysis
Point mutations in codon 12 of the K-ras oncogene were detected by mutant-enriched polymerase chain reaction (PCR) and allele-specific oligonucleotide (ASO) hybridization as described previously. Extracted DNA from two cytospin slides were subjected to PCR amplification using primers centered around codon 12. One of the primers introduces a restriction site in the PCR products derived from wild-type codon 12 alleles but not in those derived from mutant codon 12 alleles. Digestion of the PCR products was followed by a second round of PCR amplification which yields a PCR product enriched for K-ras codon 12 mutations. The resulting PCR products were denatured and dot-blotted onto nylon membranes. On each membrane the non-enriched PCR products were represented in the left lane and the mutant-enriched PCR products in the right lane. The membranes were then subjected to ASO hybridization with radioactive labeled probes, specific for the wild type codon 12 and the six possible K-ras codon 12 mutations, followed by autoradiography (figure 4). The above mutational analysis has been validated through comparison with sequence analysis in a previous study. The K-ras gene mutations in the intraoperative cytology were confirmed in the primary tumor if resected.

Figure 4 Autoradiograph of a K-ras codon 12 point mutation analysis performed in a pre-manipulation peritoneal washing. Four nylon membranes, each hybridized with a different radioactive labeled oligonucleotide specific for the sequence of the wild type codon 12 (left) and six possible K-ras codon 12 mutations, followed by autoradiography (figure 4). The above mutational analysis has been validated through comparison with sequence analysis in a previous study. The K-ras gene mutations in the intraoperative cytology were confirmed in the primary tumor if resected.

<table>
<thead>
<tr>
<th>CO</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Arg</td>
<td>Val</td>
<td>Asp</td>
<td></td>
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</tbody>
</table>

Figure 4: Autoradiograph of a K-ras codon 12 point mutation analysis performed in a pre-manipulation peritoneal washing. Four nylon membranes, each hybridized with a different radioactive labeled oligonucleotide specific for the sequence of the wild type codon 12 (left) and six possible K-ras codon 12 mutations, followed by autoradiography (figure 4). The above mutational analysis has been validated through comparison with sequence analysis in a previous study. The K-ras gene mutations in the intraoperative cytology were confirmed in the primary tumor if resected.
Statistical analysis

Results of the peritoneal washings were related to the final diagnoses and the patients' follow-up. Chi-square and Fisher exact tests were performed to determine whether there were associations among the results in cytology, K-ras, resectability, positive nodes, and positive margins at surgery. Kaplan-Meier survival analyses were performed to determine whether the following factors affected survival: positive margins at surgery; positive nodes at surgery; positive cytology and/or K-ras gene mutation. Log-rank statistics were computed to determine whether differences in survival were significant. All data were analyzed with SPSS version 10.0 for Windows.

RESULTS

Of the 136 patients, 94 patients (69%) underwent pancreaticoduodenectomy. Final histologic analysis showed that two (2%) patients were resected for a benign disease which appeared to be pancreatitis. From the 92 patients resected for a malignancy, 65 patients were margin negative and 22 patients were lymph node negative. The final diagnoses in the 94 resected patients are listed in table 1. Forty-two (31%) patients were unresectable. The reasons for unresectability were local invasion of major visceral vessels in 29 (69%) patients, liver metastases in 9 (21%) patients, and peritoneal metastases in 4 patients (10%).

<table>
<thead>
<tr>
<th>Table 1 Final pathologic diagnoses of the 94 resected patients with the concomitant findings of positive cytology and K-ras mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients (%)</strong></td>
</tr>
<tr>
<td>total (100%)</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
</tr>
<tr>
<td>Distal common bile duct adenocarcinoma</td>
</tr>
<tr>
<td>Ampullary adenocarcinoma</td>
</tr>
<tr>
<td>Duodenal adenocarcinoma</td>
</tr>
<tr>
<td>Benign disease (pancreatitis)</td>
</tr>
</tbody>
</table>

A total of 217 specimens underwent cytologic analysis, of which 134 specimens were obtained from the original washings prior to tumor manipulation, and 83 specimens were obtained after the resection was completed. In total, 10 (5%) specimens were cytologically positive for malignant cells (8 pre-manipulation, 2 post-resection) belonging to four patients with stage II disease, to two patients with stage III disease and to four patients with stage IV disease who were found to be unresectable during laparotomy. Of the 134 pre-manipulation washings, eight (6%) were positive for malignancy by cytology, 13 (10%) were atypical, and 111 (84%) were negative (table 2). Analysis of the eight patients with
Table 2 Results of conventional cytology and K-ras codon 12 mutation analysis in abdominal washings of all patients

<table>
<thead>
<tr>
<th></th>
<th>Pre-manipulation washings (n=134)</th>
<th>Post-resection washings (n=83)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive cytology</td>
<td>Atypical cytology</td>
</tr>
<tr>
<td>K-ras codon 12 mutation</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>K-ras codon 12 WT</td>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>

a positive pre-manipulation washing showed that 6 (75%) were unresectable. Pre-manipulation cytology was positive in 3 of 9 patients (33%) with liver metastases, 1 of 4 patients (25%) with peritoneal metastases, and 2 of 29 patients (7%) with local visceral vessel invasion. The final pathological diagnoses in the two resectable patients with positive pre-manipulation cytology were pancreatic carcinoma and ampullary carcinoma, both moderately differentiated, margin negative stage II tumors.

Of the 83 post-resection washings, two (2%) were positive for malignancy by cytology. In neither case was the pre-manipulation cytology positive. The final diagnoses in these patients were a poorly differentiated distal common bile duct carcinoma and a moderately differentiated pancreatic head carcinoma, both margin positive stage II tumors.

Of the original 217 cytologic specimens tested for K-ras gene mutations, six (3%) demonstrated mutations. Of these six specimens, four were obtained from the 134 pre-manipulation washings (3%), and two were obtained from the 83 post-resection washings (2%) (table 2). One sample did not amplify, and the remaining 210 samples were K-ras wild type. Of the 80 cases with both pre- and post-resection washings, four cases showed a positive cytology (n=2) or a K-ras gene mutation (n=2) in the post-resection washings only.

Five of the K-ras mutation positive specimens were negative on all cytologic analyses, whether from pre- or post-resection washings. Only two patients with a positive K-ras gene mutation underwent surgical resection. Both patients are alive without evidence of disease at 52 and 56 months' follow-up. The other three patients were found to be unresectable and underwent palliative surgery and died of their disease at 8, 11 and 11 months after diagnosis.

Analysis of the subgroup of 53 resected patients with a final histologic diagnosis of pancreatic carcinoma revealed that of the pre manipulation washings one (2%) was positive for malignancy by cytology, three (6%) were atypical, and 49 (92%) were negative. None of the pre-resection washings showed a K-ras gene mutation. Of the 38 post-resection washings, one (3%) was positive for malignancy by cytology, 5 (13%) were atypical, and 32 (84%) were negative. One
(3%) of the post-resection washings showed a K-ras gene mutation, belonging to the patient still alive after 52 months.

Of 22 patients with negative nodes after surgical resection, none showed K-ras gene mutations, and none was cytologically positive on pre- or post-resection washings (table 3). By contrast, of 70 resected patients with positive nodes at surgery, two patients showed a K-ras gene mutation in pre-resection washings, and two patients showed a K-ras gene mutation in post-resection washings. Two patients were cytologically positive on pre-resection washings, and two were cytologically positive on post-resection washings (table 3).

Table 3 Results of K-ras codon 12 mutation analysis and conventional cytology compared to node status in the 92 resected patients with a periampullary carcinoma

<table>
<thead>
<tr>
<th>Pre-manipulation washings (n=92)</th>
<th>Node positive (n=70)</th>
<th>Node negative (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive cytology</td>
<td>Atypical cytology</td>
</tr>
<tr>
<td>K-ras mutation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K-ras WT</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Positive cytology</td>
<td>Atypical cytology</td>
</tr>
<tr>
<td>K-ras mutation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K-ras WT</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post-resection washings (n=78)</th>
<th>Node positive (n=59)</th>
<th>Node negative (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive cytology</td>
<td>Atypical cytology</td>
</tr>
<tr>
<td>K-ras mutation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K-ras WT</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Positive cytology</td>
<td>Atypical cytology</td>
</tr>
<tr>
<td>K-ras mutation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K-ras WT</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Six primary tumors were available after either biopsy (n=4) or resection (n=2) from patients with a K-ras gene mutation in their peritoneal washing. In 5 of the 6 cases the mutation found in the peritoneal washing was identified in the primary tumor, in the sixth case the primary tumor was wild type at K-ras codon 12.

In the total group of 134 included patients, 2 of 8 periampullary cancers (25%) with positive cytology taken prior to resection were resectable as compared to 90 of 124 patients (73%) with negative or atypical cytology (Chi-Square p=0.011). There was also an association between K-ras codon 12 mutation and the resectability (Fisher’s Exact test, p=0.007). In the resected group of 94 patients there were no associations found, especially not between cytology or K-ras gene mutation and margin status (Fisher’s Exact test, p=1.0).

Survival analysis of all 132 patients with a periampullary carcinoma and available pre-manipulation cytologic data demonstrated median survival of 13, 15, and 6 months for patients with cytologically negative, atypical, and positive washings, respectively. Overall, these survival functions differed significantly (log rank=10.09, p=0.006) (figure 5). Given the equality in survival times for the
Figure 5 Survival curves for all patients with a periampullary carcinoma (n=134) with negative, atypical, and positive cytological examination of pre-manipulation specimens.

Figure 6 Survival curves for the resected patients with a periampullary carcinoma (n=92) with negative, atypical, and positive cytological examination of pre-manipulation specimens.
cytologically negative and atypical groups, the statistical significance of the differences in estimated survival times for the negative/atypical group, and for the positive group is more evident (log rank=10.14, p=0.0015). There was, however, no statistical difference in survival based on post-resection washings (p=0.33).

Survival analysis of the 92 patients with a periampullary carcinoma who underwent pancreaticoduodenal resection demonstrated median survival of 20, 20, and 6 months for patients with cytologically negative, atypical and positive pre-resection washings, respectively. The two patients in the resected group with a positive peritoneal cytology had a median survival of 6 and 12 months. Although a trend was seen towards a worse survival for patients with positive peritoneal cytology, the survival functions did neither differ significantly overall (log rank=3.75, p=0.15) (figure 6), nor differ significantly after combining the negative and atypical groups (log rank=3.67, p=0.055). The small number of samples harboring a K-ras gene mutation precluded survival analysis based on this factor alone. In particular, we could not demonstrate that positive K-ras mutation analysis significantly increased the predictive value of cytological examination when the two were combined. This finding is confirmed qualitatively by our observation that two out of five patients with K-ras gene mutations and negative cytologic evaluation were still alive at latest follow-up.

Histological grade of the carcinoma, size of the primary tumor, number of positive nodes, and status of surgical margins were examined for their prognostic significance. A significantly better prognosis was found in resected patients with negative surgical margins (log rank=10.78, p=0.001) and negative lymph nodes (log rank=3.69, p=0.05).

**DISCUSSION**

This study shows that the incidence of a positive cytology for malignancy in perioperative peritoneal washings in patients who undergo an exploratory laparotomy for a periampullary tumor is low. But when it is present it does convey a bad prognosis. Six of the eight (75%) patients with a positive peritoneal cytology were found to be unresectable, and the other two (25%) patients did poorly with a median survival of 6 and 14 months. Regarding all 132 specimens taken prior to manipulation, the median survival was significantly longer in patients with a cytologically negative (13 months) or atypical washing (15 months) compared to patients with a cytologically positive washing (6 months). Regarding the subgroup of 92 specimens taken prior to manipulation from patients with a periampullary carcinoma that underwent resection, the median survival was, although not statistically, longer in patients with a cytologically negative (20 months) or atypical washing (20 months) compared to patients with a cytologically positive washing (6 months).

Malignant cells were found in 6% of 134 pre-manipulation washings, which is similar to the prevalence of 3% to 7% described in some series where peritoneal washing was performed during staging laparoscopy. However, other studies have described a prevalence of 17% to
Table 4: Overview of published series on the diagnostic use of peritoneal cytologic washings in patients with periampullary cancer

<table>
<thead>
<tr>
<th>Series</th>
<th>Year</th>
<th>Patients (n)</th>
<th>Positive cytology (%)</th>
<th>Prognostic significance (independent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meszoely et al.</td>
<td>2004</td>
<td>168</td>
<td>27 (16)</td>
<td>yes (no)</td>
</tr>
<tr>
<td>Yachida et al.</td>
<td>2002</td>
<td>251</td>
<td>53 (21)</td>
<td>no (no)</td>
</tr>
<tr>
<td>Konishii et al.</td>
<td>2002</td>
<td>151</td>
<td>36 (24)</td>
<td>yes (no)</td>
</tr>
<tr>
<td>Jiminez et al.</td>
<td>2000</td>
<td>117</td>
<td>24 (21)</td>
<td>yes (n/a)</td>
</tr>
<tr>
<td>Van Eijck et al.</td>
<td>1999</td>
<td>66</td>
<td>5 (8)</td>
<td>no (no)</td>
</tr>
<tr>
<td>Nakao et al.</td>
<td>1999</td>
<td>228</td>
<td>34 (15)</td>
<td>yes (no)</td>
</tr>
<tr>
<td>Merchant et al.</td>
<td>1998</td>
<td>449</td>
<td>28 (6)</td>
<td>yes (no)</td>
</tr>
<tr>
<td>Van Dijkum et al.</td>
<td>1995</td>
<td>60</td>
<td>4 (7)</td>
<td>n/a</td>
</tr>
<tr>
<td>Leach et al.</td>
<td>1995</td>
<td>94</td>
<td>16 (17)</td>
<td>n/a</td>
</tr>
<tr>
<td>Fernandez-del Castillo et al.</td>
<td>1994</td>
<td>24</td>
<td>3 (12)</td>
<td>n/a</td>
</tr>
<tr>
<td>Rall et al.</td>
<td>1994</td>
<td>36</td>
<td>3 (8)</td>
<td>yes (n/a)</td>
</tr>
<tr>
<td>Lei et al.</td>
<td>1991</td>
<td>40</td>
<td>12 (30)</td>
<td>yes (n/a)</td>
</tr>
<tr>
<td>Warshaw</td>
<td>1986</td>
<td>23</td>
<td>5 (22)</td>
<td>n/a</td>
</tr>
<tr>
<td>Martin &amp; Goellner</td>
<td>2004</td>
<td>132</td>
<td>8 (6)</td>
<td>yes (no)</td>
</tr>
</tbody>
</table>

29% of malignant cells in patients with pancreatic head malignancies found during diagnostic laparoscopy. Table 4 summarizes the published data on peritoneal cytology in periampullary cancer. Several studies are performed at the same institution but nevertheless do have differences in published percentages of positive cytology. This is not surprising regarding the varying circumstances under which peritoneal cytology was performed (during laparotomy or laparoscopy), the different years of publication (between 1991 and 2000) and the different groups and number of patients studied. The reported differences in incidence of positive cytology may reflect different standards for peritoneal cytology collection and examination, different specimen-preparation methods, and different patient-selection criteria. For example, 57% of included patients in current study had a final diagnosis of pancreatic head adenocarcinoma, while most publications on this issue concern patients with pancreatic head carcinoma only. In our study examination of peritoneal cytology, in particular from preoperative washings, could identify a subset of periampullary cancer patients with decreased life expectancy. Furthermore, cytology findings provided an additional index of resectability. This finding confirms the results from other studies on the impact of cytological examination of abdominal washings on prognosis for periampullary cancer patients. The lack of statistical significance in the survival difference between resected patients with a positive cytology and resected patients with a negative or atypical cytology is probably due to a type II error. The trends seen are identical with those in literature and our findings may only represent the small number of patients in the individual subgroups. From all other factors examined, only the margin and lymph node status provided prognostic information in the resected patients, as has been noted in our previous reports. Once the margin
status is known for a particular patient, knowledge of the other factors does not yield additional prognostic significance regarding life expectancy for the subjects in this study.

Forty-two of the 136 (31%) included patients appeared to be unresectable during explorative laparotomy. Although all patients underwent an extensive preoperative workup consisting among other things of abdominal computed tomography, local invasion of major visceral vessels was the predominant cause for unresectability in the majority of 29 (69%) unresectable patients. This is a feature that should and probably could have been anticipated with more rigorous preoperative evaluation. In a recent study from our institution we evaluated the impact of preoperative 3D-computed tomography (3D-CT) in determining the resectability of patients with periampullary tumors. It turned out that 3D-CT was 95% accurate in determining cancer invasion of the superior mesenteric vessels.15

The finding of a mutation in codon 12 of the K-ras gene in peritoneal cytology is scarce, even in a large series of 213 specimens. Molecular analysis for K-ras did identify five cytologically negative patients with mutant DNA present in abdominal washing fluid. However, due to the small number of cytology samples that were positive by molecular analysis we could not determine whether or how the presence of a K-ras gene mutation would have an effect on the prognosis, either alone or in conjunction with conventional cytological analysis of washings.

It is important to note that there were two conversions of K-ras wild type in pre-manipulation washings to K-ras mutant in post-resection washings, raising the possibility of intraoperative spillage of mutant DNA into the abdominal fluid. The primary tumors of these two cases contained the same mutation in the K-ras codon 12 gene as were found in the post-resection washings of these patients. Since these patients were still alive at latest follow-up at 52 and 56 months after resection, if intraoperative DNA spillage did occur, at least it did not appear to affect survival in this limited analysis. Six primary tumors were available from patients with a K-ras gene mutation in their peritoneal washing. Five of the six K-ras mutations found in the peritoneal washing were confirmed in the primary tumor. The wild type K-ras codon 12 demonstrated in the sixth primary tumor might be due to sampling error. In one of the largest series known on K-ras mutational analysis by mutant-enriched PCR and ASO hybridization on cytology specimens a sampling error was seen in 7 out of 312 cytology specimens.36 Whether a sampling error was involved or not we do think that K-ras mutational analysis of peritoneal cytology in patients undergoing an exploratory laparotomy for a periampullary tumor is of dubious value.

We have demonstrated similar life expectancies for patients with negative and atypical cytology in pre-manipulation washings. This finding may reflect a conservative system for interpreting cytology specimens, with strict standards for identifying a specimen as negative (i.e. completely normal) or positive (i.e. definitely malignant), and a third, intermediate category reflecting failure to meet these strict criteria. It appears that the prognosis of patients with atypical specimens will
be comparable to the prognosis of their counterparts with negative specimens.

In conclusion, this study demonstrates the low rate of malignant cells in perioperative peritoneal washings from patients undergoing an exploratory laparotomy for a periampullary tumor. However, malignant cells in perioperative washings do convey a bad prognosis and patients with a positive cytology have a shorter median survival than patients with an atypical or negative cytology. The rate of K-ras codon 12 mutations in peritoneal cytology is even lower, and the mutational status did not always correlate with the primary tumor. These data suggest little role for standard conventional cytology or K-ras mutation analysis of peritoneal fluid in patients with potentially resectable periampullary malignancies.

REFERENCES


