Etiologic and clinical studies in primary sclerosing cholangitis
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Value of brush cytology for dominant strictures in primary sclerosing cholangitis


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Chapter X

SUMMARY

Background and study aims: Around 10% of patients with primary sclerosing cholangitis (PSC) develop cholangiocarcinoma, which is cholangiographically often indistinguishable from a benign dominant stricture. The aim of the present study was to assess the value of brush cytology in discriminating between benign and malignant dominant strictures in primary sclerosing cholangitis.

Patients and Methods: The results of all brush cytology specimens from dominant strictures from patients with established primary sclerosing cholangitis taken, at endoscopic retrograde cholangiopancreatography between 1987-1996, were compared with histological diagnosis or clinical status of the patients at least 2 years later.

Results: A total of 47 brush cytology samples, taken between 1987 and 1996, from 43 PSC patients could be included. Between 1993-1996 p53 immunocytochemical examination was done in 27 brush cytology specimens and K-ras mutation analysis in 25 patients. The sensitivity, specificity, positive predictive value, and negative predictive value of brush cytology for detection of malignancy were 60%, 89%, 59%, and 89%, respectively. These figures were not improved by adding the results of p53 and K-ras analysis. Logistic regression analysis did not reveal any additional benefit of p53 or K-ras analysis either. Prior stenting did not adversely affect specificity.

Conclusions: The sensitivity and positive predictive value of brush cytology for dominant strictures in PSC are rather poor. The specificity and negative predictive value are reasonably good. There was no additional value from p53 immunocytochemistry and K-ras mutation analysis. Prior stenting did not affect the results.
Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease of the biliary tree, whose etiology is unknown. The fibrosing inflammation leads to strictures which, when they arise in the extrahepatic bile ducts and are tight enough to impede biliary flow and increase cholestasis, are called dominant strictures. The most dismal sequela of PSC is the occurrence of cholangiocarcinoma. In larger series the incidence is estimated to be 6-8%, but this may be an underestimate as not all of the patients in these studies who died of liver failure have been autopsied (1-3). Following a diagnosis of cholangiocarcinoma the prognosis is in general very poor, and the median survival is reported to be only five months, regardless of therapy (4). The only treatment which may offer a chance of cure is orthotopic liver transplantation (OLT). However, even when a cholangiocarcinoma is found incidentally in the explanted liver, the 1-year survival rate after OLT is only 30% in most series (5-7), with the exception of that of Abu-Elmagd et al. who reported a 5-year survival rate of 26.7% (8). Most transplantation centers will reject PSC patients with known cholangiocarcinoma because of the general opinion that these poor outcomes do not outweigh the substantial morbidity and mortality that accompany OLT. This highlights the importance of accurate diagnosis whenever a suspicious dominant stricture is encountered, as well as that of early detection which might improve the above-mentioned survival rates.

The difficulty in diagnosing cholangiocarcinoma in PSC is that its presenting symptoms and cholangiographic appearance often are quite similar to those of a benign dominant stricture, see figure 1. The mainstay of discrimination between benign and malignant dominant strictures is brush cytology. However, the reliability of brush cytology is somewhat doubtful. The sensitivity of brush cytology in the detection of cholangiocarcinoma in general is reported to be around 50% (9-13). With regard to PSC-related dominant strictures, its value has, so far, not been established.

Recently, p53 tumor suppressor gene overexpression has been demonstrated in tumor tissue from 11 of 14 PSC patients with cholangiocarcinoma and not in liver tissue of PSC patients without cholangiocarcinoma (14). Moreover, several groups have described K-ras mutations at codon 12 in cholangiocarcinomas (15, 16). Detection of these tumor markers may putatively enhance the accuracy of brush cytology.
The aim of this study was to assess the value of brush cytology in discriminating between benign and malignant dominant strictures in PSC. The possible role of p53 overexpression and K-ras codon 12 permutation measurements was also evaluated.
PATIENTS AND METHODS

Between 1987 – 1996, 108 patients with established PSC underwent one or more endoscopic retrograde cholangiopancreatography (ERCP) procedures at our department. Brush cytology samples were taken from dominant strictures on 63 occasions in 43 patients. When the occasions of brush cytology sampling in one patient were separated by more than 2 years from each other, the findings were regarded as unrelated. This resulted in 47 assessable brush preparations.

A dominant stricture was defined as a narrow stricture arising either in the common bile duct, the common hepatic duct, or the main hepatic duct to the left or right liver lobe. In most cases, the indication for carrying out ERCP was suspicion of a symptomatic dominant stricture, with or without jaundice. In 12 out of 47 cases the brush cytology specimen was taken from a stricture in which a 10-Fr polyethylene endoprosthesis had been in situ within 3 weeks prior to the sampling.

Brush cytology specimens were sampled with a Geenen brush (Wilson-Cook Medical Inc., Winston-Salem, NC, USA) which has a length of 13 mm and a radial diameter of 3 mm, and they were immediately immersed in 5 ml of Roswell Park Memorial Institute solute. Shedding of the epithelial cells was promoted by vortexing for 1 minute. Several cytospin preparations on organosilan-coated slides were made and stained with Giemsa or PAP stains. Cytospin slides for tumor markers were wrapped in alufoil and stored at -20 °C.

From 1993 through 1996, nuclear overexpression of p53 protein was analyzed by immunocytochemistry on 27 brush specimens, using the rabbit polyclonal antibody CM1, as previously described (17). Results were scored in a blinded fashion. Sections of colon tumor with known p53 mutation served as positive controls. As a negative control, one slide of each patient was put through the same procedure but leaving out the primary antiserum.

From 1993 through 1996 25 brush specimens underwent K-ras mutation analysis by means of a two-step polymerase chain reaction technique as previously described (18). The results were again scored in a blinded fashion. Cell suspensions with mutant : wild type ratios of 1:100 and 1:1000 served as positive controls. Cells from the human colon cancer cell line SW 480 with a homozygous GGT to GTT mutation at codon 12, were taken as mutant in these suspensions. For the wild type control cells, the human colon cancer cell line HT 29 was applied. Water was used as control for contamination and placental DNA for aspecific hybridization. All PCR
products were hybridized with oligonucleotides with the wild type sequence to control for amplification of the DNA samples.

Histological proof of adenocarcinoma, by percutaneous puncture, at laparotomy, or at autopsy within two years from the brush sampling served as positive endpoint. When no histological findings were available, absence of cholangiocarcinoma at the time of the brush sampling was assumed when at least two years after sampling the patient was clinically well and anicteric without stent therapy.

Statistical analysis
Categorical variables were analyzed by cross tabulation and logistic regression analysis employing the SPSS package, version 7.5, (SPSS Inc., Chicago, IL, USA).

RESULTS
All 47 brush preparations contained sufficient cells for analysis. The 37 brush outcomes that stated no abnormalities, reactive changes, or some atypia were scored as 0, whereas the 10 specimens that were staged as adenocarcinoma or suspicious for carcinoma were scored as 1. The correlation between the brush cytology results and the definitive outcome is shown in table 1. Follow-up disclosed 10 malignancies. The sensitivity, specificity, positive and negative predictive values according to Bayes’ theorem (which takes into account the influence of prevalence) are presented in table 2.

<table>
<thead>
<tr>
<th>Final outcome</th>
<th>1</th>
<th>0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brush result</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>33</td>
<td>37</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>37</td>
<td>47</td>
</tr>
</tbody>
</table>
Table 2.

Diagnostic performance of brush cytology alone or in combination with p53 and K-ras analysis.

<table>
<thead>
<tr>
<th></th>
<th>Brush alone</th>
<th>Brush with p53 and K-ras</th>
<th>Brush alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=47</td>
<td>n=23</td>
<td>n=23</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.60</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.89</td>
<td>0.79</td>
<td>0.95</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.59</td>
<td>0.49</td>
<td>0.80</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.89</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Tumour prevalence</td>
<td>0.21</td>
<td>0.17</td>
<td>0.17</td>
</tr>
</tbody>
</table>

With regard to the total of 63 brush specimens, in seven patients repeated sampling (up to 5 times) was done within 6 months. Two of these patients had inconsistent results. These numbers were too limited to draw any conclusion.

Table 3 shows the distribution of results when the outcomes of the p53 and K-ras analyses were added.

A brush cytology result was scored as 1 when either the cytology, the p53 or the K-ras was positive, and scored as 0 when none of the three examinations was positive. The diagnostic performance is shown in table 2.

Stepwise forward logistic regression analysis showed no additional diagnostic value from either tumor marker variable.

The differences, especially in sensitivity and negative predictive value, are explained by selection bias. To demonstrate this, the performance of the cytology results alone in the subgroup of 23 patients in which all three diagnostic tests had been performed, is also shown in table 2.

There was no dependent relation between prior stenting and cytology result (Fisher’s exact test, p=1.0), and forward logistic regression revealed no influence of the presence or absence of prior stenting on the predictive value of the brush cytology result.
Table 3.
Cross-table for pooled positive results from brush cytology, p53, and K-ras.

<table>
<thead>
<tr>
<th>Combined result</th>
<th>Final outcome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>19</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Our results show that the sensitivity and positive predictive value of brush cytology investigation for detection of cholangiocarcinoma in dominant strictures in PSC patients are rather poor. The specificity and negative predictive value are reasonably good. The occurrence of false-negative results may be a result of the sampling method. False-positive brush cytology results may be explained by the fact that the bile duct epithelium is inherently inflamed in PSC, giving rise to reactive cells in the brush specimen that may not always be easily differentiated from neoplastic cells. No additional accuracy was gained by determination of the tumor markers p53 and K-ras. In our patient group the presence of prior stenting did not give rise to an increased false-positive rate.

Our results are in accordance with those of earlier studies investigating common bile duct strictures in general. Most studies of brush cytology in bile duct strictures report sensitivities around 50% and specificities close to 100% (9-13), although it is difficult to group these studies together, because of differences in design and in the proportion of the various underlying malignancies. Moreover, most studies contain methodological weaknesses, which are immanent with regard to a diagnostic issue which lacks a uniformly assessable “gold standard”. Finally, it is difficult to compare the results of a diagnostic test for cholangiocarcinoma arising in PSC with those in the setting of primary cholangiocarcinoma or extrinsic bile duct malignancies such as pancreatic carcinoma or metastatic carcinoma.

What can be inferred from these studies is that brush cytology is the method of choice for the detection of biliary malignancy. It is clearly superior to exfoliative bile cytology (9, 12), and equals endobiliary forceps biopsy, probably because the success rate of the latter technique is less than that of brush cytology (11, 13). In PSC, the extrahepatic bile duct is often narrowed entirely or at multiple levels, which will even more adversely affect this success rate. Moreover, because of the
presence of thin-walled sacculations, biopsy-taking may be hazardous. On the other hand, when forceps biopsy seems feasible and safe, for instance in a distal common bile duct stricture, it may be worthwhile to also attempt this.

The finding of K-ras mutations and p53 tumor suppressor protein in cholangiocarcinoma attains biological significance when detection of these mutations can augment the detection of malignancy. Unfortunately, we could not demonstrate this in our study. Both measurements gave rise to false-negative and false-positive results. K-ras mutations have also been observed in premalignant disease states such as pancreatic ductal cell mucinous hyperplasia and chronic pancreatitis (19). Since PSC can be regarded as a premalignant condition, this would imply that K-ras codon 12 mutation analysis is not a useful marker for malignant degeneration. As mentioned earlier, overexpression of p53 has already been demonstrated in cholangiocarcinomas arising in PSC, and was found negative in a control group of end-stage PSC patients without cholangiocarcinoma (14). To ascertain whether the occurrence of false-negative and false-positive results arises because of the sampling method or abundant overexpression of the normal protein, which is theoretically possible, would require concomitant histology taking and gene sequencing.

How can the diagnostic yield of brush cytology in dominant strictures in PSC be improved? As for cholangiocarcinoma in general, repeated brushing up to three times on the same occasion may increase the sensitivity, as suggested by Rabinovitz et al. (10). The additional value of p53 immunocytochemistry and K-ras mutation analysis, and of newer tumor markers such as aspartyl(asparaginyl)beta-hydroxylase is yet to be established (20).

At present, the moderate sensitivity of brush cytology for cholangiocarcinoma precludes its use as a surveillance technique for the detection of early cancer. However, the specificity and negative predictive value are reasonably good. This implies that when the decision on whether to carry out a liver transplant is to be made, brush cytology can be used to rule out malignancy in dominant strictures.

REFERENCES


Chapter X


