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Endoscopic Approaches to Diagnose Cholangiocarcinoma in Patients with Primary Sclerosing Cholangitis

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INTRODUCTION

Patients with PSC have a lifetime risk of developing cholangiocarcinoma (CCA) of 10-15%.[1] The ability to distinguish between benign strictures and CCA can be challenging as they may have similar appearances on imaging with endoscopic retrograde cholangiopancreatography (ERCP) and magnetic resonance cholangiopancreatography. Visualization and definitive sampling of a dominant mass lesion is diagnostic, but mass lesions often are not always seen in patients with early CCA.[2] In addition, up to 37% of patients with PSC and elevated CA 19-9 do not have CCA.[3] Brush cytology, fluorescence in situ hybridization (FISH), cholangioscopy, probe-based confocal laser endomicroscopy (pCLE), and endoscopic ultrasound with fine needle aspiration (EUS FNA) can be used to obtain a more definitive diagnosis. This manuscript will review the different endoscopic techniques to diagnose CCA in patients with PSC, as well as their success rates, risks and benefits.

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Brush Cytology

Brush cytology is the most common method for tissue acquisition during ERCP as some lesions are too proximal in the biliary tree (or are in ducts too small) to biopsy. Brush cytology has a high specificity (97-100%) to detect biliary malignancy, but has traditionally had a low sensitivity.[4,5] In a recent meta-analysis that included 11 studies and 747 patients with PSC, the sensitivity of brush cytology was 43% and the specificity was 97%.[6]

The inherent benefit of brush cytology lies in its high specificity when positive for malignancy. However, due to its low sensitivity, the primary drawback of cytology is the frequent inability to rule out malignancy.[7] The chronic inflammation inherent with PSC can also lead to reactive atypia, which can lead to malignancy, making the diagnosis of cholangiocarcinoma challenging.[8, 9]

Biliary brushing results are classified into one of three cellular categories: benign, malignant, or "atypical." In a cohort study of 86 patients with atypical biliary brushings (many of whom, but not all, had PSC), Witt et al. sought to identify factors predictive of malignancy. Sixty of these patients were ultimately found to have confirmed cancer of pancreatobiliary origin. In the setting of an atypical biliary brushing result, the risk of malignancy was significantly correlated with

Table 1. Atypical biliary brushing score (ABBS) for evaluation of patients with biliary strictures. A score > 4 suggests patients are at high risk for malignancy.

	Score
Age 60	+1
Endoscopic impression malignant	+2
Procedure indication pancreatic mass	+1
Stricture in Common Hepatic Duct	+2
Stricture in Distal Common Bile Duct	+1
Presence of PSC	+2
CA 19-9 above 300 U/mL	+1

age ≥ 60, suspicious/malignant endoscopic impression, the presence of a pancreatic mass, indications for ERCP including jaundice and/or dilated bile ducts, stricture within common bile duct, PSC, and CA 19-9 greater than 300 U/ml. For patients with a CBD stricture, 45/59 (76%) were diagnosed with malignancy. The authors created a scoring model to predict malignancy called the Atypical Biliary Brushing Score (ABBS), made up of the above factors predictive of malignancy. (Table 1) A score ≥ 4 suggests patients are at high risk for malignancy. The PSC subgroup had a 29% rate of malignancy.[10]

In a review of 107 biliary brushings from 51 patients with PSC, sensitivity and specificity were 62.5% and 100%, respectively. With a CA 19-9 cutoff of 186 IU/ml for CCA, sensitivity and specificity were 100% and 94%, respectively.[11]

In a large population of patients referred for their first ERCP due to suspicion for PSC, PSC was diagnosed by brush cytology in 261 patients, 211 (80.8%) of whom were asymptomatic at the time of diagnosis. The ERCP findings were categorized by a modified version of the Amsterdam endoscopic retrograde cholangiography (mERC) score defined by Ponsioen et al.[6] Symptoms of PSC included jaundice, pruritis, fatigue, weight loss, fever, or cholangitis. The authors found 42.9% of patients with PSC had advanced disease and 6.9% had suspicious or malignant brush cytology at first ERCP. Patients with advanced PSC (mERC score > 3) were not significantly more symptomatic (p = 0.303) than patients with early PSC (mERC 2-3). CA 19-9 levels did not correlate with brush cytology results (p = 0.751).[12]

A meta-analysis of 747 patients found that the pooled sensitivity and specificity of bile duct brushings for diagnosis of CCA in patients with PSC were 43% and 97%, respectively. Pooled diagnostic odds ratio was 20.23, meaning that if a bile duct brushing in a PSC stricture shows CCA, the patient has a 20 times higher likelihood of a final, positive pathological diagnosis. Pooled positive likelihood ratio was 8.87 and the pooled negative likelihood ratio was 0.56. This again demonstrates bile duct brushing is reliable in the diagnosis of CCA as well as in the exclusion of benign strictures.[13]

Although ERCP is generally very safe when performed by experienced endoscopists, it is not without risks and complications. In a multicenter study of 83 patients who underwent a total of 106 ERCPs for suspected PSC, complications occurred in 10 cases (9%). Complications include pancreatitis (n = 3), cholangitis (n = 2), increase of cholestasis (n = 2), postsphincterotomy bleeding (n = 1), cystic duct perforation (n = 1), and venous thrombosis (n = 1). All of these resolved quickly with medical therapy. Complications occurred in 16% of ERCPs with biliary intervention (ex: sphincterotomy or stent placement) compared to 4% in ERCPs without interventions (RR 4.5, 95% CI 0.94-30, p = 0.04).[14]

A retrospective cohort study of 185 ERCPs performed on 75 patients with PSC examined 30-day post-ERCP adverse event rates and found that the endoscopist with the highest ERCP volume had the lowest lower complication rate, arguing for PSC ERCPs to be done at high volume centers or by those

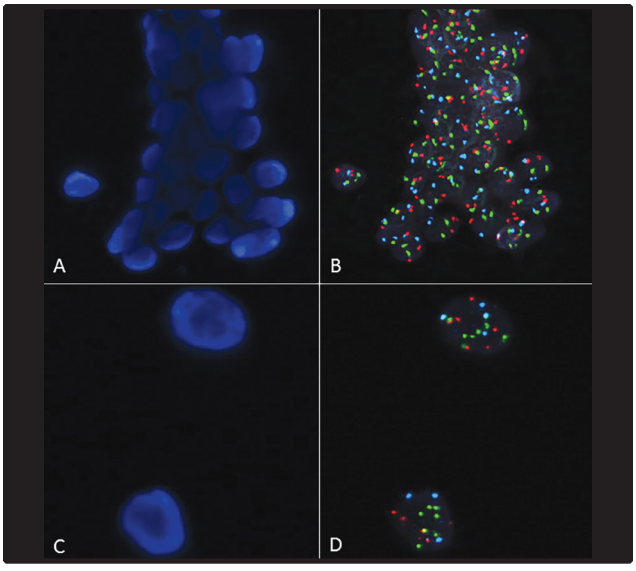

Figure 1. Urovysion FISH performed on biliary brushing samples showing a morphologically normal biliary epithelial group (A) with a normal diploid pattern for chromosomes 3, 7 and 17 (B) cytologically abnormal cells (C) with combined FISH probes showing abnormal numbers of red, green, and blue signals (D). *Image courtesy of Barbara Chadwick, MD*

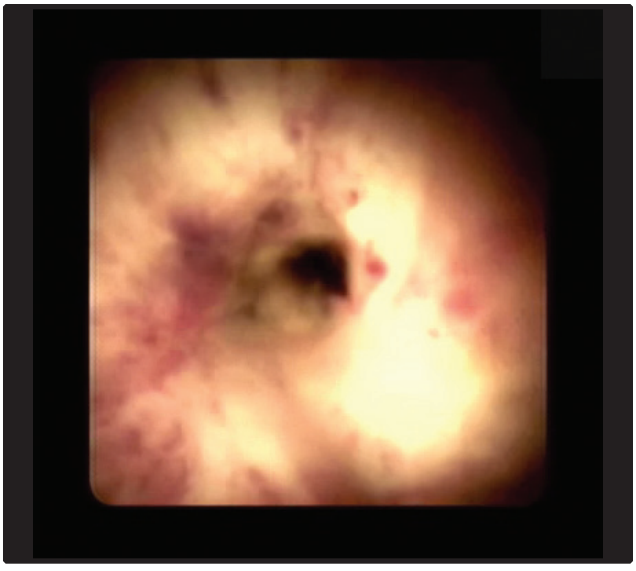

Figure 2. Digital cholangioscopy image of the bile duct in a patient with PSC. Note the circumferential ductal narrowing, inflammation, and edema. This is overall benign appearing and brushings did not disclose evidence of malignancy.

experienced with PSC cases. Multivariate analysis also revealed statistically significant associations with biliary dilation, sphincterotomy, presence of cirrhosis, Crohn's disease and autoimmune hepatitis. They did not find an increased adverse event rate when looking at gender, the placement of a stent during the procedure, the presence of a dominant stricture, or cholangitis.[15]

FISH

Routine cytology, despite its ease of use and low cost, has limited sensitivity, which is problematic in the diagnosis of cholangiocarcinoma (CCA). Many patients with CCA are not diagnosed by routine cytology alone. Fluorescent in situ hybridization (FISH) probes are used to target the centromeric regions of chromosomes 3, 7, and 17 and the 9p21 band (p16) to examine for evidence of aneuploidy and aid in the diagnosis of CCA. (Figure 1) FISH testing has been available commercially in the United States for over a decade, but many endoscopists still have limited knowledge of and experience with its role in diagnosing biliary malignancies.

In a study of 235 patients with PSC, 120 (51%) had evidence of aneupoidy by FISH, but only one third of these positive patients had CCA. Sensitivity and specificity for FISH polysomy were 46% and 88%, respectively; for trisomy/tetrasomy, they were 25% and 67%, respectively. Survival analysis of 120 patients with PSC with FISH polysomy had outcomes similar to patients with CCA. If patients had evidence of a dominant stricture as well as FISH polysomy, the specificity was 88%. The authors proposed the following set of guidelines: 1) FISH testing should not be used as a screening modality in unselected PSC patients undergoing ERCP. However, in patients with clinical or laboratory suspicion of CCA, such as weight loss, abdominal pain, dominant stricture, or elevated CA 19-9, FISH can be extremely helpful given the limitations of routine cytology. In patients with clinical or laboratory suspicion of CCA, such as weight loss, abdominal pain, dominant stricture, or elevated CA 19-9, FISH can be helpful.[16]

A dysplasia-carcinoma sequence has been proposed in the pathophysiology of PSC. Patients with history of or current CCA were more likely to have polysomy in dysplasia results by FISH than patients without CCA (70% versus 14%; $p = 0.05$). Patients with biliary dysplasia and CCA have evidence of polysomy and homozygous 9p21 loss. Cytogenetic abnormalities demonstrated in CCA are also seen in precursor lesions. High-grade dysplasia is found disproportionately in PSC patients with CCA. Overall, these findings could

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help delineate the grading of biliary dysplasia in this group of patients.[17]

In a study of 102 patients with PSC, 30 (29%) with an equivocal cytology developed carcinoma within 2 years. Serum CA 19-9 ≥ 129 U/ml (HR 3.19, P = .001) and polysomy (HR 8.70; P < 0.001) were each found to be predictive of future malignancy by univariate analysis. Polysomy FISH was the only significant predictor of malignancy in a multivariable analysis (HR 6.96). In a subgroup analysis of ten patients with both an elevated CA 19-9 and polysomy, all developed cancer (nine within two years). In this subgroup analysis, the combined finding of CA 19-9 ≥ 129 U/ml and polysomy by FISH was found to put patients at high risk of malignancy (HR 10.92; P < 0.001). The investigators suggested that regular lab monitoring with alkaline phosphatase, total bilirubin, and serum CA 19-9 levels does not adequately predict malignancy in patients with PSC. Based on their findings, they found polysomy by FISH is able to identify patients at risk for malignancy without evidence of mass lesion on imaging and with equivocal cytology.[18] Regarding bilirubin specifically in PSC, Haseeb et al. performed a retrospective cohort study of 81 patients with PSC and found that an initial bilirubin more than two times the upper limit of normal was significantly associated with the development of CCA, subsequent liver transplantation, and death (p < 0.017). In addition, hyperbilirubinemia correlated with increased severity of biliary ductal disease (p < 0.0001).[19]

In a retrospective review of 30 patients with PSC who had polysomy FISH result and no radiological or pathological evidence of malignancy at the time of first polysomy, Barr Fritcher et al. demonstrated that 9 of 13 patients (69%) with serial polysomy FISH results were diagnosed with CCA compared with 3 of 17 patients (18%) with subsequent non-polysomy FISH results (PPV 69% vs 18%, p = 0.008). Furthermore, patients with serial polysomy developed CCA in a shorter period of time than those patients with serial non-polysomy results. Interestingly, 47% of patients with PSC with a polysomy FISH result did not have evidence of malignancy by ERCP at the time FISH was obtained.

In a retrospective review of 371 patients with PSC, multifocal polysomy (MFP) was found to be the strongest predictor of CCA compared to patients with unifocal polysomy (UFP)

Compared to patients with UFP, patients with MFP had an increased likelihood of weight loss (32% vs 9%), suspicious cytology (45% vs 13%), and develop serial polysomy (91% vs 35%). MFP was strongly correlated with CCA (HR 82.42). However, patients with UFP and suspicious cytology are still at an increased risk of CCA.[20]

Overall, FISH has limited sensitivity but high specificity. A meta-analysis with 8 studies and 828 patients demonstrated pooled sensitivity and specificity of FISH for diagnosis of CCA in patients with PSC were 68% and 70%, respectively. Pooled likelihood ratio was 2.69 and negative likelihood ratio was 0.47. Pooled odds ratio was 7.24. Pooled sensitivity and specificity for FISH polysomy (6 studies with 690 patients) were 51% and 93%, respectively. The authors recommend that FISH be employed if clinical suspicion of malignancy remains high despite an inconclusive brushing cytology result.[21]

Cholangioscopy

Diagnosing malignancy in patients with PSC with dominant bile duct strictures has historically been challenging. Cholangiocarcinomas tend to be fibrotic, hypocellular, and often display significant desmoplasia, all of which complicate adequate tissue acquisition. Cholangioscopy, performed in the context of ERCP, can aid in tissue diagnosis in patients with (and without) PSC. (Figure 2) In 53 patients with PSC with dominant bile duct strictures, when compared with brush cytology, cholangioscopy had increased sensitivity (92% vs 66%; p = 0.25), specificity (93% vs 51%; p < 0.001), accuracy (93% vs 55%; p < 0.001), PPV (79% vs 29%, p < 0.001) and NPV (97% vs 84%; p < 0.001). In 75% of the PSC patients with CCA, an intraductal mass was visualized on cholangioscopy, which allowed these patients to be differentiated from those with benign strictures. The authors recommend cholangioscopy with repeat tissue sampling in patients with suspected malignancy but benign tissue biopsies.[22]

Cholangioscopy assists in localizing sites for tissue acquisition in patients with PSC with biliary strictures suspicious for malignancy. (Figure 3) In a retrospective study of 18 patients with PSC who underwent cholangioscopy for suspected CCA, the overall operating characteristics were a sensitivity of 75%, specificity of 55%, PPV of 23%, and a NPV of 92%. Results of cholangioscopy-directed biopsies correlated well with brush cytology and FISH brush cytology. Cholangioscopy increased visualization

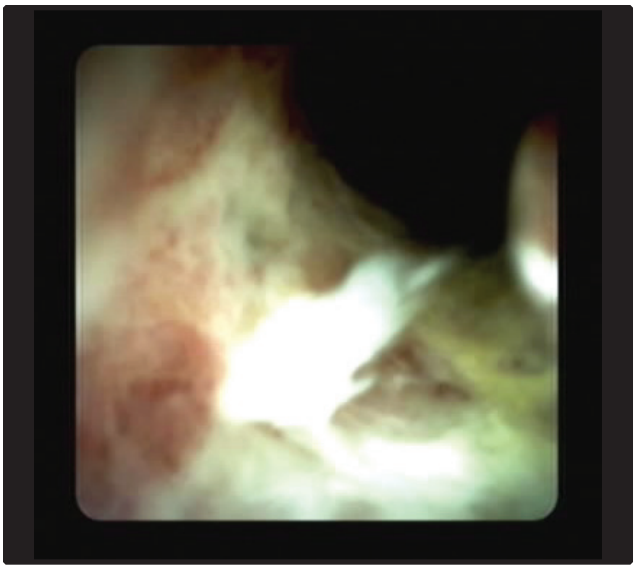

Figure 3. Digital cholangioscopy image of the bile duct in a patient with PSC and cholangiocarcinoma. Note the papillary projections at the 7 o'clock position. Biopsies revealed malignancy.

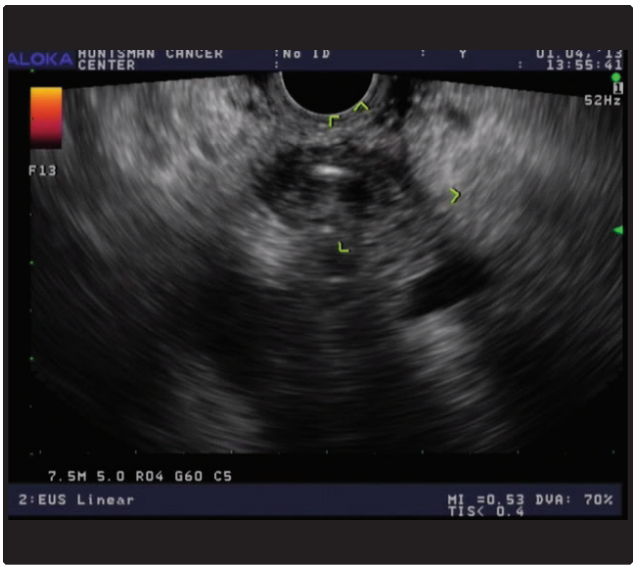

Figure 4. 7.5MHz EUS image of cholangiocarcinoma manifesting as diffuse bile duct wall thickening around a biliary stent.

of fine intra-ductal details allowing for improved tissue acquisition with brushings, FISH studies, and cholangioscopy-directed biopsies. Due to its high sensitivity, cholangiosocopy could be used to screen for malignancy in patients with and without PSC suspected of having CCA. Advantages of cholangioscopy included improved visualization of bile duct tissue when compared to cholangiogram and highly targeted biopsies and brushings in all patients, allowing specific locations within strictures to be marked for tissue acquisition. Disadvantages of cholangioscopy included increased cost and procedure time, with an average of 20 minutes for cholangioscopy time.[23]

In another study of 62 patients with indeterminate strictures who underwent 72 cholangioscopies (16 for stricture in setting of PSC), Shah et al. demonstrated that cholangioscopy with and without biopsy had a high accuracy in diagnosing and excluding CCA. Overall, sensitivity was 89%, specificity 96%, PPV 89%, and NPV 96%.[24]

In a prospective cohort of 41 patients with PSC who underwent 60 cholangioscopy procedures, Awadallah et al. noted an increased rate of biliary stone detection with cholangioscopy compared to cholaniogram; 30% of stones had been missed by cholaniogram. Cholangioscopy-directed biopsies were able to exclude CCA in the majority of patients; biopsies were positive for malignancy in one patient and excluded malignancy in 31 patients at a median follow up of 17 months (range 1-56 months). However, the investigators had difficulty accessing 25% of desired strictures in patients with PSC using cholangioscopy.[25]

In 47 patients with PSC, single-operator peroral cholangioscopy (SOC) was performed to evaluate 64 biliary strictures and technical success occurred in 96% (45/47) patients. Sample quality was adequate in 98% (62/63) of the cytology brushings and in 95% (21/22) of the mini-forceps biopsies. When evaluating for malignancy, sensitivity, specificity, accuracy, and NPV were 33%, 100%, 96%, and 95%, respectively. A key advantage of SOC in PSC is the ability to visually direct guidewire placement in patients with complex anatomy in whom a specific duct needs to be accessed. In four patients (9%), reaching the target lesion would not have been possible without SOC. Complications occurred in 15% (7/47) of patients; these included pancreatitis (n = 4), cholangitis (n = 2), extravasal contrast leakage (n = 1), stent due to suspected bile duct perforation (n = 1). The vast majority of complications (71%, 5/7) occurred in the first 15 patients included in the study.[26]

In another study, SpyGlass imaging and brush cytology with directed biopsies were performed in 29 of 31 (93.%) patients, 19 of whom had known PSC, and 10 with non-PSC strictures. SpyGlass directed biopsies demonstrated an increased diagnostic yield when compared to brush cytology as the SpyGlass biospies showed more inflammatory characteristics and also obtained more tissue material.[27]

From a limitations point of view, Sethi et al. examined interobserver agreement (IOA) with single operator choledochoscopy among 7 interventional endoscopists who examined 38 SpyGlass choledochoscopy video clips and found that it was slight to fair. They felt that SOC could not replace tissue diagnosis currently due to the low level of IOA; they suggested that a standardized scoring system should be developed.[28]

Sethi et al. performed a second follow up study looking at IOA for single operator cholangioscopy. Specifically, they found that IOA was "slight" for scoring of surface strictures as well as for characterization of blood vessels and lesions. In addition, IOA was only "slight" for describing cholangioscopy findings and for providing a final diagnosis. They found that the diagnostic accuracy by visual impression was less than 50%. The authors concluded that high IOA agreement and reproducibility are necessary to establish a valid imaging-based diagnostic sytem for cholangioscopy. Currently, the fair to poor agreement on the above criteria is an impediment for establishing definitive cholangioscopic criteria for accurate diagnosis.[29]

The SpyGlass single-operator cholangioscope has been shown to aid tissue diagnosis in patients with PSC, but it is not without limitations. SpyGlass was performed in 11 consecutive patients to monitor progression of PSC in a single tertiary center. SpyGlass directed biopsies were adequate for cytological and histological diagnosis in 9 (82%) and 10 patients (91%), respectively. Two cases of post-ERCP pancreatitis were observed.[30]

Probe Based Confocal Laser Endomicroscopy (pCLE)

Probe-based confocal laser endomicroscopy (pCLE) enables endoscopists to view the biliary tree using live microscopic imaging. pCLE requires the injection of contrast, typically fluorescein, which stains the extracellular matrix of the surface epithelium and allows the endoscopist to view the architecture of the surface mucosa and examine for neoplastic changes.[31] In addition, the smaller diameter of the pCLE probe compared to the cholangioscopy probe (3F vs. 10F), allows it to be advanced more easily into strictures without pre-dilation.[32]

In a single center chart review of 15 patients with PSC with 21 dominant strictures evaluated by pCLE, Heif et al. successfully visualized strictures in 95% of the procedures. Sensitivity was 100% (95% CI 19.3-100%), specificity was 61.1% (95% CI 35.8-82.6%), PPV was 22% (95% CI 3.5-59.9%), and NPV was 100% (95% CI 71.3-100%) for detection of malignancy. The low specificity was likely due to ductal inflammation in setting of PSC. However, the high NPV of pCLE may be able to rule out malignancy. Given the limited number of patients, the authors concluded that pCLE could be used to risk stratify dominant strictures in patients with PSC if validated on a larger scale.[33]

In a single center retrospective review of 35 patients (13 with PSC, 22 without PSC) with histologically proven inflammatory strictures (IS), Karia et al. examined pCLE images for each of the Paris Classification (PC) criteria for descriptive criteria of IS:

1. vascular congestion
2. dark granular pattern
3. increased inter-glandular space
4. thickened reticular structures (TRS)

Each of the PC criteria was found more often in patients without PSC. TRS was found in 95% of patients without PSC versus 62% of patients with PSC ($p = 0.01$). Presence of TRS has a 13-fold increase in predicting non-PSC etiology as the cause of IS.[34]

A consensus report by 16 physicians, some of whom are on the Mauna Kea Technologies advisory board, on the use of pCLE in biliary strictures determined the following six statements:

1. CLE can be used to evaluate biliary strictures and the probe can be delivered via a biliary catheter or a cholangioscope
2. CLE is more accurate than ERCP with brush cytology and/or forceps biopsy in determing malignant or benign strictures, using established criteria
3. The NPV of CLE is very high
4. The use of CLE can assist clinical decision-making such as excluding malignancy
5. CLE should be cited in official guidelines as a valuable tool for an increased diagnostic yield
6. The 'black bands' that can be seen in pCLE images have been shown to be collagen fibrils that predictably increase in pathologic tissue[35]

The limitations of pCLE are:

1. Costs of pCLE devices is high

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2. Limited number of clinical uses
3. Low number of centers specialized for pCLE procedures
4. Generalizability of increased diagnostic needs to be validated by more studies and endoscopists
5. Incremental diagnostic yield should be cost effective[36]
6. The interreader reliability of pCLE is not well defined.

EUS FNA

EUS FNA is another method to attempt to diagnose malignancy in patients with PSC. DeWitt et al. performed EUS-FNA on 24 patients with PSC who had ERCP brush cytology studies that were either negative/non-diagnostic or unable to be performed. They were able to visualize a mass with EUS in 23 (96%) patients, including 13 in whom prior imaging did not demonstrate a lesion. EUS-FNA was positive for malignancy in 17 (71%) patients. Sensitivity was 77%, specificity was 100%, PPV was 100%, NPV was 29%, and overall accuracy of EUS-FNA was 79%. The authors concluded that the sub-optimal NPV does not allow for exclusion of malignancy after a negative biopsy result.[37] Of note, FNA of suspected cholangiocarcinoma is discouraged given the risk of tumor seeding at some centers.

EUS is not the first line imaging choice for identification of CCA when compared to other imaging and sampling techniques. It can be technically difficult as early CCA, in patients with and without PSC, can be laterally spreading along the duct with minimal to no demonstration of a mass or wall thickening. FNA of a thin-walled mass is typically not diagnostic. Intraductal ultrasonography during ERCP may provide additional information in the evaluation of suspected CCA, but accuracy in differentiation between benign and malignant strictures appears poor.[38] Intraductal ultrasound, while once more popular, is now rarely used in clinical practice.

CONCLUSION

Although the diagnosis of CCA remains clinically challenging, brush cytology, FISH, cholangioscopy, pCLE, and EUS FNA can add to our armamentarium. Brush cytology has been the mainstay for tissue diagnosis of CCA due to its high specificity and ability to exclude malignancy, but its low sensitivity is problematic. When clinical suspicion for CCA remains high, FISH allows for detection of aneuploidy to aid in diagnosis. It has been shown to have increased sensitivity compared to brush cytology while retaining a high specificity. Cholangioscopy allows for specific locations within strictures to be accessed for tissue acquisition, but its high cost and procedure time remain limiting factors. pCLE enables endoscopists to analyze surface mucosa for evidence of neoplasia in real-time, but it seems likely that the interreader reliability needs to be improved before it disseminates into widespread practice. EUS is limited by its sub-optimal NPV as well as the risk of tumor seeding of suspected CCA with FNA. ■

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