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Comparison of the novel quantitative ARMS assay and an enriched PCR-ASO assay for K-ras mutations with conventional cytology on endobiliary brush cytology from 312 consecutive extrahepatic biliary stenoses

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ABSTRACT

Background
Extrahepatic biliary stenosis (EBS) can be caused by malignant and benign diseases. Patients with EBS comprise a group of patients at risk for having or developing a malignancy. There is a need for accurate diagnostic tests for early detection and surveillance. The sensitivity of biliary cytology for a malignancy is relatively low. K-ras mutation analysis on brush cytology is a valuable adjunct, but the specificity remains a concern. A novel quantitative test for K-ras mutations has been developed: the Amplification Refractory Mutation System (ARMS) assay. The aim of this study was to assess the test characteristics and additional value of the ARMS assay in diagnosing the cause of EBS.

Methods
Brush samples obtained during endoscopic retrograde cholangiopancreatography (ERCP) were prospectively collected from 312 consecutive patients with EBS. K-ras mutation analysis was performed using the ARMS assay, in which allele specific amplification was coupled with real time fluorescent detection of PCR products. The results were compared to both conventional cytology and K-ras mutation analysis using Allele Specific Oligonucleotide (ASO) hybridization, and evaluated in view of the final diagnosis.

Results
The test characteristics of the ARMS and ASO assays were largely in agreement. The sensitivity for detecting a malignancy was 49% and 42%, the specificity 93% and 89%, and the positive predictive value 96% and 92%, respectively. The sensitivity of the ARMS assay and cytology combined was 71% with a positive predictive value of 93%. The specificity of the quantitative ARMS assay could be increased to 100% by setting limits for the false positives. This reduced the sensitivity of ARMS from 49% to 43%.

Conclusions
ARMS can be considered supplementary to conventional cytology, and comparable to ASO in diagnosing patients with malignant EBS. Additionally, a specificity of 100% can be acquired with ARMS. The ARMS assay deserves also consideration as an adjunct in the surveillance of patients at risk for pancreatic cancer.
INTRODUCTION

Differentiation between malignant and benign causes of extrahepatic biliary stenosis (EBS) is often difficult, but very important. Patients with EBS comprise a group at risk for having or developing pancreatic cancer. Another group at risk are family members of patients with pancreatic cancer, which was been recognized for decades. Both groups have an increased risk of developing pancreatic cancer, and the application of a screening test for early detection would therefore be very helpful. Since the incidence of pancreatic cancer in the general population is relatively low, screening tests will be limited to selected groups that carry an increased risk, as mentioned above.

Although the specificity of brush cytology for a malignant cause of EBS obtained during endoscopic retrograde cholangiopancreatography (ERCP) is high, its sensitivity is relatively low. Previously we showed that the addition of a K-ras mutation analysis using Allele Specific Oligonucleotide (ASO) hybridization to brush cytology improved the sensitivity for diagnosing a malignancy. Brush sampling during ERCP has a high probability of yielding sufficient cells for DNA analysis and may contain mutations that originated in malignant cells preferentially shed from pancreatic ducts. This sample location also raises the possibility of detecting cells originating from other malignant causes of EBS such as distal bile duct carcinoma, in which K-ras mutations have been reported as well. The 89% specificity of K-ras mutation analysis based on data from as many as 312 consecutive patients with EBS reported in our studies, is one of the highest. Nevertheless, a diagnostic test with a specificity of 89% remains sub-optimal, especially considering the major therapeutic consequences in case of a malignant diagnosis.

Despite its limitations we think that K-ras still can be a promising marker in the diagnostic procedure of EBS. After all, the cause of malignant EBS is mostly pancreatic head carcinoma, which has the highest incidence of RAS mutations in human tumors identified to date. Furthermore, the localization of the majority of K-ras mutations to the single codon 12 makes them relatively easy to detect. Dependent on the used technique the reported frequencies of codon 12 mutations range from 20% to 100% and occur as early events in the tumor progression model. Recently, a novel quantitative real-time assay for K-ras mutations has been developed: the Amplification Refractory Mutation System (ARMS) assay. The real-time quantitative approach of this assay with its sensitive detection and mutant sequence quantification allows the determination of the true detection limit in any clinical application. It routinely provides quantitative data relating to the amount of K-ras mutations in positive samples. In this way a threshold can be set above which the specificity will be a 100%.

The ARMS assay might be a valuable adjunct to early detection in patients with EBS, and also to sensible surveillance strategies in family members of patients with pancreatic cancer. The genetic transmission of pancreatic cancer in hereditary familial syndromes and the patients at high risk are increasingly better defined. There is a need for a molecular diagnostic test in members from
families with certain pancreatic cancer syndromes, which should be able to translate recent molecular genetic discoveries into improved surveillance measures. The ARMS assay for K-ras mutation analysis might be such a test.

The aim of this study was to assess the value of the quantitative ARMS assay for K-ras mutations compared to conventional cytopathology and the established enriched PCR-ASO assay for the diagnosis of a malignancy in patients with EBS in a large series of consecutive patients with complete follow-up.

MATERIALS AND METHODS

Patients

Two earlier studies have been published on the same patient cohort. The study population consisted of 312 consecutive patients who underwent ERCP with endobiliary brush cytology for the evaluation of EBS at the Academic Medical Center in Amsterdam from January 1993 to February 1996. The mean age of the 312 included patients was 63 (19-99) years and the male:female ratio was 173:139. The follow-up was updated and four additional final diagnoses were encountered that were previously unspecified. For this study a final diagnosis could be made in 298 patients, of which 223 (75%) had a malignant and 75 (25%) a benign stenosis. The spectrum of the different etiologies of EBS is given in Table 1.

Table 1 Spectrum of the different causes of EBS in 298 patients with a final diagnosis

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Number of patients (n = 298)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant stenosis</td>
<td></td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td>98 (33%)</td>
</tr>
<tr>
<td>Bile duct carcinoma</td>
<td>73 (24%)</td>
</tr>
<tr>
<td>Gall bladder carcinoma</td>
<td>7 (2%)</td>
</tr>
<tr>
<td>Ampullary carcinoma</td>
<td>8 (2%)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>11 (4%)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>Unspecified</td>
<td>25 (8%)</td>
</tr>
<tr>
<td>Benign stenosis</td>
<td>75 (25%)</td>
</tr>
<tr>
<td>Inflammatory</td>
<td></td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>27 (9%)</td>
</tr>
<tr>
<td>Cholelithiasis</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Mirizzi syndrome</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>26 (9%)</td>
</tr>
<tr>
<td>Postsurgical</td>
<td>13 (4%)</td>
</tr>
<tr>
<td>Unspecified</td>
<td>5 (2%)</td>
</tr>
</tbody>
</table>

Samples and DNA isolation

The methods for collecting the required samples and isolating the DNA have been described previously. In summary: after brushing of the EBS four cytology smears from each patient were
stained by conventional Giemsa and Papanicolaou and independently evaluated by an experienced cytopathologist. The following diagnostic categories were used: positive for carcinoma, negative for carcinoma, suspect for carcinoma, and material insufficient or not suitable for diagnosis. The remaining brush specimen was suspended in 10 ml of DNA buffer, fixed with 10 ml 100% ethanol, and stored at 4°C for subsequent K-ras mutation analysis. The archival tissue blocks, available from 71 patients with a malignant EBS and 10 patients with a benign EBS, were analyzed for K-ras mutations.

One ml of each brush cytology suspension was used for DNA isolation. Careful microdissection of the tissue blocks was performed to ascertain a sample of which at least 50% of the cells comprised the tissue of interest. DNA was extracted as described previously.22

K-ras mutation analyses

Two different methods were used for K-ras mutation analysis: the PCR-ASO hybridization based assay and the novel ARMS allele-specific amplification assay.

The protocol for the first method has been described and validated previously. With this assay, DNA is subjected to PCR amplification using primers around codon 12. Digestion of the PCR products with a restriction enzyme is followed by a second round of amplification, which yields a PCR product enriched for K-ras codon 12 mutations. The resulting DNA fragments are denatured and dot-blotted onto nylon membranes and subjected to allele-specific oligonucleotide hybridization with radioactively labeled probes specific for each possible K-ras codon 12 mutation, followed by autoradiography. Controls for positive and negative outcomes, contamination, specific and non-specific hybridization were employed. Both enriched and non-enriched PCR products were dot-blotted next to each other to check the digestion and mutant enrichment.

The second method was based on ARMS allele specific amplification for mutant K-ras sequence discrimination. This was undertaken using the ABI 7700 machine (PE Applied Biosystems, Foster City CA, USA) to detect amplification products by fluorescence in real time. This assay has been described in detail. In summary: 5ml of each of the 11 brush cytology DNA samples were added to each of 7 ARMS reactions and a control reaction for DNA amount in a 96 tube format. An additional control reaction without DNA for each ARMS mix was included to detect possible contamination. The control reaction amplified all K-ras exon 1 sequences irrespective of mutational status, in order to quantify the total amount of DNA in each sample. Reactions were thermocycled in the ABI 7700 and the relative fluorescence (Rn) was measured after each cycle. The point at which it exceeded a threshold baseline signal was called the threshold cycle (Ct). Ct values from the control and ARMS reactions were plotted against statistically validated data obtained using wild type K-ras exon 1. These data were obtained to establish background signal resulting from wild type K-ras exon 1 over a wide dynamic range of starting DNA concentration (100 fold). The
dynamic range of starting DNA concentrations (6-600ng DNA) was chosen in relation to the yield of DNA typically obtained in 5ml of DNA solution extracted from all clinical samples (tissue and cell suspensions in bodily fluids) entering our laboratory. Any clinical sample giving a signal above background in an ARMS reaction had a <1% probability of containing wild type sequence alone and was therefore interpreted as positive for that mutation. A similar collection of data was obtained using each of the 7 mutant K-ras sequences over the same concentration range. In this way the amount of K-ras mutant sequence in each positive sample could be calculated as a proportion of the total amount of K-ras exon 1 in that particular sample.

Definitions of test characteristics
Sensitivity was defined as the percentage of patients with malignancy and positive test results. Specificity was defined as the percentage of patients without malignancy and negative test results. Positive predictive value was defined as the percentage of patients with positive test results who had a malignancy. Negative predictive value was defined as the percentage of patients with negative test results who did not have a malignancy.

RESULTS
Eighty-one of 223 (36%) patients with malignant EBS were detected as positive by cytology alone. By combining the suspicious cytology results with the positive cytology results, 111 of 223 (50%) malignancies could be detected. Ninety-four of 223 (42%) patients with malignant EBS were detected by K-ras analysis using the enriched PCR-ASO assay alone, and 109 (49%) were detected by ARMS alone. A summary of the test results is listed in table 2. When the results from ARMS assay and cytology were combined, 129 of 223 (58%) and 159 of 223 (71%) confirmed malignancies were detected, depending on whether the cytology results were restricted to the positive samples or included the suspicious samples, respectively.

Table 2 Distribution of ERCP cytology samples based on diagnostic procedure results and final diagnosis of malignancy*

<table>
<thead>
<tr>
<th>Diagnostic procedure</th>
<th>Diagnostic result</th>
<th>Malignancy present</th>
<th>Malignancy absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>positive</td>
<td>81/223 (36%)</td>
<td>2/75 (3%)</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>105/223 (47%)</td>
<td>67/75 (89%)</td>
</tr>
<tr>
<td></td>
<td>suspicious</td>
<td>30/223 (14%)</td>
<td>5/75 (7%)</td>
</tr>
<tr>
<td></td>
<td>insufficient material</td>
<td>7/223 (3%)</td>
<td>1/75 (1%)</td>
</tr>
<tr>
<td>ASO</td>
<td>positive</td>
<td>94/223 (42%)</td>
<td>9/75 (12%)</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>129/223 (58%)</td>
<td>66/75 (89%)</td>
</tr>
<tr>
<td>ARMS</td>
<td>positive</td>
<td>109/223 (49%)</td>
<td>5/75 (7%)</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>114/223 (51%)</td>
<td>70/75 (93%)</td>
</tr>
</tbody>
</table>

*Final diagnosis of malignancy was established by histological and/or clinical findings (symptomatology, imaging studies and course of the disease)
The ability of the two different methods to detect K-ras mutations, the enriched PCR-ASO assay and the ARMS assay, were largely in agreement. The frequency of occurrence of each mutation detected by the two assays is visualized in Figure 1. The most frequently observed mutations in both assays resulted in the codon 12 Gly-to-Asp and Gly-to-Val amino acid substitutions. No mutations were detected in the codon 12 Gly-to-Ser test by enriched PCR-ASO. An additional test for the codon 13 Gly-to-Asp mutation in the ARMS assay detected 8 mutations. There was no equivalent test in the enriched PCR-ASO assay and a zero was recorded for this mutation.

**Table 3** Summary of results for the 15 non-malignant* samples which gave positive results by cytology, PCR-ASO or ARMS

<table>
<thead>
<tr>
<th>Patient Sex - Age</th>
<th>Cytology positive</th>
<th>Cytology suspicious</th>
<th>ASO positive</th>
<th>ARMS positive*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female - 31</td>
<td>x</td>
<td>x</td>
<td>12 Asp</td>
<td>x</td>
</tr>
<tr>
<td>Male - 60</td>
<td>x</td>
<td>x</td>
<td>12 Asp</td>
<td>x</td>
</tr>
<tr>
<td>Male - 43</td>
<td>x</td>
<td>check</td>
<td>Ala</td>
<td>x</td>
</tr>
<tr>
<td>Male - 46</td>
<td>x</td>
<td>check</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Female - 85</td>
<td>x</td>
<td>check</td>
<td>Cys</td>
<td>x</td>
</tr>
<tr>
<td>Female - 44</td>
<td>x</td>
<td>check</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Female - 54</td>
<td>x</td>
<td>check</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Male - 72</td>
<td>x</td>
<td>check</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Male - 41</td>
<td>x</td>
<td>check</td>
<td>12 Asp</td>
<td>x</td>
</tr>
<tr>
<td>Male - 38</td>
<td>x</td>
<td>check</td>
<td>Ala</td>
<td>x</td>
</tr>
<tr>
<td>Male - 44</td>
<td>x</td>
<td>check</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Female - 60</td>
<td>x</td>
<td>x</td>
<td>12 Asp</td>
<td>x</td>
</tr>
<tr>
<td>Male - 55</td>
<td>check</td>
<td>x</td>
<td>12 Asp</td>
<td>x</td>
</tr>
<tr>
<td>Male - 51</td>
<td>x</td>
<td>check</td>
<td>Val</td>
<td>x</td>
</tr>
<tr>
<td>Female - 40</td>
<td>check</td>
<td>x</td>
<td>12 Asp</td>
<td>Val (0.24%)</td>
</tr>
</tbody>
</table>

* Based on a final diagnosis of malignancy which was established by histological and/or clinical findings (symptomatology, imaging studies and course of the disease)
samples is shown in figure 2. Representation by this means has been described previously in lung cancer. The majority of samples in each test are negative for K-ras mutations. The data points appear above the 98% CI established using wild-type K-ras exon 1, or fail to give ARMS products during cycling and are recorded as 50 cycles. Samples appearing below the lower limit of the 98% CI have a <1% chance of containing wild-type sequence alone and are therefore recorded as positive. Duplicate analysis using the same DNA samples confirmed the samples appearing as positives (data not shown).

All three diagnostic methods gave positive results in cases of confirmed non-malignancy. False-positivity was 7% (5/75) for the ARMS assay, 12% (9/75) for the PCR-ASO assay, and 3% (2/75) to 7% (5/75) for conventional cytology (table 2). Table 3 summarizes the results for the 15 samples that had any false-positive diagnostic test. Since the results of the ARMS assay were quantitative, cut-off limits were set for each of the four mutations found. These were 12.5%, 0.95%, 1.25% and 0.24% for the codon 12 Gly-to-Ala, -Asp, -Cys and -Val.

Figure 2 This figure shows the real time ARMS analysis of a hundred ERCP brush cytology samples for K-ras mutations. Appearance of products in the ARMS and control reactions (CT) for each cytology sample were plotted against data obtained from wild type control DNA. ERCP samples were analyzed in each of 7 K-ras tests: (A) codon 12 GGT to GCT (Gly to Ala); (B) codon 12 GGT to CGT (Gly to Arg); (C) codon 12 GGT to GAT (Gly to Asp); (D) codon 13 GGC to GAC (Gly to Asp); (E) codon 12 GGT to TGT (Gly to Cys); (F) codon 12 GGT to AGT (Gly to Ser);and (G) codon 12 GGT to GTT (Gly to Val). ERCP DNA samples are labeled as either mutation positive (■) or negative (○). The 2 regression lines represent mean Y (—) and the 99% confidence limit for Yi data (⋯).
tests, respectively. This means that a specificity of 100% was established for diagnosis of malignancy using the adjusted ARMS assay. This reduced the sensitivity of the assay from 49% to 43%, because mutations were detected at equivalent or lower levels in samples from patients with clinically confirmed malignancy. Results of sensitivity, specificity, positive predictive values and negative predictive values for all of the methods used are summarized in table 4. The biggest problem with any of the approaches used in isolation is that their negative predictive values are low and they simply do not detect enough malignancies. When ARMS and cytology were combined, however, they were able to detect 159 of 223 (71%) malignancies with a positive predictive value of 93%. Individually they detected 109 of 223 (49%) and 111 of 223 (50%) malignancies, with positive predictive values of 96% and 94%, respectively.

### DISCUSSION

This study shows that the novel quantitative ARMS assay for K-ras mutation analysis is a valuable adjunct to conventional cytology and non-quantitative PCR-ASO assay in diagnosing EBS. By setting cut-off limits, the ARMS assay was 100% specific and 43% sensitive in diagnosing a malignant cause in 312 consecutive endobiliary brush samples obtained from patients with EBS. Furthermore, a diagnostic combination of conventional cytology and the ARMS assay was able to predict a malignant cause in 93%.

Cytology is highly specific in diagnosing the cause of EBS, but its sensitivity is routinely low. Although it has been suggested that repeated brushings increase the sensitivity, cytology alone is not good enough to differentiate between malignant and benign EBS. Our study population comprises patients at risk for having or developing a malignancy, mostly pancreatic or distal bile duct carcinoma. So far, early detection of pancreatic cancer is the only realistic option for cure. Although it is unlikely that screening for pancreatic cancer in the general population would be justified at this moment, there is certainly a need for accurate diagnostic tests in families with
genetic disorders known to predispose to pancreatic cancer. Sensitive molecular assays offer the chance to improve test characteristics when used as an addition to cytology. Of course this largely depends on the prevalence of the candidate marker in the disease and the technique used for detection of molecular changes.

K-ras mutations are highly prevalent in pancreatic carcinoma and common in bile duct carcinoma, the two main malignant causes of EBS. K-ras encodes a protein located on the inner side of the plasma membranes, which have intrinsic GTPase activity. A mutation in the K-ras gene at codon 12 or 13 results in inappropriate growth signal. Several studies have been conducted to elucidate the clinical utility of K-ras mutation analysis in the diagnostic and therapeutic process of EBS. Reported rates of K-ras mutations and outcomes of the numerous studies vary widely. For example, Ponsioen et al. could not demonstrate any additional value of K-ras mutation analysis in discriminating between benign and malignant strictures from patients with primary sclerosing cholangitis. We showed in previous studies, however, that K-ras codon 12 mutation analysis could be considered supplementary to light microscopy evaluation of ERCP brush cytology specimens for the diagnosis of malignant EBS.

Variations in reported diagnostic K-ras mutations can be attributed to the nature of the clinical material being investigated and the sensitivity and specificity of the used assay. In a previous study we used the established fine needle aspiration (FNA) of pancreatic or duodenal juice to demonstrate that a molecular panel including the K-ras, p53, and DPC4 (MAD4) genes can supplement traditional cytologic diagnosis. FNA has the potential advantage of the ability to enrich for malignant cells. When secretin is administered as part of an exocrine function test prior to sampling, K-ras mutations can be prevalent in juice samples obtained from patients with benign disease. In current study we used brush samples obtained during ERCP, performed routinely in patients with EBS in our institution, as an indirect sampling method to reduce the chance of detecting cells from benign tissue. There are a number of theories as to why this type of sampling may be beneficial. One theory is that malignant cells, growing uncontrollably in the pancreatic ducts, are more likely to be shed from their site of origin than benign cells. Cancer cells can therefore be detected at a distant site. Another theory is that all cells are shed with equal likelihood into the ductal system but malignant cells are more likely to remain intact, because they have reduced apoptotic potential and consequently less degradation of DNA compared to normal cells. Either theory means that a molecular assay for K-ras oncogene mutations would be of limited use in direct sampling of pancreatic tissue.

Unfortunately, mutations in the K-ras oncogene also occur in non-malignant pancreatic tissue. K-ras gene mutations have been observed in premalignant disease states such as PanIN lesions and chronic pancreatitis. In the recently developed progression model for pancreatic ductal adenocarcinoma, mutations in the K-ras oncogene seem to be an early event in the series of
architectural and cytological changes.\textsuperscript{36-38} In a previous study on the same 312 consecutive patients, we performed a long-term follow-up of the eight patients with a K-ras mutation detected in brushings of clinically benign EBS.\textsuperscript{11} After a median follow-up of 65 months all these eight had to be considered as confirmed false-positives. Few publications exist on patients who developed pancreatic cancer after an even longer interval than 65 months.\textsuperscript{39,40} Although a false-positive result was infrequent and in theory these eight patients still could develop a malignancy, a diagnostic test with a specificity of 89% remains sub-optimal.

The novel real-time ARMS assay used in current study provided quantitative data allowing determination of the true detection limit above which the specificity was 100%.\textsuperscript{20} It is a convenient and homogeneous method, which facilitates high-throughput sample analysis for a range of clinical materials. The enriched PCR-ASO assay requires separate amplification steps and will always carry the risk of PCR contamination. In contrast, the ARMS assay is “closed-tube” and the amplification products can be disposed with a vastly reduced risk of contamination. The additional data provided by this sensitive technique used in conjunction with cytology increased the detection of clinically confirmed malignancy from 111/223 (50%) to 159/223 (71%). The 100% specificity in the 298 ERCP samples analyzed by ARMS alone was based on the appearance of false positives in the clinically confirmed non-malignant cases. Occurrence of mutations in subsequent samples taken from patients with a clinical diagnosis of non-malignant stenosis will redefine the cut-off limits depending on the quantities of mutation. This raises questions about the significance of mutations detected at equivalent or lower levels in confirmed malignant patients. However, the intention with any diagnostic assay is to achieve the highest specificity when distinguishing between two clinical scenarios, whilst maintaining a reasonable sensitivity.

Knowledge about well-defined high-risk groups that might benefit from sensible surveillance strategies is rapidly increasing.\textsuperscript{41} Particularly noteworthy in this regard are the hereditary syndromes that include pancreatic cancer.\textsuperscript{41,42} In order to determine which unaffected relatives in the direct cancer-prone lineage are at an increased risk of pancreatic cancer, a genetic marker of sufficiently high sensitivity and specificity is required.\textsuperscript{43} The ARMS assay could be a valuable adjunct in this category, since it can be set to a specificity of 100%. Members of pancreatic cancer families are felt to be at high risk for the development of pancreatic cancer and therefore ideal candidates for surveillance.\textsuperscript{44} We believe that a symptomatic kindred of patients with a mutation known to be associated with familial susceptibility warrant an aggressive approach that incorporates our growing knowledge of the genetics of pancreatic cancer. The goal for surveillance of familial pancreatic cancer patients is to diagnose them in the dysplasia or carcinoma in situ stage, before the development of invasive cancer. Some authors even advise to perform a complete pancreaticoduodenectomy in these patients.\textsuperscript{45} It has already been shown that thorough screening of patients with a family history of pancreatic cancer is feasible.\textsuperscript{46} In the near future, the ARMS assay for K-ras mutation
analysis on brush cytology could form a suitable diagnostic panel with other molecular markers in pancreatic cancer recently discovered by global gene expression technology. 

In conclusion, this study shows that the novel, real-time ARMS assay for K-ras mutations can be considered supplementary to conventional cytology and the non-quantitative PCR-ASO assay in diagnosing patients with malignant EBS. The quantitative nature of the ARMS assay makes it possible to diagnose a malignant stenosis with a specificity of 100%. We believe that in the near future the ARMS assay could become of additional value in the surveillance strategy of presymptomatic patients at risk for hereditary pancreatic cancer.

REFERENCES


