Bile Duct Brush Cytology

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Introduction

The majority of biliary strictures are malignant, the most common malignancies being pancreatic ductal adenocarcinomas, cholangiocarcinomas, and peri-ampullary carcinomas. Benign etiologies for bile duct strictures include chronic pancreatitis, choledocholithiasis, primary sclerosing cholangitis, IgG4-related sclerosing cholangitis, and iatrogenic bile duct injury (following cholecystectomy and liver transplantation) [1, 2].

The malignances of the pancreatico-biliary tract, ductal adenocarcinomas and cholangiocarcinomas, are associated with a very poor prognosis. Based on the most recent Surveillance, Epidemiology, and End Results data, the overall 5-year survival for pancreatic cancer is 8.5%. However, for cancer which is localized, that is, confined to the pancreas, the 5-year survival is 34.3% [3]. The prognosis for extrahepatic biliary cancer is similar with the 5-year survival being approximately 30% if the cancer is resectable. A definitive diagnosis of malignancy aids in early treatment if the tumor is resectable and, if not, helps in planning palliative care [4].

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The determination of biliary strictures as benign or malignant is challenging in clinical practice. Several imaging modalities have been studied for the differentiation between benign and malignant biliary strictures. Endoscopic retrograde cholangiopancreatography (ERCP) has shown comparable sensitivity (85%) and specificity (75%) to magnetic resonance cholangiopancreatography (MRCP) (sensitivity 85%, specificity 71%) in the diagnosis of malignant biliary strictures. ERCP not only provides the location and extent of the biliary stricture but has the added advantage of allowing sampling for pathologic examination. Histologic sampling in this area is difficult due to high rate of complications and is also associated with significant artifacts from tissue crushing and distortion. For evaluation of pancreatic duct and bile duct strictures, endoscopic retrograde cholangiopancreatography (ERCP)-guided brush cytology is considered the preferred method. The brushings sample a much larger area as compared to a biopsy and are associated with fewer complications [5]. Bile duct brush cytology has special value in the surveillance of patients with primary sclerosing cholangitis. This condition incurs a high risk of developing cholangiocarcinoma with the lifetime occurrence varying from 5% to 36%. The early detection of malignancy in these patients may improve survival by enabling a liver transplantation [6].

The specificity of brush cytology for the diagnosis of pancreatico-biliary tract malignancy is >95%, but the sensitivity is suboptimal, ranging from 30% to 85% [7–11]. A 2015 meta-analysis of nine studies found that the sensitivity of brush cytology and intraductal biopsy in diagnosing malignancy was 45% and 48%, respectively. A combination of the two techniques resulted in a modest increase in sensitivity to 59.4%. However, the specificity of both techniques approached 100% [12].

The distinct advantage of biopsies lies in their ability to provide subepithelial stroma that can help in recognizing stromal invasion. Mucosal atypia can be difficult to categorize as reactive or neoplastic, especially with a history of an indwelling stent. The value of biliary biopsies has been variably reported. Different approaches for tissue acquisition have been found useful including combined brushing and biopsy, biopsy follow-

ing negative cytology, and biopsy only. The varying results could be related to differences in sampling and interpretation [13–16].

Role of EUS-FNA

Radiologically evident pancreatic masses are usually sampled by fine needle aspiration under endoscopic ultrasound guidance. For bile duct strictures, the sensitivity of EUS-FNA is much higher for diagnosing distal malignant bile duct strictures as compared to that for proximal ones. The overall sensitivity of EUS-FNA for the diagnosis of cholangiocarcinoma has been shown to be 73%, being significantly higher in distal compared to proximal cholangiocarcinoma (81% vs. 59%, respectively). It is also superior to ERCP in tissue sampling, especially for pancreatic masses with an overall accuracy and sensitivity of 94% and 94% for EUS-FNA and 53% and 50% for ERCP sampling, respectively [17].

EUS-FNA may provide a diagnosis of malignancy when ERCP sampling is negative or indeterminate and in patients in whom cross-sectional imaging does not reveal a mass. It is also important in sampling of lymph node metastases. Therefore, EUS-FNA can also prove useful to the diagnostic armamentarium in patients with suspected cholangiocarcinoma [18].

Sample Preparation Methods

Typically, the brush is guided through a stricture over a wire and positioned across the stricture. The brush scrapes material from the superficial mucosa, and it is retracted into a sheath. To retrieve material from the brush, the brush is opened outside of the sheath to expose the bristles. The brush may be placed against a glass slide to prepare direct smears. Thereafter, the brush is cut from the catheter and placed in a fixative solution. In the laboratory, the sample is agitated, and usually a ThinPrep preparation is prepared. Any residual material may be employed for cell block preparation following fixation in formalin [19].

The main advantage of the ThinPrep over direct smears has been the elimination of air-drying artifact and diminished effect of obscuring blood elements and inflammation that are frequently encountered with smears. In addition, it results in a greater diagnostic cell yield; and due to enhanced alcohol fixation, the cells are better preserved with improved chromatin detail [20].

Cytologic Features

Normal Bile Duct Epithelium

Normal biliary epithelium is composed of cohesive groups of cuboidal to columnar cells with retained polarity, that is, basally located nuclei. The cells are seen in an orderly arrangement in flat honeycomb sheets or in a picket fence arrangement. The cytoplasmic borders are well defined. The nuclear-cytoplasmic ratio is low. The nuclear membranes are smooth, and the chromatin is finely distributed with indistinct nucleoli.

Reactive Bile Duct Epithelium

As a consequence of inflammatory changes as in the presence of stones, stent, and primary sclerosing cholangitis, the biliary epithelium can demonstrate reactive changes that can be very pronounced. These changes can be present to a varying degree in the epithelium, resulting in a spectrum of cell populations. The background may show acute inflammation and even necrosis. The cells are seen in flat to mildly disorganized groups. Mild degree of nuclear enlargement and anisonucleosis may be seen; however, the nuclear-cytoplasmic ratio is usually low. The chromatin is finely distributed, the nuclear membranes are smooth, and nucleoli may be prominent (Figs. 11.1, 11.2, 11.3, and 11.4) [21].

We frequently receive bile duct brushings for evaluation from patients with an indwelling stent or a history of stent placement in our practice. In such an instance, the cytomorphologic features that are associated with malignancy include anisonucleosis (with at least sixfold variation in nuclear size), three-dimensional archi-

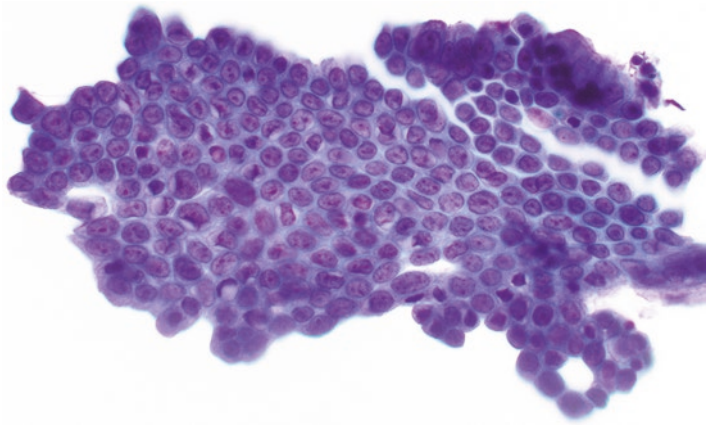

Fig. 11.1 Reactive bile duct epithelium reveals flat to mildly disorganized groups with mild degree of anisonucleosis, fine chromatin, and smooth nuclear membranes (ThinPrep, Papanicolaou stain, 600×)

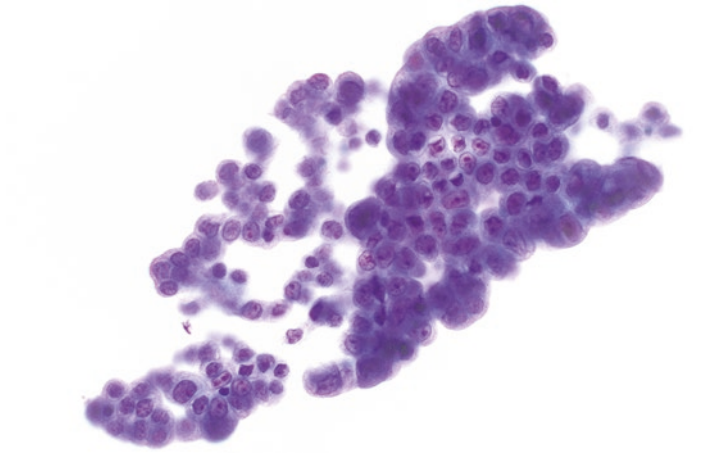

Fig. 11.2 The reactive features can be pronounced in the setting of a stent and of primary sclerosing cholangitis. Anisonucleosis can exceed 1:3. However, there is usually only mild disorganization of architecture, nuclear membranes are smooth, and chromatin is finely distributed (ThinPrep, Papanicolaou stain, 600×)

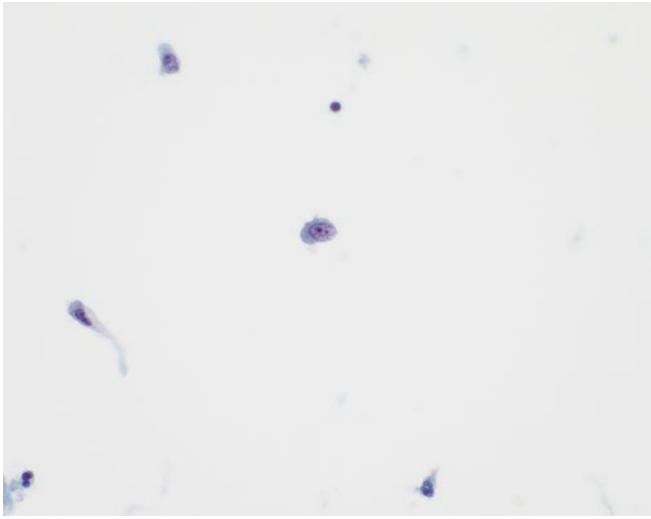

Fig. 11.3 Single atypical cells with enlarged nuclei can occasionally be seen in primary sclerosing cholangitis with reactive changes (ThinPrep, Papanicolaou stain, 600×)

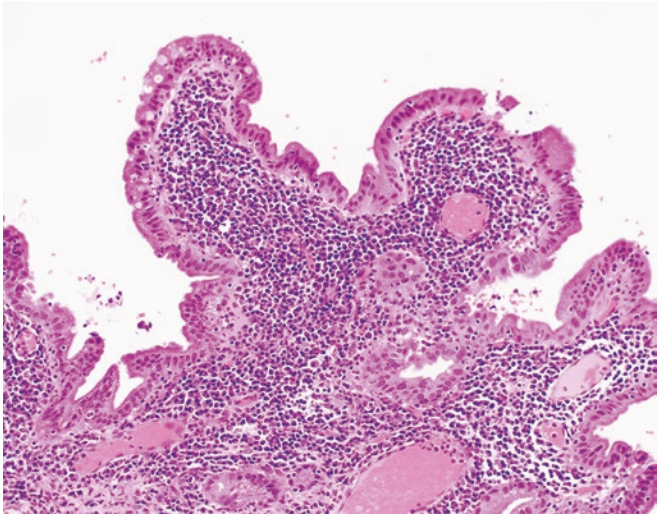

Fig. 11.4 Bile duct biopsy in primary sclerosing cholangitis with reactive, inflamed epithelium (hematoxylin-eosin, 200×)

tecture, coarse chromatin distribution, and the presence of single malignant cells [22].

Dysplasia

Cytologic criteria for biliary intraepithelial neoplasia (BiIN) are not well defined. The biliary epithelium shows cytologic atypia, ranging from low grade to high grade. Low-grade dysplasia results in nuclear stratification with mild nuclear crowding and hyperchromatic and elongated nuclei. While there is nuclear enlargement in these cells, the overall nuclear-cytoplasmic ratio remains low. High-grade dysplasia, in contrast, reveals three-dimensional cell arrangement, nuclear enlargement, irregular nuclear membranes, and coarse chromatin distribution. High-grade dysplasia can be indistinguishable from adenocarcinoma on cytology [23, 24].

Adenocarcinoma

Several studies have attempted to identify cytologic criteria that can better predict malignancy in bile duct brushings. Cohen et al. showed that nuclear molding, chromatin clumping, and increased nuclear-cytoplasmic ratio were key cytologic features that were associated with malignancy. The presence of two of these features resulted in 83% sensitivity and 98% specificity for carcinoma detection [25]. Renshaw and colleagues demonstrated that an overall assessment of malignancy or the criteria of chromatin clumping, increased nuclear-cytoplasmic ratio, and either nuclear molding or loss of honeycombing accurately predicted malignancy in bile duct brushings [26]. Fritcher et al. examined cytologic criteria associated with malignancy in pancreatobiliary brushings with corresponding positive fluorescence in situ hybridization (FISH) and found that abnormal single cells, nuclear membrane irregularity, and nuclear enlargement were independent predictors of malignancy on logistic regression. Their study also showed that the presence of single abnormal cells (defined

as single cells with at least one atypical nuclear feature) was the most significant finding on multivariate and univariate analysis (76.9% in malignant vs. 10.0% in benign samples) [7]. Salomao et al. also reported that on multivariate exact logistic regression, only the finding of single vacuolated cells was predictive of malignancy in biliary strictures (70.58% in malignant vs. 16.07% in benign samples) [27]. In a recent study, Avadhani et al. reported that 11 cytologic characteristics were significantly associated with malignancy on statistical analysis in bile duct brushings. These included three-dimensional clusters, pleomorphism, two-cell population, chromatin pattern changes, high nuclear-cytoplasmic ratio, cytoplasmic vacuoles, nuclear irregularity, cellular discohesion, hypercellularity, nuclear molding, and prominent nucleoli. They found that the identification of 3 of these 11 features improved the pathologists' performance greatly in predicting malignancy [28].

As is depicted above, different studies have highlighted various key features for diagnosing malignancy in bile duct brushings. My approach toward diagnosing malignancy in bile duct brushings is based on the constellation of cytologic features identified in the specimen. Renshaw et al. have also shown that an overall cytologic assessment of malignancy was a better predictor of malignancy as compared to any other criteria with a sensitivity of 36.2% and a specificity of 95% [26].

The bile duct brushings from adenocarcinoma usually reveal two distinct cell populations—benign and malignant. However, one can see a spectrum of malignant cells in the sample when the tumor exhibits a range of differentiation. The malignant groups show nuclear crowding to a varying degree, ranging from mild nuclear overlap to more pronounced three-dimensionality. Nuclei are enlarged with a greater variation ($\geq$1:3) in nuclear size within the same cell group. Nuclear membranes can be irregular, chromatin distribution is abnormal (mostly coarse but can be hypochromatic), and nucleoli may be prominent. An important finding that points toward malignancy is the presence of single malignant cells in the background. Necrosis and inflammation are nonspecific findings that can also be identified in benign samples (Figs. 11.5, 11.6, 11.7, 11.8, and 11.9).

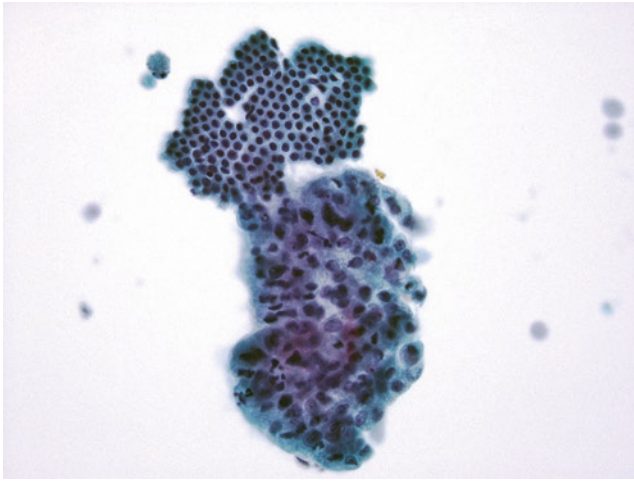

Fig. 11.5 Two contrasting populations are typical of malignant non-stented bile duct brushings. The well-organized group of normal biliary epithelium shows a striking contrast to the three-dimensional malignant group (ThinPrep, Papanicolaou stain, 400×)

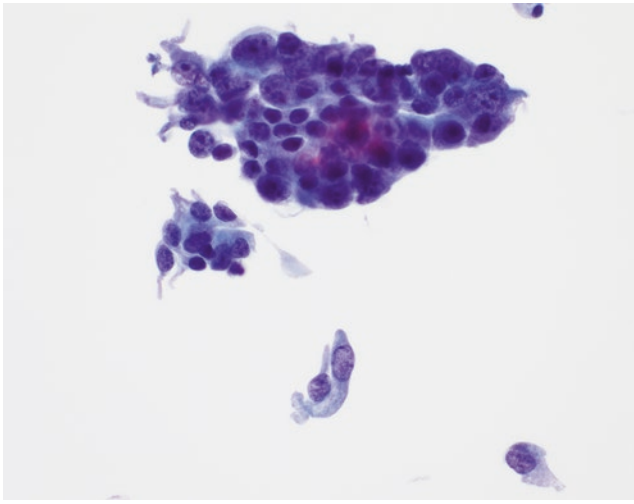

Fig. 11.6 Adenocarcinomas in bile duct brushings show irregular chromatin distribution, significant nuclear crowding, and anisonucleosis (at least to the extent of ≥1:3) (ThinPrep, Papanicolaou stain, 600×)

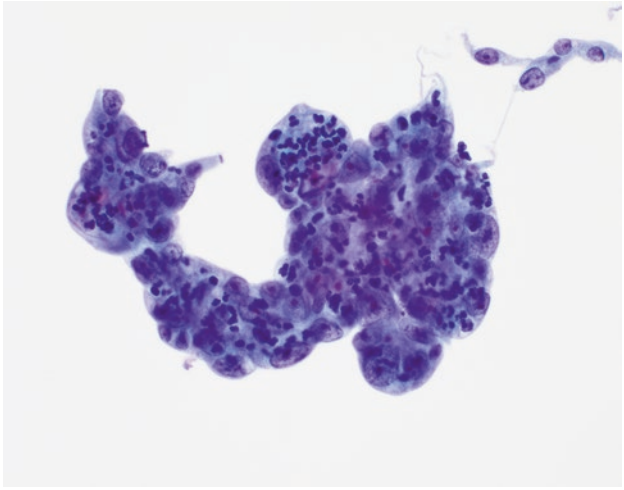

Fig. 11.7 Malignant groups in bile duct brushings can show hypochromatic nuclei with markedly prominent nucleoli and presence of intracytoplasmic neutrophils. However, the presence of neutrophils has not been observed as a specific feature of malignancy (ThinPrep, Papanicolaou stain, 600×)

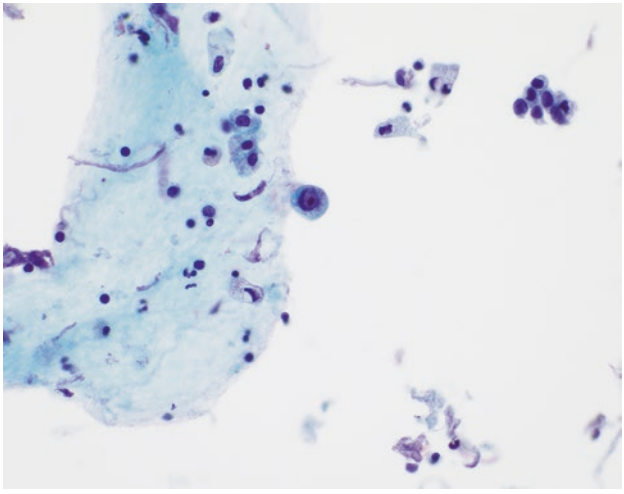

Fig. 11.8 Single malignant cells in the background are a helpful clue to the diagnosis of malignancy. However, the overall morphology of the lesion should be considered to make a diagnosis of malignancy (ThinPrep, Papanicolaou stain, 600×)

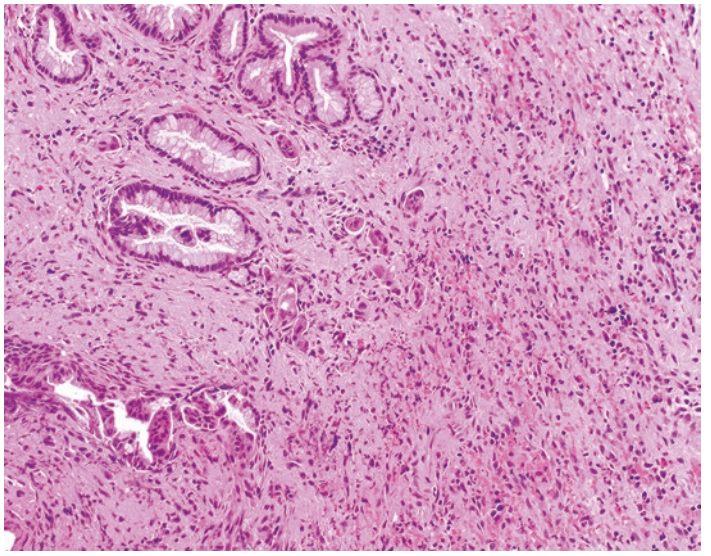

Fig. 11.9 Intraductal forceps biopsy aids in the diagnosis of malignancy as it can demonstrate the presence of stromal invasion (hematoxylin-eosin, 400×)

Intraductal Papillary Neoplasm of the Bile Ducts (IPN-B)

Intraductal papillary neoplasm of the bile ducts (IPN-B) is a rare disease that is seen primarily in patients from Far Eastern areas, such as Taiwan, Japan, and Korea, where hepatolithiasis and clonorchiasis are endemic. Majority of the patients are between 50 and 70 years of age [29].

It is characterized by an intraductal, predominantly papillary proliferation with a distinct fibrovascular stalk. The lining cells usually show intracellular mucin production. Many authors have considered IPN-B to represent the biliary counterpart of IPMN. Also similar to IPMN, four histologic subtypes of IPN-B have been recognized: pancreatobiliary, intestinal, gastric, and oncocytic. Approximately 40–80% of IPN-Bs contain a component of invasive carcinoma—tubular or mucinous adenocarcinoma—suggesting that IPN-B is a disease with a high potential for malignancy. It

is difficult to make an accurate diagnosis of IPN-B preoperatively due to its low incidence and lack of a specific clinical manifestation. Computed tomography (CT) scan and magnetic resonance imaging (MRI) usually fail to detect minor tumors and mucin. Therefore, cholangiography and cholangioscopy are needed for pathologic confirmation by biopsy and to demonstrate the extent of the lesions. In multifocal disease, different foci may be at different stages, indicating that the pathologic diagnosis by biopsy cannot reflect the actual stage in many cases. An approach for the cytologic evaluation of these neoplasms has not been established [30, 31].

Diagnostic Challenges

The low sensitivity of BDB cytology is mainly attributed to sampling difficulties, interpretation errors, and suboptimal slide preparations. In a retrospective analysis of 1832 pancreatobiliary brushings, Logrono and colleagues found that sampling errors were the major cause of false-negative diagnoses (67%), followed by interpretation errors (17%) and technical issues (17%) [10]. In an assessment of 267 bile duct brushings, Kocjan and Smith also concluded that improved sampling, preparation, and interpretation could result in better diagnostic accuracy of bile duct brushings [32].

Interpretation can be difficult due to limited cellularity specimens, well-differentiated adenocarcinomas, mucinous carcinomas, and cytologic atypia that falls short of a malignant diagnosis and in the presence of confounding factors such as primary sclerosing cholangitis and stent-related changes. Primary sclerosing cholangitis with marked reactive atypia of the biliary epithelium and scant cellularity with degenerative atypia can result in a false-positive diagnosis of malignancy [33]. Therefore, the threshold for malignant diagnosis is generally high in BDB cytology.

Ancillary Studies

Fluorescence in situ hybridization (FISH) has shown promise as an adjunct in improving the sensitivity of cytology for the detection of malignant biliary strictures. Using the UroVysion probe

set (Abbott Molecular Inc., Des Plaines, IL), it has been shown to enhance the sensitivity of BDB cytology to 42.9–63.89% for detection of malignancy [27, 34]. The FISH probes detect aneuploidy in the centromeric regions of chromosomes 3, 7, and 17 and homozygous or heterozygous deletion of locus 9p21. The results of FISH testing need to be correlated with clinical and imaging findings in patients with primary sclerosing cholangitis. Polysomy in the presence of a dominant stricture has a higher positive predictive value for cholangiocarcinoma in patients with PSC [34]. More recently, Fritcher et al. reported that a different set of FISH probes 1q21, 7p12, 8q24, and 9p21 identified malignancy in pancreatobiliary samples with a higher sensitivity (64.7%) as compared to the UroVysion probes (45.9%) or routine cytology analysis (18.8%) [35].

In terms of immunohistochemistry, various markers have been studied to assist in the identification of malignancy in bile duct brushings including p53, S100P, maspin, and claudin-18. Maspin is a mammary serine protease inhibitor that potentially plays a role in cell growth, invasion, and metastases and is overexpressed in cholangiocarcinomas. S100P is a calcium-binding protein that belongs to the S100 protein family and is overexpressed in biliary dysplastic epithelium and cholangiocarcinoma. Claudins are tight junction transmembrane proteins present in epithelial and endothelial cells. Claudin-18 immunohistochemical expression reportedly has a sensitivity of 89% for the detection of pancreatobiliary carcinomas [36, 37].

Recently, targeted next-generation sequencing along with cytology revealed a sensitivity of 85% for the detection of malignancy in bile duct brushings by revealing driver mutations in 30% of cases, including *KRAS*, *TP53*, *SMAD4*, and *CDKN2A* [38].

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