

CYTOLOGY OF THE PANCREATO-BILIARY TRACT

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Objectives:

- Review variety of sampling techniques for the pancreato-biliary tree.
- Discuss the diagnostic criteria for adenocarcinoma
- Illustrate different diagnostic pitfalls
- Present data on ancillary studies to help in the diagnosis of adenocarcinoma.

Cytology plays an important role in the evaluation of biliary and pancreatic strictures and mass lesions.

Collecting Methods and Sampling: A number of different sampling techniques can be used. By ERCP, cells can be obtained via aspiration of bile or pancreatic juice, brushing of the bile or pancreatic duct, biopsy of the bile or pancreatic duct, and fine needle aspiration. Fine needle aspiration can also be performed under endoscopic ultrasound, abdominal ultrasound, CT guidance, or direct visualization and palpation in the operating room. Key to all of these techniques is the use of imaging to guide the needle or brush to the area of interest. *If the needle is not in the mass or the brush not on the tumor, the entire procedure is completely worthless and may even cause harm.* Sampling is one of the key components in cytology.

Aspiration of Bile or Pancreatic Juice: In general aspiration has the lowest cellular yield. Bile duct cancers often have the highest yield. Dilating a stricture or manipulating the area of interest can increase the yield. Currently our endoscopists do the aspiration last, after the brush and biopsy.

Technique: To aspirate bile or pancreatic juice, collect in a sterile container and send to the lab. An alternative is to place in a fixative solution (95% alcohol) and send to the lab.

Slide preparation: Performed at the cytology laboratory. The specimen is centrifuged onto a slide and then stained.

Brush: Increased cellularity compared to aspirate. There is lower yield with pancreatic or metastatic malignancies.

Technique: The brush is applied to the area of interest, either pancreatic duct or bile duct (typically a stricture). This can be difficult in obstructed ducts.

Slide preparation: The brush is smeared onto a slide or placed into ThinPrep fixative. The slide should be immediately fixed in 95% alcohol or fixed with spray fixative.

Complications: There is an increased risk of pancreatitis. This is especially true of pancreatic duct brushing. One study²⁰ showed a 21% chronic pancreatitis rate, which was reduced with the use of a pancreatic stent.

- Bile Duct Brushing
 - Lower Yields with pancreatic or metastatic malignancies
 - Easiest and most frequently performed
 - Lower yields with small tumors
- Pancreatic Duct Brushing
 - High incidence of pancreatitis
 - 21% no stent
 - Decreased with stent
 - Often difficult secondary to obstruction

Forceps Biopsy: Allows one to get tissue fragments for histologic examination. Many pathologists are more comfortable with histologic examination compared to cytologic examination. It allows more architectural relationships to be seen.

Technique: The biopsy forceps is introduced up the duct and a portion of tissue is torn off.

Slide preparation: The tissue is fixed in formalin and sent to the lab. These fragments can be very small. Our lab spins the formalin and creates a cellblock to cut the histologic section.

Complications: Perforation has been reported in biopsy above the stricture.

Diagnostic Utility of Biliary Sampling Techniques:

In general sampling by ERCP has a low sensitivity, a high specificity, a high positive predictive value, and a low negative predictive value. Aspirates tend to be less cellular than brushings or biopsy. Inflammatory atypical can mimic carcinoma. Most institutions use at least two if not more of the above collection techniques⁶. A study in our institution⁴ of biliary strictures is typical of the literature. This involved 94 patients (68 malignant and 28 benign).

Cellularity	Biopsy (93 pts)	Brush (94 pts)	Aspirate (76 pts)	
Adeq/limit	88% / 7%	95% / 4%	65% / 22%	
Inadeq	5%	1%	13%	
Dx in 68	with Ca			Total
Positive	28%	29%	7%	47%
Suspicious	8%	13%	3%	12%
Atypia	9%	12%	15%	10%
Cumulative	45%	54%	25%	69%

A summary of the diagnostic criteria for bile brushings is presented in the next chart:

Criteria	Content	Sensitivity	Specificity
Iowa, Cohen ^{2,3}	Nuclear molding, chromatin clumping, increased N/C ratio	83	98
Japan, Nakajima ¹⁴	3 of following: Loss of honeycombing, enlarged nuclei, loss of polarity, bloody background, flat nuclei, cell-in-cell arrangement	86	77
Boston, Renshaw ¹⁵	Chromatin clumping, loss of polarity, nuclear molding		
Duke, Layfield ¹¹	Degree of atypia	37	100
Korea, Jin ⁹	Loss of honeycombing, hyperchromatism, increased N/C ratio, coarse chromatin	56	100

The next chart reveals Renshaw et al's¹⁵ evaluation of the various criteria with a Kappa value for three cytologists.

Criteria	Kappa	Sensitivity	Specificity
Nuclear molding/cell-in-cell arrangement	0.41	35	83
Chromatin clumping	0.63	36	92
Increased N/C ratio	0.5	41	88
Enlarged nuclei	0.57	49	68
Loss of polarity	0.2	36	97
Loss of honeycombing	0.59	51	73
Bloody background	0.19	15	88
Flat nuclei	0.08	7	100
Overall assessment	0.7	36	95

Our institution⁸ looked a bile duct brushing with ThinPrep using similar criteria.

- Criteria
 - Loss of polarity
 - Nuclear enlargement
 - Nuclear membrane irregularity
 - Nuclear overlapping/molding
 - Nuclear chromatin clumping
 - Macronucleoli
- 68 specimens
 - 37% malignant
 - Sensitivity 71%
 - Specificity 96%

Ancillary Techniques: Due to the low sensitivity of most cytology specimens, a number of ancillary immunohistochemical and molecular techniques have been tried to increase the sensitivity. The most commonly tried are K-ras mutation detection^{13,18,19} and p53 immunohistochemical staining¹⁷. Neither works well for biliary lesions. K-ras mutation is a relatively uncommon event in biliary tract cancers, less than 33% of the tumors have the mutation. p53 immunohistochemical staining also does not seem to add any information.

Fine Needle Aspiration of Pancreatic Lesions

Fine Needle Aspiration: Use of a fine needle to remove a sample of cells. There must be a target, a “mass”, which can be identified by ultrasound, CT, or palpation.

Technique: A fine needle, typically 22 gauge or smaller is introduced into the lesion under imaging guidance. The stilet is removed from the needle. Suction is typically applied. A cutting motion is crucial with long “piston-like” excursions and quick strokes (3 strokes per second). One wants to take alternate paths through the lesion to increase the sampling of the lesion. Aspiration by suction alone, with the needle at rest, is not sufficient to draw tissue into the needle. The needle should not be rotated, nor the syringe pumped. Typically only the end of the needle is filled with tissue, one does not need to get blood in the syringe.

After multiple excursions through the lesion, the suction should be released and the needle removed from the lesion and the endoscope.

Slide preparation: The cells must be delicately and thinly smeared, with minimal distortion, and fixed quickly. Once the needle has been removed from the endoscope, the syringe must be removed from the needle and filled with air, and then reattached to the needle. The bevel of the needle should be placed adjacent to the glass slide near the frosted end. The next step is to express the aspirated material, using the air-filled syringe to blow out the needle, onto a slide (a second slide can be used at a 45-degree angle to “block” and prevent making a mess. The aspirate is next inspected grossly:

- Adequate?
- Fluid?
 - Drain, reaspirate if any residual mass
- Purulent?
 - Culture
- Necrotic?
 - Reaspirate periphery
- Bloody?
 - Stop or Reaspirate.

A drop of the aspirated material, about the size of a pin and with tissue fragment, is placed near the frosted end of a slide (the diagnostic slide). A second, or spreader slide is gently lowered, crosswise, over the droplet, which will spread out slightly by capillary action. The spreader slide is then gently pulled straight back, in one smooth motion, down the length of the diagnostic slide. This should create a nice oval smear. The slide is then fixed quickly, usually in 95% alcohol.

Complications: A number of possible complications exist including: pain and anxiety, hematoma, hemorrhage, vasovagal reaction, seizures, nerve damage, fever, infection, tumor necrosis, pneumothorax, air embolism, pancreatitis, bile peritonitis, sepsis, and local anaphylaxis. Two studies, one with 218 patients⁷ and one with 520 patients⁵ had the following results:

Complication rate 2%, 2 pts with bleeding, 2 patients with pancreatitis (1.2% rate) Complication rate 5%, 1 hematoma, 3 vasovagal reactions, 21 pain

(This was an abdominal ultrasound guided FNA)

Contraindications: Include patients with bleeding problems, a highly vascular lesion, suspected hydatid cyst (typically liver), or an uncooperative patient.

FNA can be performed intraoperatively, by CT or ultrasound (abdominal or endoscopic) guidance, or from ERCP. The specimens are identical from a pathologist's perspective as seen in the following study by Mallery et al¹².

Sampling Method	Sensitivity	NPV	Accuracy
EUS	74%	27%	76%
CT	80%	23%	81%
Surgical	78%	50%	82%
Combined	77%	28%	79%

Impact of On-site Cytologist Immediate Adequacy Determination: Two studies suggest that an on-site cytologist improves accuracy and adequacy rate. In the Mallery study the accuracy rate improved from 68% to 83% and the adequacy rate from 91% to 96% with an on-site cytologist. Klapman¹⁰ stated that on on-site cytologist increased the frequency of a positive or negative (versus an equivocal) diagnosis and was less likely to have an unsatisfactory specimen.

Types of Pancreatic FNA's: At MUSC the vast majority of fine needle pancreatic aspirates are for adenocarcinoma (64%). Approximately 25% are for reactive lesions or pseudocysts, less than 5% islet cell tumors, and the rest a variety of other lesions. Less than 2% of the FNA's are non-diagnostic.

Special Procedures:

Electron Microscopy: A technique that allows examination of intracellular architecture. It allows much higher power magnification of individual cells and can be helpful in determining the type of tumor. It is rarely done due to the availability of immunohistochemical stains. However for optimal specimens, the specimen should be fixed in glutaraldehyde fixative.

Flow Cytometry: A technique for measuring multiple characteristics of single cells in a moving fluid stream. Cell size, internal structures, antigens, DNA ploidy, and cell cycle analysis. It is very important for the classification of lymphomas, as it allows for the determination of clonality, and cell type (B cell v T cell). It requires the specimen to be collected fresh or in a balanced media (RPMI) and run within 24 hours of collection.

Criteria for Adenocarcinoma in Pancreatic FNA's: Robins et al¹⁶. summarized the following criteria for malignancy. Two or more major criteria or one major and three minor criteria gave them a sensitivity of 90% and a specificity of 100%.

Major Criteria	Minor Criteria
Overlapping nuclei/ Crowded groups	Single epithelial cells
Nuclear contour irregularity	Necrosis
Chromatin clearing and/or clumping	Mitosis
	Nuclear enlargement

Chen et al.¹ at MUSC examined the false negative cases, 30 of 402 cases. Of these 30 cases, 18 had malignant cells. The criteria used were three of the following: Loss of polarity, Nuclear enlargement (1.5 x size of RBC), Nuclear membrane irregularity, Pleomorphism, Chromatin pattern, Confluence, Increased cellularity, Hyperchromasia, Macronucleoli, and Necrosis. The first three criteria were the most common seen. Persistent false negative cases had a paucity of cells and little cytologic aberration, perhaps representing a sampling error.

Summary of Diagnostic Features of Malignancy:

Cells	Nucleus	Cytoplasm	Background
Usually numerous	Disorderly: loss of polarity, piled up, crowded	Pleomorphism	Necrosis
Disorganized, crowded groups (chaotic architecture)	Enlargement	Loss of cell boundaries	Blood, hemosiderin
Single intact atypical cells	Pleomorphic size and shape, including abnormal shapes	Abnormal staining	Tumor diathesis
Cannibalism (cell-in-cell pattern)	Multinucleation	Abnormal cellular products	
Abnormal shapes	Naked nucleus (no cytoplasm)		
Increased nuclear/cytoplasmic ratio	Molding		
	Irregular nuclear membrane		
	“Thick” nuclear membrane		
	Hyperchromatic		
	Irregular, abnormal chromatin pattern		

Artifacts: A number of artifacts can be induced (usually at the time of collection) that make a cytologic diagnosis impossible²¹.

Air Drying: With Pap stained slides that are alcohol fixed, air-drying can enlarge the cells and make them look malignant. From the time the sample is collected until it is placed in alcohol it is being air-dried.

Obscured Smear: The malignant cells on a slide can be obscured by excessive blood (an ideal specimen has very little blood) or by other cells (a thick smear).

Displace Epithelium: Cells can be picked up from another site. Examples:

Fine Needle Aspirations, the needle goes through the gut wall. The surface epithelium of the gut can contaminate the sample even with the stilet in place. Even worse, if a lymph node is being sampled through the tumor, malignant cells can be picked up. Currently, lymph nodes are not sampled through a tumor.

Brush sample may brush the end of the endoscope and pick up epithelium.⁽¹⁻³⁾

References:

1. Chen RYM. Fraig MM. Hoda RS. Lewin D. Hoffman BJ. Hawes RH. Wallace MB. A new cytological criteria of fine needle aspiration (FNA) by endoscopic ultrasound (EUS) of pancreatic adenocarcinoma decreases the false negative rate. *Gastrointestinal Endoscopy*. 55(5):AB242, 2002.
2. Cohen MB. Wittchow RJ. Johlin FC. Bottles K. Raab SS. Brush cytology of the extrahepatic biliary tract: comparison of cytologic features of adenocarcinoma and benign biliary strictures. *Modern Pathology*. 8(5):498-502, 1995.

3. Cohen MB, Wittchow RJ, Johlin FC, et al. Brush cytology of the extrahepatic biliary tract: comparison of cytologic features of adenocarcinoma and benign biliary strictures. *Modern Pathology*. 8:498-502, 1995.
4. Cunningham JT, Lewin D, Tarnasky P, Hoffman B, Hawes R, Cotton PB. A prospective study of multiple sampling methods in the assessment of biliary strictures. *Gastrointest Endoscopy* 1997;45(4):AB128.
5. Di Stasi M, Lencioni R, Solmi L, Magnolfi F, Caturelli E, De Sio I, Salmi A, Buscarini L. Ultrasound-guided fine needle biopsy of pancreatic masses: results of a multicenter study. *American Journal of Gastroenterology*. 93(8):1329-33, 1998.
6. Farrell RJ, Jain AK, Brandwein SL, Wang H, Chuttani R, Pleskow DK. The combination of stricture dilation, endoscopic needle aspiration, and biliary brushings significantly improves diagnostic yield from malignant bile duct strictures. *Gastrointestinal Endoscopy*. 54(5):587-94, 2001.
7. Gress FG, Hawes RH, Savides TJ, et al. Role of EUS in the preoperative staging of pancreatic cancer: a large single-center experience. *Gastrointest Endosc* 1999;50:786-91.
8. Hoda RS, Minamiguchi S, Makie T, McEvoy R, Houser PM, Fraig MM, Lewin D, Wallace M. Bile Duct Brushings on ThinPrep: Experience with 68 Specimens. *Modern Pathology*. 16(1):69A, 2003.
9. Jin YH, Kim SH, Park CK. Diagnostic criteria for malignancy in bile cytology and its usefulness. *Journal of Korean Medical Science*. 14(6):643-7, 1999.
10. Klapman JB, Logrono R, Dye CE, Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. *Am J Gastroenterol* 2003;98:1289-94.
11. Layfield LJ, Wax TD, Lee JG, Cotton PB. Accuracy and morphologic aspects of pancreatic and biliary duct brushings. *Acta Cytologica*. 39(1):11-8, 1995.
12. Mallery JS, Centeno BA, Hahn PF, Chang Y, Warshaw AL, Brugge WR. Pancreatic tissue sampling guided by EUS, CT/US, and surgery: a comparison of sensitivity and specificity. *Gastrointestinal Endoscopy*. 56(2):218-24, 2002.
13. Muller P, Ostwald C, Puschel K, Brinkmann B, Plath F, Kroger J, Barten M, Nizze H, Schareck WD, Hauenstein K, Liebe S, Lohr JM. Low frequency of p53 and ras mutations in bile of patients with hepato-biliary disease: a prospective study in more than 100 patients. *European Journal of Clinical Investigation*. 31(3):240-7, 2001.
14. Nakajima T, Tajima Y, Sugano I, et al. Multivariate statistical analysis of bile cytology. *Acta Cytologica*. 38:51-55, 1994.
15. Renshaw AA, Madge R, Jiroutek M, Granter SR. Bile duct brushing cytology: Statistical analysis of proposed diagnostic criteria. *American Journal of Clinical Pathology*. 110(5):635-40, 1998.
16. Robins DB, Katz RL, Evans DB, Atkinson EN, Green L. Fine needle aspiration of the pancreas. In quest of accuracy. *Acta Cytol* 1995;39:1-10.
17. Stewart CJ, Burke GM. Value of p53 immunostaining in pancreatobiliary brush cytology specimens. *Diagnostic Cytopathology*. 23(5):308-13, 2000.
18. Sturm PD, Rauws EA, Hruban RH, Caspers E, Ramsoekh TB, Huibregtse K, Noorduin LA, Offerhaus GJ. Clinical value of K-ras codon 12 analysis and

- endobiliary brush cytology for the diagnosis of malignant extrahepatic bile duct stenosis. *Clinical Cancer Research*. 5(3):629-35, 1999.
19. van Heek NT. Rauws EA. Caspers E. Drilenburg P. Gouma DJ. Offerhaus GJ. Long-term follow-up of patients with a clinically benign extrahepatic biliary stenosis and K-ras mutation in endobiliary brush cytology. *Gastrointestinal Endoscopy*. 55(7):883-8, 2002.
 20. Vandervoort J. Soetikno RM. Montes H. Lichtenstein DR. Van Dam J. Ruymann FW. Cibas ES. Carr-Locke DL. Accuracy and complication rate of brush cytology from bile duct versus pancreatic duct. *Gastrointestinal Endoscopy*. 49(3 Pt 1):322-7, 1999.
 21. Young NA. Mody DR. Davey DD. Misinterpretation of normal cellular elements in fine-needle aspiration biopsy specimens: observations from the College of American Pathologists Interlaboratory Comparison Program in Non-Gynecologic Cytopathology. *Archives of Pathology & Laboratory Medicine*. 126(6):670-5, 2002.