

**VM102 Biliary Brush Malignancy: Cytomorphology, with
Correlation to FISH and DNA Ploidy by Digital Image**

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Case Presentations

Case 1

Clinical History: 81-year-old male with a history of long standing chronic renal failure, aortic stenosis, abdominal aortic aneurysm, and congestive heart failure. The patient is referred to gastroenterology for workup of ascites. CT exam reveals dilated left portal vein and mildly dilated intrahepatic bile ducts. Gallstones are also noted. ERCP is performed. Biliary brush specimen is obtained and sent for routine cytology, DIA and FISH analysis.

Cytologic Findings: Negative for malignancy. Reactive biliary epithelium.

DIA Results: Negative.

FISH Results: Negative.

Outcome/Follow up: Ascites felt to be related to congestive heart failure. Patient continues treatment for multiple medical problems. No evidence of malignancy 2 years in follow-up.

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Case 2

Clinical History: 79-year-old female without prior history of disease, presents with jaundice, several weeks of fatigue and 30 pound weight loss. CT exam reveals dilated ductal system and fullness in the pancreatic head. Gallstones are noted. CA 19-9 is not elevated. ERCP is performed to rule out malignancy. Biliary brushing specimen is obtained and sent for routine cytology, DIA and FISH.

Cytologic Findings: Positive for malignancy. Adenocarcinoma.

DIA Results: Positive. Aneuploid population detected (DNA index, 1.7)

FISH Results: Positive. Polysomic cells detected.

Outcome/Follow up: Tumor was not deemed resectable. Patient declined chemotherapy and succumbed to pancreatic adenocarcinoma 3 months after diagnosis.

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Case 3

Clinical History: 33-year-old male with multiple medical problems including recurrent urinary tract infections and pyelonephritis, chronic anemia, S/P small bowel resection for obstruction with resulting short bowel syndrome and chronic pain. Patient presents with biliary colic symptoms. CT exam reveals gallstones with impacted stone in the distal common bile duct. ERCP is performed and the stone is extracted. Biliary brush cytology is obtained and sent for routine cytology, DIA and FISH. A J stent is placed.

Cytologic Findings: Atypical. Markedly atypical biliary epithelial cells.

DIA Results: Negative.

FISH Results: Negative.

Biliary Calculator Interpretation:

These results are equivocal.

The finding of atypical on cytology, negative on DIA, and negative on FISH, in a patient without PSC, aged 33, resulted in a cancer rate of 23% (confidence interval, 9% to 46%) in a cohort of 356 patients undergoing evaluation for possible biliary tract malignancy at the Mayo Clinic; 48% of whom had a final diagnosis of malignancy. This risk estimate is our best approximation given currently available data. The likelihood of cancer in an individual patient will vary based on other factors.

Outcome/Follow up: Biliary colic symptoms one year later. Cholecystectomy is performed. Pathologic exam reveals chronic cholecystitis and cholelithiasis. No evidence of neoplasm.

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Case 4

Clinical History: 60-year-old female, previously healthy, presents with obstructive jaundice. CT exam reveals findings that suggest an impacted biliary stone. ERCP reveals a hilar stricture that extends into the biliary ductal system. Biliary brush specimen is collected for routine cytology, DIA and FISH.

Cytologic Findings: Atypical, favor reactive etiology.

DIA Results: Positive. Aneuploid population detected (DNA index 1.3)

FISH Results: Positive. Greater than 4 polysomic cells detected.

Biliary Calculator Interpretation:

These results are suspicious for malignancy.

The finding of atypical on cytology, positive on DIA, and polysomy on FISH, in a patient without PSC, aged 60, resulted in a cancer rate of 97% (confidence interval, 85% to 100%) in a cohort of 356 patients undergoing evaluation for possible biliary tract malignancy at the Mayo Clinic; 48% of whom had a final diagnosis of malignancy. This risk estimate is our best approximation given currently available data. The likelihood of cancer in an individual patient will vary based on other factors.

Outcome/Follow up: Additional imaging studies reveal occlusion of the left branch of the portal vein with splenic hilar varices, hilar lymphadenopathy and ascites. Abdominal fluid cytology is positive for malignancy. Patient undergoes chemotherapy, but unfortunately dies of disease 14 months after diagnosis.

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Case 5

Clinical History: 52-year-old female with history of chronic ulcerative colitis (9 years) and primary sclerosing cholangitis (8 years). She presents for routine surveillance screening by ERCP for cholangiocarcinoma. ERCP is performed with biliary brush sample taken for routine cytology, FISH and DIA.

Cytologic Findings: Suspicious for Malignancy.

DIA Results: Negative. Diploid cell population

FISH Results: Negative. Chromosomal abnormalities not detected.

Biliary Calculator Interpretation:

These results are equivocal.

The finding of suspicious on cytology, negative on DIA, and negative on FISH, in a patient with PSC, aged 52, resulted in a cancer rate of 43% (confidence interval, 16% to 75%) in a cohort of 356 patients undergoing evaluation for possible biliary tract malignancy at the Mayo Clinic; 48% of whom had a final diagnosis of malignancy. This risk estimate is our best approximation given currently available data. The likelihood of cancer in an individual patient will vary based on other factors.

Outcome/Follow up: Three additional ERCP procedures performed over a two-year period. Cytology, DIA and FISH are all negative on subsequent biliary brush specimens. MRI demonstrates no mass lesion or dominant stricture. Patient is currently on liver transplantation list for end stage primary sclerosing cholangitis.

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Case 6

Clinical History: 71-year-old female without prior medical concerns presents with jaundice, fatigue, abdominal pain and diarrhea for several weeks duration. CT exam reveals gallstones and intrahepatic bile duct dilatation. ERCP is performed to rule out malignancy. A long stricture is noted. Biliary brushing specimen is obtained and sent for routine cytology, DIA and FISH.

Cytologic Findings: Negative for malignancy. Reactive biliary epithelium.

DIA Results: Positive. Aneuploid cell population detected (DNA index 1.2)

FISH Results: Positive. Polysomic cells detected.

Biliary Calculator Interpretation:

These results are suspicious for malignancy.

The finding of negative on cytology, positive on DIA, and polysomy on FISH, in a patient without PSC, aged 71, resulted in a cancer rate of 97% (confidence interval, 87% to 100%) in a cohort of 356 patients undergoing evaluation for possible biliary tract malignancy at the Mayo Clinic; 48% of whom had a final diagnosis of malignancy. This risk estimate is our best approximation given currently available data. The likelihood of cancer in an individual patient will vary based on other factors.

Outcome/Follow up: Diagnosis of cholangiocarcinoma made. Patient started on chemotherapy. Patient succumbs to disease 7 months after diagnosis.

Biliary Tract Malignancy: Cytologic Criteria			
	Architectural Features	Nuclear Features	Atypical Single Cells
Benign	<ul style="list-style-type: none"> - Flat Sheets - Evenly Spaced Nuclei - Preserved Polarity 	<ul style="list-style-type: none"> -Round to Oval -Smooth Nuclear Membranes -Relatively Low N/C -Uniform, fine chromatin -Inapparent Nucleoli 	None
Reactive	<ul style="list-style-type: none"> - Flat Sheets - Increased Crowding of Nuclei - Preserved Polarity 	<ul style="list-style-type: none"> -Moderate increase in N/C Ratio -Nuclear Enlargement -Smooth Nuclear Membranes -Uniform, Fine Chromatin -One or more Variable Nucleoli 	None
Atypical/Indeterminate	<p>Mild:</p> <ul style="list-style-type: none"> - Crowded Sheets - Disordered Nuclei-variably crowded - Altered nuclear polarity-variable - Increased depth of focus within group 	<ul style="list-style-type: none"> -Moderate increase in N/C ratio -Moderate Nuclear Enlargement -Mildly Irregular Nuclear Membranes -Anisonucleosis (up to 2x) -Prominent Nucleoli-variable -May have Coarse Chromatin 	Rare
Malignant	<p>Moderate-Marked:</p> <ul style="list-style-type: none"> - Crowded Sheets - Nuclear Disarray - Altered Nuclear Polarity - 3D Micropapillae 	<p>Moderate-Marked:</p> <ul style="list-style-type: none"> -Increased N/C ratio -Nuclear enlargement -Irregular Nuclear Membranes -*Anisonucleosis (up to 3x) -Single, Large, Irregular, Prominent Nucleoli -Chromatin Variably Dense and Irregular 	*Frequent

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References:

Boberg KM, Jebsen P, Clausen OP, Foss A, Aabakken L, Schrupf E. Diagnostic benefit of biliary brush cytology in cholangiocarcinoma in primary sclerosing cholangitis. *Journal of Hepatology* 2006;45:568-574.

Furmanczyk PS, Grieco VS, Agoff SN. Biliary brush cytology and the detection of cholangiocarcinoma in primary sclerosing cholangitis. Evaluation of specific cytomorphic features and CA19-9 levels. *American Journal of Clinical Pathology*. 2005;124:355-360.

Govil H, Reddy V, Kluskens L, Treaba D, Massarani-Wafai R, Selvaggi S, Gattuso P. Brush cytology of the biliary tract: retrospective study of 278 cases with histopathologic correlation. *Diagnostic Cytopathology* 2002;26:273-7.

Kipp BR, Stadheim LM, Halling SA, Pochron NL, Harmsen S, Nagorney DM, Sebo TJ, Therneau TM, Gores GJ, de Groen PC, Baron TH, Levy MJ, Halling KC, Roberts LR. A comparison of routine cytology and fluorescence in situ hybridization for the detection of malignant bile duct strictures. *American Journal of Gastroenterology* 2004;99:1675-1681.

Krishnamurthy S, Katz RL, Shumate A, Strohle K, Khanna A, Tucker SL, Rajman I, Lahoti S. *Cancer* 2001;93:229-235.

Krishnamurti U, Sasatomi E, Swalsky PA, Finkelstein SD, Ohori P. Analysis of loss of heterozygosity in atypical and negative bile duct brushing cytology specimens with malignant outcome. Are "false-negative" cytologic findings a representation of morphologically subtle molecular alterations? *Archives of Pathology and Laboratory Medicine* 2007;131:74-80.

Lal A, Okonkwo A, Schindler S, De Frias D, Nayar R. Role of biliary brush cytology in primary sclerosing cholangitis. *Acta Cytologica* 2004;48:9-12.

Layfield JL, Wax TD, Lee JG, et al. Accuracy and morphologic aspects of pancreatic and biliary duct brushings. *Acta Cytologica* 1995;39:11-18.

Lee JG, Leung JW, Baillie J, Layfield LJ, Cotton PB. Benign, dysplastic, or malignant –making sense of endoscopic bile duct brush cytology: results in 149 consecutive patients. *American Journal of Gastroenterology* 1995;90:722-726.

Logrono R, Kurtycz DF, Molina CP, Trivedi VA, Wong JY, Block KP. Analysis of false-negative diagnoses or endoscopic brush cytology of biliary and pancreatic duct strictures: the experience at 2 university hospitals. *Archives of Pathology and Laboratory Medicine* 2000;124:387-92.

Logrono R, Wong JY. Reporting the presence of significant epithelial atypia in pancreaticobiliary brush cytology specimens lacking evidence of obvious carcinoma: impact on performance measures. *Acta Cytologica* 2004;48:613-21.

Moreno Luna LE, Gores GJ. Advances in the diagnosis of cholangiocarcinoma in patients with primary sclerosing cholangitis. *Liver Transplantation* 2006;12:S15-S19.

Moreno Luna LE, Kipp B, Halling KC, Sebo TJ, Kremers WK, Roberts LR, Barr Fritcher EG, Levy MJ, Gores GJ. Advanced cytologic techniques for the detection of malignant pancreatobiliary strictures. *Gastroenterology* 2006;131:1064-1072.

Okonkwo AM, De Frias DV, Gunn R, Diaz L, Schindler S, Lal A, Nayar R. Reclassification of "atypical" diagnoses in endoscopic retrograde cholangiopancreatography-guided biliary brushings. *Acta Cytologica* 2003;47:435-42.

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Stewart CJ, Mills PR, Carter R, O'Donohue J, Fullarton G, Imrie CW, Murray WR. Brush cytology in the assessment of pancreatico-biliary strictures: a review of 406 cases. *Journal of Clinical Pathology* 2001;54:449-455.

Trent V, Khurana KK, Pisharodi LR. Diagnostic accuracy and clinical utility of endoscopic bile duct brushing in the evaluation of biliary strictures. *Archives of Pathology and Laboratory Medicine* 1999;123:712-5.

Volmar KE, Vollmer RT, Routbort MJ, Creager AJ. Pancreatic and bile duct brushing cytology in 1000 cases: review of findings and comparison of preparation methods. *Cancer* 2006;108:231-8.

Ylagan LR, Liu LH, Maluf HM. Endoscopic bile duct brushing of malignant pancreatic biliary strictures: retrospective study with comparison of conventional smear and ThinPrep® techniques. *Diagnostic cytopathology* 2003;28:196-204.