Introduction

Abnormalities of the extrahepatic biliary tract and pancreatic ductal system may come to clinical attention following patient presentation with unexplained jaundice, abdominal pain or weight loss. These abnormalities may be secondary to a variety of processes including auto-immune disease, inflammatory lesions, malignancy and calculus disease. While most benign strictures are managed by ductal dilatation and/or stenting, malignant strictures when operable are treated by Whipple resection or bile duct resection. In patients with unresectable disease, simple stenting may be undertaken or neoadjuvant chemo and radiation therapy performed to shrink the tumor to an operable size. Thus, confirmation of the benign or malignant nature of the stricture is of utmost clinical importance.

Minimally invasive techniques for investigating extrahepatic biliary tract and pancreatic duct strictures as well as pancreatic masses include endoscopic ultrasound guided (EUS) fine needle aspiration (FNA) and duct brushing cytology. Unfortunately, the majority of biliary and pancreatic duct lesions are not accessible to forceps or clamshell biopsy. Brushing cytology is the predominant initial technique for obtaining a tissue diagnosis for strictures. This, despite the fact that diagnostic sensitivity of brushing cytology is disappointingly low.

A large number of authors have reported their experience with the diagnostic sensitivity, specificity and accuracy of brushing cytology. The sensitivity of the technique has varied from approximately 25% to 90% with most studies revealing a sensitivity of between 40% and 60%. Specificity is superior to sensitivity and has been reported to range between 80% and 100%. A number of clinical conditions including inflammation and primary sclerosing cholangitis are known to decrease diagnostic
A variety of ancillary techniques have been utilized in an attempt to improve overall diagnostic sensitivity, specificity and accuracy.\textsuperscript{39-49}

While clinicians treating pancreatic biliary strictures favor a definitive classification into benign or malignant, most cytopathologists favor a diagnostic scheme utilizing one or more intermediate categories.\textsuperscript{16,18,31,35} Terminology for these intermediate categories has varied and includes the terms dysplasia, atypical cells, atypical cells suspicious for malignancy, atypia not otherwise specified and atypia favor reactive.\textsuperscript{16,18,31} The Papanicolaou Society of Cytopathology is in the process of developing a set of guidelines for diagnostic techniques, diagnostic criteria and categories along with recommendations for the use of ancillary studies and post-FNA follow-up and therapy. The proposed categorization for the diagnosis of pancreatic and biliary lesions contains the categories: 1) non-diagnostic, 2) benign, 3) atypical (including reactive), 4) suspicious for malignancy and 5) malignant. A number of studies have investigated the diagnostic criteria for the separation of benign from malignant pancreatico-biliary duct brushings.\textsuperscript{2,16,18,19,31,32,50-55} Many of these studies have utilized linear regression analysis to determine which criteria are most accurate in separating benign from malignant lesions. Some studies have established primary and secondary criteria for this separation.\textsuperscript{2,18,53} Primary criteria have included nuclear moulding, chromatin clumping and increased nuclear cytoplasmic ratio. Secondary criteria included anisonucleosis, irregular nuclear membranes, loss of polarity, chromatin clearing, nuclear enlargement, macronucleoli and disruption of the normal honeycomb pattern. The sensitivity and specificity of these criteria have varied.\textsuperscript{31} In many of these studies proposals have been made to use multiple diagnostic categories because many times the identified criteria are difficult to recognize or apply uniformly. Hence, the inclusion of atypical and suspicious for malignancy categories in many diagnostic schemes. This is similar to the Bethesda system for squamous lesions of the cervix and the system for thyroid FNA. Characteristics of benign biliary epithelium, atypical, suspicious for
malignancy and malignant are listed in tables I through IV. In the diagnostic categorization used in the PSC guidelines, major criteria for malignancy include increased nuclear cytoplasmic ratio, chromatin clumping, nuclear moulding, loss of honeycomb pattern or polarity, cell-in-cell arrangements and a fourfold or greater variability of nuclear size within a single cell group or cluster.

Major diagnostic pitfalls exist for the cytologic diagnosis of pancreatico-biliary brushings. These include sampling, special tumors types, smear background, dysplasia, primary sclerosing cholangitis, the presence of stones or stents, cloaking of malignant epithelium by overlying benign epithelium and degeneration of the cellular content due to bile or duodenum contamination. Well differentiated adenocarcinomas of the pancreatico-biliary system also represent a problematic area for the cytologic diagnosis of strictures. Biliary tract stones and stents may be associated with marked reactive atypia. Helpful clues in differential diagnosis include the fact that atypical cells often lack abnormal chromatin patterns and membrane irregularities are usually mild. Importantly, the nuclear cytoplasmic ratio remains near normal. Acute inflammation involving the biliary tract is also associated with nuclear atypia which can be distinguished from malignancy by recognizing the presence of acute inflammation. Other helpful features in separating the atypia of inflammation from true neoplasia are that reactive atypia due to acute or chronic inflammation maintains a near-normal nuclear-cytoplasmic ratio. Additionally, the nuclear membrane changes seen with inflammatory atypia are usually less than those seen in true malignancy. Moreover, in reactive atypias two distinct cell populations are not seen but there is a merging of features between cells with significant atypia and clearly benign cells. This range of morphologies favors benign while two distinctly separate populations (atypical versus benign) favors malignancy.
As described above the sensitivity and specificity of pancreatico-biliary brushing cytology is well known and published a large number of studies.\textsuperscript{1-37} Follow-up for the atypical and suspicious for malignancy categories is less well described. In a study of a thousand cases, Volmar, et al\textsuperscript{35} documented that clinical or pathologic follow-up of cases designated “suspicious for malignancy” revealed a malignancy in 79.2%. For the category “atypical/inconclusive” follow-up revealed malignancy in 43.6% of cases. Our personal experience demonstrates that atypical/favor reactive is associated with an absolute risk of 0.20 and a relative risk of 0.8 for malignancy. The atypical/suspicious in our experience has an absolute risk of 0.74 and a relative risk of 3.0 for malignancy. From the results of these two studies the risk of malignancy in the “suspicious for malignancy” category closely approaches that of a definitive diagnosis of malignancy.

The “atypical (favor reactive)” category has a risk of malignancy which varies between studies. In the study by Volmar, the atypical/inconclusive category had a significant risk of malignancy (43.6%) and was similar to the risk of malignancy in the benign category reported in a number of studies. In the studies at the University of Utah, the “atypical (favor reactive)” category had an absolute risk and relative risk of malignancy less than that seen for the benign category. The absolute risk and relative risk for the benign category were 0.248 and 1.0 respectively. From this data it appears that the category “atypical (favor reactive)” can be followed/treated similarly to the benign category while the “suspicious for malignancy” category should be followed/treated like the malignant period.

Because of the poor diagnostic sensitivity of pancreatic biliary brushing cytology a number of ancillary testing methods have been utilized including mutational analysis for KRAS mutations, p53 mutations as well as ploidy analysis and loss of heterozygosity. More recently, ancillary testing has included fluorescence in-situ hybridization for changes in chromosomal numbers.\textsuperscript{39} Evaluation of current ancillary testing appears to indicate that fluorescence in-situ hybridization (FISH) is the most diagnostically useful in separating benign from malignant pancreatico-biliary strictures. This technique
should be utilized on all equivocal cytologies to improve diagnostic sensitivity without loss of specificity.39

References


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Table I - Morphologic Characteristics of Benign Biliary Epithelium

- Regular “honeycomb” sheets
- Retention of cell polarity
- Smooth nuclear membranes
- Even chromatin pattern
- Small or indistinct nucleoli
- Mitotic figures and necrosis absent
- Low nuclear/cytoplasmic ratio
- Variability in nuclear size less than threefold in any single group

Table II – Morphologic Characteristics of “Atypia”

- Mild variation in nuclear size less than threefold in any single group
- Nuclear hyperchromasia
- Distinct nucleoli
- Normal mitotic figures may be present
- Relatively normal n/c ratio
- Mild nuclear membrane irregularities
- Squamous metaplasia

Table III – Morphologic Characteristics of “Suspicious for Malignancy” Category

- Loss of “honeycomb” pattern
- Loss of polarity
- Nuclear crowding and overlap
- Coarse chromatin
- Enlarged nucleoli
- Increased n/c ratio
- Nuclear membrane irregularities
- Three to fourfold variability in nuclear size within a single cell cluster

*some but not all the features of malignancy present

Table IV – Morphologic Characteristics of “Malignant”
- Cell groups with prominent nuclear overlap and crowding
- Coarse chromatin
- Enlarged nucleoli
- Increased n/c ratio
- Irregular nuclear membranes
- Three to four or more fold variability in nuclear size within a single cell group