Bile Duct Brushing Cytology: A Morphologic and Molecular Approach

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Introduction

Strictures of the bile and pancreatic duct along with masses impinging on the bile or pancreatic ducts represent difficult clinical management and diagnostic problems. While imaging and endoscopic finding are often helpful in the diagnosis of these lesions, cytology remains an important modality for determining the precise nature of these strictures and masses. Cytologic examination may include evaluation of aspirated bile or brushing of the stricture or epithelium overlying the mass. Examination of aspirated bile has been shown to have a very low sensitivity due to the corrosive nature of bile on the epithelial component as well as the overall low cellularity. Bile duct brushings yield specimens of higher cellularity, but are associated with a number of technical challenges resulting in relatively low sensitivity for the procedure. Diagnostic accuracy has varied considerably in the reported literature, but most authors have reported a sensitivity between 30% and 60% and a specificity of 87% to 100%. A number of issues have been suggested as being responsible for the low sensitivity. These include the destructive nature of bile (making evaluation of the cellular component difficult), the scirrhous reaction surrounding tumor cells resulting in low cellularity specimens and normal mucosa overlying a deeper carcinoma precluding sampling of the malignancy. Given the low sensitivity but high specificity many authors have suggested that a negative result is essentially equivalent to non-diagnostic and has little impact on patient management while a positive result is highly valuable for initiation of further patient management and therapy. Due to the low sensitivity of the technique, a number of authors have used multivariant statistical analysis to determine what morphologic features are useful in the diagnosis of bile duct carcinoma. These studies have shown that nuclear molding, chromatin clumping, increased nuclear/cytoplasmic ratio, loss of honeycomb pattern, greater than three or four-fold variation in nuclear size within a single cell cluster, cell-in-cell arrangements and a bloody background are the most useful diagnostic criteria. Despite these findings, any single one of these criteria is associated with a diagnostic sensitivity of between 35% and 51%. Loss of the honeycomb pattern is the most sensitive (51%), but has a relatively low specificity (73%). The use of multiple criteria appears to increase diagnostic accuracy.

In addition to the issue of poor sensitivity, a number of biological phenomenon complicate the interpretation of bile duct brushings. These changes include reactive atypia secondary to inflammation, ulceration, stents or stones. Each of these can result in considerable reactive atypia which may be mistaken for carcinoma or dysplasia. The issue of dysplasia is also a significant problem that must be addressed in the evaluation of biliary cytology. The presence of pre-malignant lesions within the biliary system variably designated as dysplasia or biliary intraepithelial neoplasia (BilLN) complicates cytologic interpretation of bile duct brushings. The concept of biliary intraepithelial neoplasia is well-accepted and defined histopathologically. However, diagnostic criteria are less developed for the cytologic recognition of dysplasia/biliary intraepithelial neoplasia. In an attempt to better stratify the risk of
malignancy and to incorporate the concept of biliary intraepithelial neoplasia into cytologic diagnosis and classification, several authors have introduced intermediate categories between benign and malignant.6,12,15,16 Despite these attempts, consensus as to terminology is difficult to achieve. The Papanicolaou Society of Cytopathology proposed a six tier classification system comprising the categories non-diagnostic, negative, atypical, neoplasm, suspicious for malignancy, and malignant.17 These categories are associated with defined risks for malignancy as well as post cytologic diagnosis follow-up and treatment. Using the definitions of the Papanicolaou Society of Cytopathology scheme, lesions designated “atypical” demonstrated a malignancy risk of between 44% and 62%.8,13 The category “suspicious for malignancy” is associated with a malignancy risk of 74%.13,17 The malignant category has a malignancy risk of nearly 100%. The category atypical contains bile duct brushings with cells showing greater dysmorphology than a cytologist is comfortable assigning to the negative (for malignancy) category, but less than that necessary for assignment to the “suspicous for malignancy” category. Follow-up histology revealed that this category contained specimens showing reactive atypia and low grade dysplastic (BilIN 1) lesions. Because this is an indeterminate category, it should be used sparingly. The “suspicious for malignancy” category contains lesions with greater dysmorphology than can be assigned to the “atypical” category, but less than that necessary for assignment to the malignant category. The “suspicious for malignancy category” is useful in maintaining the high positive predictive value of the malignant category. Histologic follow-up of lesions assigned to the “suspicious for malignancy” category demonstrate high-grade dysplasia (BilIN 2 and 3) as well as carcinomas of the bile duct. The categories “atypical” and “suspicious for malignancy” are associated with high inter- and intraobserver variability. A significant percentage of lesions assigned to the “atypical” category will be downgraded by other cytopathologists to the “negative” category while a significant percentage of the “suspicious for malignancy” category might be upgraded by review to the fully malignant category.

Ancillary testing can be of considerable value in establishing the diagnosis of malignancy for bile duct brushing specimens. Ancillary testing is especially useful in the category “suspicous for malignancy”. Ancillary methods potentially useful for the interpretation of negative and indeterminate cytologic results include digital image analysis, immuno-labeling, fluorescence in situ hybridization, and genetic molecular analysis for neoplasia specific mutations (KRAS).

Digital image analysis of samples from bile duct strictures has been used for a number of years to identify abnormalities of nuclear DNA content. Digital image analysis utilizes the Feulgen reaction which stoichiometrically binds the Feulgen dye to the nucleic acids. Using this technique, DNA ploidy can be assessed by a variety of commercially available image analyzers. This technique generates histograms which characterize specimens into diploid, aneuploid, and tetraploid results. Aneuploid and tetraploid results are more likely to support the presence of a malignancy. However, the sensitivity of digital image analysis does not appear to improve diagnostic accuracy beyond that achievable by routine cytology. This is especially true for specimens derived from primary sclerosing cholangitis. In the absence of sclerosing cholangitis, digital image analysis does appear to slightly improve diagnostic sensitivity. The technique has excellent specificity, but only moderate sensitivity for malignancy. As such, digital image analysis does not appear to have sufficient sensitivity to be employed in routine clinical analysis.

A number of immunocytochemical markers exist which are associated with the presence of dysplasia or malignancy. Among these are CD10, IMP3, and potentially SMAD4. CD10 positivity is present in the overwhelming majority of benign lesions, but absent in high-grade dysplasia and malignancies of the bile
ducts. Adenocarcinomas frequently stain for MUC4 and IMP3. IMP3 staining is present in approximately 78% of adenocarcinomas. Nonetheless, for a variety of technical reasons, these immunocytochemical techniques are rarely utilized in the diagnosis of bile duct brushings.

KRAS mutational analysis has been proposed as an ancillary testing procedure for the analysis of pancreatic juice, bile duct brushings and fine needle aspirations of biliary strictures and solid and cystic pancreatic masses. While early studies suggested a high utility for KRAS mutational analysis, subsequent studies have shown it to have limited applicability. While KRAS mutations are present in the majority of adenocarcinomas, they are also found in some reactive atypias and low grade dysplasias. It appears that KRAS mutations are an early event in the development of pancreatic and bile duct carcinomas limiting its diagnostic value.

Fluorescence in situ hybridization appears to be the optimal ancillary test for the investigation of bile duct brushings. The procedure appears to be most useful for the analysis of specimens designated as “suspicious for malignancy”. FISH analysis investigates specimens for the present of polysomy using a commercially available DNA probe set (UroVysion). This probe set targets pericentromeric regions of chromosomes 3 (CEP3), 7 (CEP7), and 17 (CEP17) as well as chromosomal band 9p21. The method may be automated, potentially facilitating reproducibility. Authors have shown the FISH method to have 100% specificity and a sensitivity of at least 60% for the identification of carcinoma. A specimen is considered positive for malignancy by FISH when five or more cells show polysomy (greater than two signals in at least two of the four probes). FISH analysis have been showed to out-perform routine cytology for the evaluation for brush specimens. Studies have shown that the sensitivity of FISH is approximately 90% with a related specificity of 94%. Its positive predictive value is 98% and negative predictive value is 75%. The method can be performed on liquid-based preparations as well as cell block materials.

The evaluation of bile duct strictures and peri-ductal masses requires a multidisciplinary approach. Clinical findings, imaging findings, endoscopic findings and cytologic diagnoses should all be reviewed in a multidisciplinary conference to select appropriate management and patient follow-up procedures. The cytologic diagnosis of “suspicious for malignancy” is not equivalent to the diagnosis of malignancy. A cytologic diagnosis of “suspicious for malignancy” should not be used as the sole result for the initiation of radical surgery. If ancillary testing and/or clinical and imaging features also indicate the presence of malignancy, the suspicious category can be used as one component of the diagnostic set for the initiation of pancreaticobiliary surgery.
References:


