

Review Article

Cytology of the Biliary Tree

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Abstract

The biliary tree is composed of intrahepatic and extrahepatic ducts and is lined by simple columnar/cuboidal epithelium. Particularly in evaluation of extrahepatic bile ducts, cytology is commonly utilized. Herein we review common indications, methodology and microscopic features as relates to biliary tract cytologic diagnoses. Additionally, we briefly discuss newer techniques that can be used in conjunction with routine procedures, with reported increase in the overall diagnostic yield.

ABBREVIATIONS

EHBDS; PTC; EUS; FNA; MRCP; BDB; PSC; GAG; N/C; FISH; LOH

INTRODUCTION

In humans the biliary network is subdivided into two portions, the intra-hepatic bile ducts and extra-hepatic bile ducts (EHBDS). The biliary tract begins with the canals of Herring. From there on its anatomical progression follows interlobular, septal and segmental ducts, eventually merging to form the extra-hepatic biliary tract with graduated increase in the bile ducts' diameter. Canals of Herring are in direct continuity with terminal cholangiols. The biliary tract is lined by a simple columnar/cuboidal epithelium, with basement membrane and tight junction between the adjacent cells. Larger and septal ducts have embryological origin and histologic features that can resemble the EHBDS with mucus producing glands in the duct wall, aka peri-biliary glands. The EHBDS include the common hepatic duct which joins the cystic duct to form the common bile duct. The common bile duct enters the duodenum through the Papilla of Vater, and in majority of individuals, the main pancreatic duct joins the CBD just before entering the duodenum [1,2].

Biliary cytology gained popularity in 1960s when the percutaneous transhepatic cholangiography (PTC) and endoscopic retrograde cholangiopancreatography (ERCP) came into clinical use. In 1975, endobiliary brush cytology was introduced by Osnes *et al.* More recently, the use of endoscopic ultrasound (EUS) guided fine-needle aspiration (FNA) has provided a supplemental technique for acquiring cytology from the biliary tract [3,4]. At the current time, sampling of the biliary tree by cytology is the mainstay for evaluation and management of patients with biliary disease. While tissue biopsies are infrequently performed due to complications including risk of scar with stricture, hemorrhage and direct bile leak leading to peritonitis [4].

A set of guidelines to include indications for EUS guided

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- Bile duct brushing
- Fish

FNA, techniques for the ERCP, terminology and nomenclature of the pancreaticobiliary disease has been developed by the Papanicolaou Society of Cytopathology.⁴ American Medical Association mandated pre-procedural informed consent with recommendations to disclose the diagnosis (if known), nature, purpose, risks and benefits of the methodology, alternate options, their risks, and risk or benefits if not receiving treatment or procedure. All to be performed in a way that the patient understands [5].

A commonly utilized methodology now for evaluating biliary epithelium is brushings obtained during the ERCP, which provide an ultimate sampling source [4]. Although certain indications for ERCP have been recently replaced by use of magnetic resonance cholangiopancreatography (MRCP) or EUS, it is still the most useful clinically for providing relief of obstructing jaundice by biliary stent placement [4,5]. Aspiration of bile juice during ERCP can be utilized, retrieving exfoliated cells with expectedly low sensitivity for detection of malignancy- but can put to use passive biliary drainage collections. In parallel, biliary stent cytology can provide additional opportunity for diagnosis when prior sampling is inconclusive, but is not useful when there is a need for an immediate diagnosis [3].

Despite a very high specificity, sensitivity of cytologic methods remains generally low and variable. Sensitivity of bile duct brushing (BDB) is reported to range from less than 30% to over 80% with median of 50%, while its specificity is reported greater than 80% up to 100% with median of 98%, according to different reviews. The reported accuracy of BDB is somewhat comparable to biliary aspirations, but significantly better than that of bile cytology [6-10]. Although brush cytology by ERCP is widely performed, according to Adler *et al.*, it has a lower yield

than EUS-FNA for suspected cholangiocarcinoma, in particular in undetermined hilar-based strictures. The sensitivity of EUS-FNA is reported higher in detection of the distal compared to the proximal lesions [10]. The main indication for evaluation of bile duct epithelium is the presence of a stricture or a mass in common bile duct or pancreatic duct. While many strictures of the biliary system are due to malignancy, strictures may also have a non-malignant source. These changes could be due to inflammatory conditions, choledocholithiasis, chronic pancreatitis, surgical trauma, ischemia, primary sclerosing cholangitis, infection and/or idiopathic processes [11] (Figures 1,2).

Reporting of biliary cytology is reflective of the findings. The diagnosis usually falls into one of four categories: negative for malignant cells, suspicious for malignancy, positive for malignant cells and unsatisfactory for evaluation due to scant cellularity, poor preservation or air drying, followed by an explanation of the findings.

In brushing specimens, non-diseased ductal epithelium appears as flat monolayer sheets with maintained polarity. These epithelial cells have a centrally located nucleus, dense cytoplasm, and well-defined cell borders. Chromatin is finely granular and pale stained, and nucleoli are inconspicuous. Bile may be seen in the background as amorphous material that sometimes appears yellow. In thin layer preparations and bile juice aspirates the fragments are often smaller than direct smears (Figures 3,4) [6,12].

Adenocarcinoma, while overall uncommon, is the most common malignant neoplasm affecting the extra-hepatic biliary ductal system. It is most frequent in the distal portion of the biliary tree, and as expected symptoms mostly relate to obstruction of the biliary system, with painless jaundice and pruritus being the most common. Cholangiocarcinoma most commonly presents in 6th decade, affecting men more than women; ulcerative colitis, biliary stones and particularly in Asia, parasitic infections are recognized as risk factors. Most adenocarcinomas are advanced at the time of diagnosis and are not amenable to surgical resection [13]. Thus, palliation, often in the form of stent placement is the major form of therapy. Prognosis is dismal, with many patients dying within the first year after the diagnosis.

In brushing smears, adenocarcinoma presents as solitary, intact malignant cells and/or three-dimensional aggregates. Within the clusters, the abnormal nuclei are piled up and

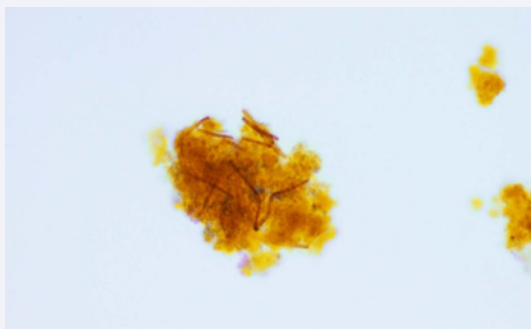


Figure 1 Biliary Brushing- Typical pseudohyphae of *Candida* admixed with bile (400x).

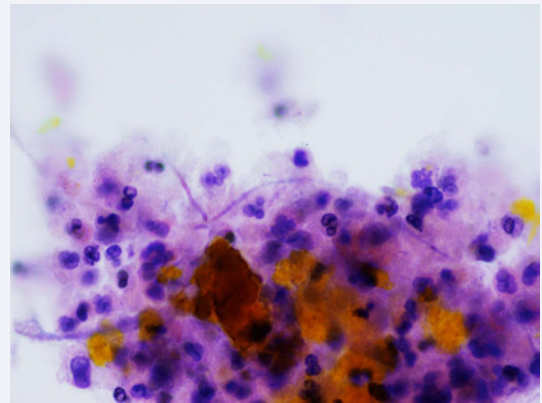


Figure 2 Biliary Brushing- Fungal hyphae admixed with acute inflammatory exudate and bile (400x).

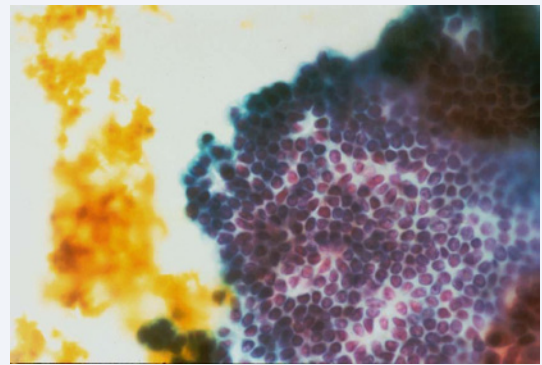


Figure 3 Benign bile duct epithelium, with cellular uniformity and even "honeycomb-like" distribution. Bile is noted in the background (200x).

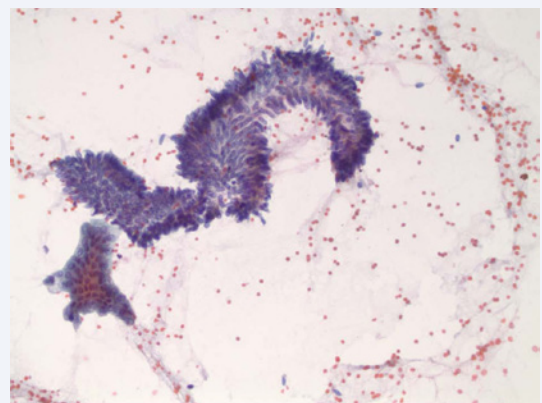


Figure 4 Benign duct epithelium obtained by direct brushing of the bile ducts. Columnar cells are uniform, even in shape and have maintained polarity (100x).

crowded. Individual cells have enlarged nuclei with smooth to irregular contours, coarse hyperchromatic chromatin, and often well-developed nucleoli. The nuclear to cytoplasmic (N/C) ratio is variable but generally high [6]. Chromatin clumping, increased nuclear to cytoplasmic ratio, and either nuclear molding or loss of honeycombing are reproducible cytologic criteria that accurately

predict malignancy in bile duct brushing (Figures 5,6). The major differential diagnosis is benign reparative atypia. According to Vadmal *et al*, even when few in number, small, three dimensional epithelioid clusters with marked atypia signify malignancy and warrant the diagnosis of malignancy in brushing cytology, whereas single cells, cytoplasmic vacuoles and prominent nucleoli are not essential for diagnosis of malignancy [6,12,14].

False negative results in biliary cytology for malignancy can be secondary to well-differentiated/ special tumor types such as mucinous or papillary subtypes (bland cellular morphology), smear concealment of rare tumor cells, dysplasia, sampling limitations due to anatomic difficulties, extensive fibrosis, benign epithelium overlying cancer, or extrinsic tumors that surround the bile ducts but do not infiltrate them. Fritcher *et al*, using quantitative nuclear morphology and in situ hybridization, confirm many false negatives attributable to inadequate sampling secondary to poor visibility of the lesion or increased fibrosis, rather than the pathologist's interpretation. While in the subset with adequate sampling, misinterpretation was more likely seen in tumors with a well-differentiated morphology [6,15].

Reactive atypia typically has cohesive monolayer sheets; intact atypical and individually dispersed cells are extremely rare. Generally, a honeycomb arrangement with slight overlapping of cells, mildly enlarged round-to-oval nuclei with fine chromatin and smooth nuclear contour is present [6]. Nucleoli are generally inconspicuous, although macro-nucleoli can occur (Figure 7). Polarity is generally maintained, streaming cytoplasm with intact cell borders is commonly present. The smear background may contain bile pigment, cholesterol, crystals, and varying degrees of inflammation.

Marked reactive change may be seen in biliary brushings of patients with strictures of primary sclerosing cholangitis (PSC), which can be difficult to distinguish from a malignant process. This is further complicated by the increased incidence of dysplasia and cholangiocarcinoma in patients with PSC. Dysplastic cell groups usually show more significant cellular crowding and overlapping than reactive reparative cells [6]. More significant nuclear atypia with increased N/C ratio and abnormal chromatin

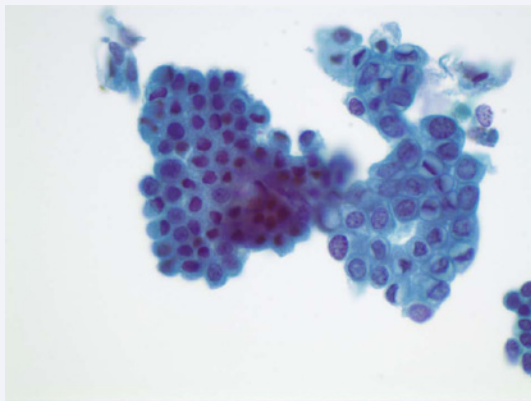


Figure 5 Well differentiated adenocarcinoma on the right in comparison with benign biliary epithelium on the left side. The neoplastic cells display loss of polarity, nuclear irregularity and size variation (400x).

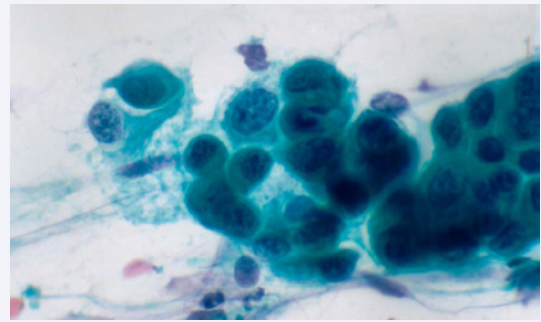


Figure 6 Moderately differentiated adenocarcinoma, the haphazard neoplastic cells are hyperchromatic with chromatin clumping, occasional prominent nucleoli and range in size 1:4 (400x).

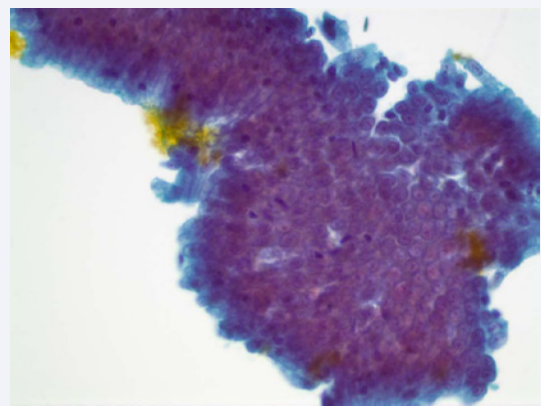


Figure 7 Atypical bile duct epithelium with slight crowding, enlargement of nuclei and notable nucleoli (200x).

may be present in dysplasia. Finally, although biliary strictures can be due to primary malignancy, strictures may rarely be secondary to a metastatic disease (Figures 8,9).

Various techniques have been introduced to improve the sensitivity of biliary cytology. These include processes to optimize the yield and/or use of concurrent sampling/detection venues. Between liquid based preparations and usual direct smears, diagnostic criteria for malignancy are maintained and comparable, while a better cellular quality can be achieved by liquid based methods. For instance, ThinPrep® can decrease variability due to air-drying artifact, reduce obscuring clotting secondary to lysing solution, and homogenize the sample to an even epithelial monolayer. Pre-wash is reported to further enhance the liquid-based yield. Campion *et al*, reports higher detection sensitivity upon Glacial acetic acid (GAC) pre-wash thin prep processing compared with standard non-gyn ThinPrep®, similar to observations from cervicovaginal sampling of earlier studies [16-18]. When feasible, concurrent biopsy and/or dilation of bile duct stricture before obtaining bile samples, may improve detection sensitivity [19,20]. Based on the concept that majority (approximately 80%) of biliary cancers exhibit aneuploidy, two advanced methods, digital image analysis (DIA) and fluorescent in situ hybridization (FISH), been shown to notably increase the diagnostic sensitivity of the biliary tract malignancies, while maintaining a high specificity [11]. DIA technique uses a microscope and camera to quantify the amount

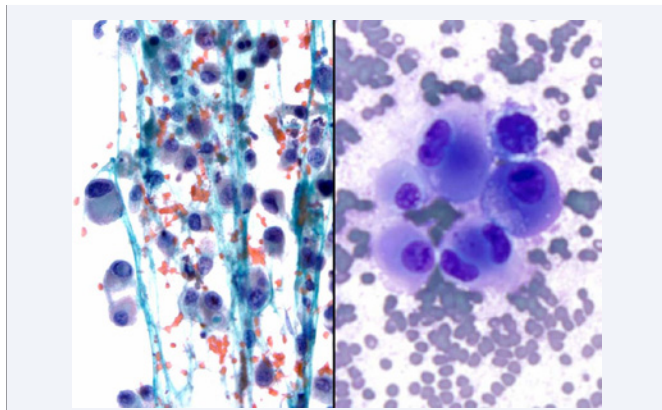


Figure 8 Metastatic melanoma, with discohesive neoplastic cells containing large prominent macro-nucleoli, features should be confirmed with immunostaining for definitive diagnosis (400x).

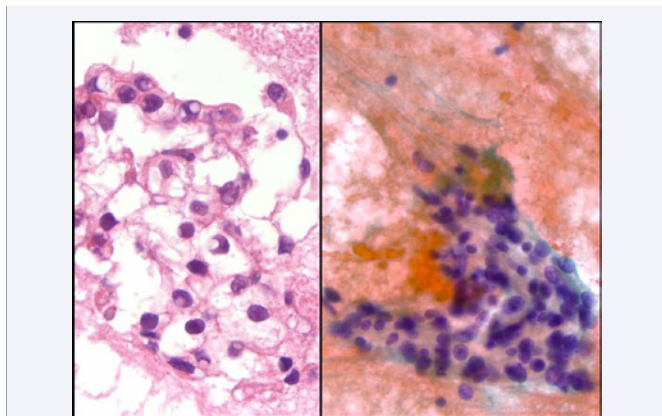


Figure 9 Metastatic renal cell carcinoma, tumor cells are polygonal with clear cytoplasm and prominent nucleoli. These features are suggestive of renal cell carcinoma but should be confirmed with the clinical history and immunostain work up (200x).

of DNA by measuring intensity of nuclear Feulgen dye and FISH utilizes fluorescently labeled DNA probes to detect chromosomal anomalies [11,21].

Using cell imaging analysis, to evaluate parameters such as nuclear area, nuclear DNA content and chromatin distribution, Yeaton *et al.* achieved a 95% sensitivity and 100% specificity for identification of biliary malignancy [22]. Moreno *et al.* reports increased sensitivity utilizing DIA, yet only intermediate to routine cytology in those with aneuploidy (DNA index from 1.12 to 1.89). Although DIA improved detection accuracy of routine cytology, this review points to a superior sensitivity of FISH in comparison with DIA [11,22].

FISH has been consistently reported to improve sensitivity in detection of biliary malignancy. Examples in literature where FISH increased detection sensitivity of brush sampling, while retaining a high specificity, are numerous. Hence, it is recommended to use FISH in combination with routine cytology as a valuable adjunct to achieve the best diagnostic outcome [9,11,15,23,26]. Probes for chromosomes 3, 7, and 17 and a locus specific indicator probe for 9p21 band are typically utilized. Positive FISH is reported as polysomy, trisomy or tetrasomy. The

most frequent anomalies are polysomy and trisomy 7. Patients with polysomy FISH results are reported 70 times more likely to harbor carcinoma than those with normal FISH. Moreno *et al.*, suggests that detection of polysomy in pancreaticobiliary lesions to be viewed as equivalent to diagnosis of cancer in cytology. Yet trisomy of a single gene (trisomy 7 specifically) should be interpreted with caution, as it can be either neoplastic or non-neoplastic [11]. Fritcher *et al.* recommends detection of trisomy 7 to be followed with a timely re-evaluation rather than directly interpreted as a sign of biliary malignancy [15]. Multivariable modeling using routine cytology, FISH, age, and PSC status may be used to estimate risk of carcinoma in an individual [9].

For better distinction between reactive atypia and malignancy, the multiprobe FISH UroVysion combined with automated relocation of atypical cells is reported offering a powerful technique to clarify inconclusive pancreaticobiliary cytology. The targeted analysis of the atypical cells of interest, through use of automated relocation, allows for a more precise evaluation, even in paucicellular specimens. The 9p21 deletion alone or in combination with polysomy provides compelling evidence for malignancy, with sensitivity of greater than 60% and nearly 100% specificity and positive predictive value [26]. According to the recent literature, evaluating loss of heterozygosity (LOH) has shown promise to improve sensitivity along with routine biliary cytology. Studies of LOH from microsatellite allelic loss at 1p, 3p, 5q, 9p, 10q, 17p, and 22q report that multiple mutations are frequent in carcinoma while single mutations may be associated with benign lesions [27]. These results indicate that molecular alterations are related to, but do not necessarily represent the carcinoma itself. Larger studies are needed to better understand this concept. Isolated interpretation of LOH must be taken with caution and correlation with morphology is necessary.

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