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Next-Generation Sequencing and Fluorescence *in Situ* Hybridization Have Comparable Performance Characteristics in the Analysis of Pancreaticobiliary Brushings for Malignancy

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Cytological evaluation of pancreatic or biliary duct brushings is a specific, but insensitive, test for malignancy. We compared adjunctive molecular testing with next-generation sequencing (NGS) relative to fluorescence *in situ* hybridization (FISH) for detection of high-risk neoplasia or malignancy. Bile duct brushings from 81 specimens were subjected to cytological analysis, FISH using the UroVysion probe set, and targeted NGS. Specimens were placed into negative/atypical (negative) or suspicious/positive (positive) categories depending on cytology and negative or positive categories on the basis of FISH and NGS results. Performance characteristics for each diagnostic modality were calculated on the basis of clinicopathologic follow-up and compared in a receiver operating characteristic analysis. There were 33 high-risk neoplasia/malignant strictures (41%) and 48 benign (59%). NGS revealed driver mutations in 24 cases (30%), including *KRAS* (21 of 24 cases), *TP53* (14 of 24 cases), *SMAD4* (6 of 24 cases), and *CDKN2A* (4 of 24 cases). Cytology had a sensitivity of 67% (95% CI, 48%—82%) and a specificity of 98% (95% CI, 89%—100%). When added to cytology, NGS increased the sensitivity to 85% (95% CI, 68%—95%), leading to a significant increase in the area under the curve in a receiver operating characteristic analysis ($P = 0.03$). FISH increased the sensitivity to 76% (95% CI, 58%—89%), without significantly increasing the area under the curve. These results suggest that ancillary NGS testing offers advantages over FISH, although studies with larger cohorts are needed to verify these findings. *(J Mol Diagn 2016, 18: 124—130; http://dx.doi.org/10.1016/j.jmoldx.2015.08.002)*

Strictures of the biliary and pancreatic ducts detected on imaging may reflect reactive or neoplastic processes. Reactive causes include primary sclerosing cholangitis, cholecystitis, and pancreatitis; benign neoplastic causes include intraductal papillary mucinous neoplasms (IPMNs) of the pancreatic ducts and biliary intraepithelial neoplasia of the biliary ducts; and malignant causes include pancreatic ductal adenocarcinoma, cholangiocarcinoma, and metastases. The etiology of ductal strictures is typically evaluated cytologically using brushing specimens obtained via endoscopic retrograde cholangiopancreatography, thus directing clinicians to management options such as stent placement, surveillance, or surgery.[1,2]

Pancreaticobiliary brushing cytology has a high specificity for malignancy, but its sensitivity is usually moderate

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Table 1 Patient Demographics

Characteristic	Value
Total No. of patients	74
Total No. of cases	81
No. of bile duct brushings	73
No. of pancreatic duct brushings	8
Mean (range) age (years)	63 (19–93)
Male/female ratio	42:32
Average follow-up (months)	6.4

to poor, ranging from 8% to 62%.[1,3,4] Adjunctive tests used to improve on the sensitivity of cytology have included digital image analysis, assessment of *KRAS* mutation status, and fluorescent *in situ* hybridization (FISH) for polysomy. Digital image analysis, by itself, achieves sensitivities of 14% to 48% and only marginally improves sensitivity when combined with FISH.[5,6] *KRAS* mutation detection by quantitative PCR achieved a moderate sensitivity of 47% in one study,[7] although restriction to analysis of one gene limits specificity and the ability to detect nonpancreaticobiliary cancers. Multicolor FISH using the UroVysion probe set (Abbott Molecular Inc., Des Plaines, IL) has enjoyed the most widespread clinical implementation, yet this test still achieves only moderate sensitivities, typically between 35% and 60%.[5–8] Multicolor FISH is also expensive, is labor intensive, requires morphological expertise, and can be technically challenging because of nuclear overlap.

Next-generation sequencing (NGS) is capable of interrogating large panels of genes simultaneously and detecting small quantities of mutant DNA in much larger quantities of normal DNA.[9] Recent studies have documented its potential to screen for cancer DNA in Papanicolaou test fluid,[10] bladder washings,[11] stool samples,[12] pancreatic cyst fluid,[13] and blood,[14,15] among other sources. NGS also has the potential for reduced cost and complexity because brushing samples can be batched together with routine solid tumor specimens. We hypothesized that an NGS panel, originally developed to genotype solid tumor specimens, would be as sensitive as FISH in the detection of malignancy or high-risk neoplasia (HRN; main duct IPMN, high-grade dysplasia, or carcinoma) in bile duct brushing specimens. The goal of this study was to compare the performance characteristics of targeted NGS and FISH as adjunctive tests for HRN in pancreaticobiliary brushing cytology specimens.

Materials and Methods

Study Cohort

We analyzed bile duct ($n = 73$) and main pancreatic duct ($n = 8$) brushing specimens from 74 patients who underwent endoscopic retrograde cholangiopancreatography at the Massachusetts General Hospital (Boston, MA) from April 2014 to January 2015 (Table 1). Each patient sample was received as a ThinPrep vial (Hologic Corp., Marlborough, MA) containing a brush. Cells were suspended in 40 mL of CytoLyt and divided in one pass, with 20 mL going for cytological analysis, 10 mL for FISH using the UroVysion probe set (Abbott Molecular Inc.), and 10 mL for targeted NGS. Specimens were placed into negative/atypical (negative) or suspicious/positive (positive) categories on the basis of cytology and negative or positive categories on the basis of FISH and NGS results. Performance characteristics for each diagnostic modality were calculated using follow-up or concurrent radiological and/or pathological data as the gold standard (Supplemental Table S1). Follow-up was considered positive (or malignant) if the duct brushed had evidence of involvement by a high-grade dysplastic process, a main-duct IPMN, or a malignant process.

FISH

FISH analysis was performed as a reference test at the Mayo Clinic Department of Laboratory Medicine and Pathology (Rochester, MN). Specimens were shipped in 10 to 20 mL of PreservCyt (Hologic Corp.). According to the laboratory, results were determined by evaluating the number of centromere probe (CEP)3, CEP7, CEP17, and 9p21 probe signals in cytologically atypical cells. Specimens were classified as negative (disomy for all four probes), equivocal (at least 10 cells with tetrasomy or trisomy for CEP7 but disomy for the other probes), or positive for malignancy (a gain of two or more of CEP3, CEP7, and/or CDP17 in at least five cells). For the purposes of statistical analysis in this study, equivocal FISH results were considered negative.

NGS

Mutation hotspots and exons from 39 genes were targeted using Anchored Multiplex PCR.[16] Briefly, DNA was isolated

Table 2 Follow-Up Diagnoses in 74 Patients

Follow-up diagnoses	No. (%) of patients
Neoplastic	31 (42)
Pancreatic adenocarcinoma	14 (19)
Cholangiocarcinoma	2 (3)
Gallbladder carcinoma	1 (1.4)
Bile duct carcinoma	6 (8)
Acinar cell carcinoma	1 (1.4)
Pancreaticobiliary carcinoma, NOS	2 (3)
Metastatic SCC	1 (1.4)
Metastatic esophageal carcinoma	1 (1.4)
IPMN	3 (4)
Nonneoplastic	43 (58)
Cholecystitis	5 (7)
Pancreatitis	10 (14)
Primary sclerosing cholangitis	5 (7)
Choledocholithiasis	3 (4)
Cholangitis	4 (5)
Papillary stenosis	1 (1.4)
Non-specific/other	15 (20)

IPMN, intraductal papillary mucinous neoplasm; NOS, not otherwise specified; SCC, squamous cell carcinoma.