ERCP tissue sampling

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It could be said about ERCP tissue sampling that the more things change, the more they stay the same. Numerous advances in both procedural technology and sample analysis methods (such as fluorescence in situ hybridization [FISH]) have been made since *Gastrointestinal Endoscopy* last published a major review of the topic in 2002.[1,2] However, despite its limitations in sensitivity, brush cytology remains the first-line method of obtaining tissue at ERCP. Its ease, safety, and diagnostic specificity make brush cytology a sensible go-to approach, but as we strive to more quickly and reliably diagnose biliary (and in some cases pancreatic) strictures, the search to find and fine tune new methods continues. Many of these new techniques involve advances in imaging, even down to the cellular level with probe-based confocal laser endomicroscopy (pCLE), for example, or molecular specimen analysis methods, but as is oft quoted and remains true, "tissue is the issue." Ideally, any tissue sampling technique should have high sensitivity for detecting malignancy while maintaining absolute specificity, and as with any procedure, ERCP-based sampling techniques should be safe, simple, and relatively inexpensive so they can be widely used.

Biliary strictures that are suspected to be malignant but lack a tissue diagnosis confirming as much, termed indeterminate strictures, often pose a diagnostic dilemma. There is a need to obtain a timely diagnosis without subjecting patients who harbor benign disease to major surgery. Although EUS-guided FNA (EUS-FNA) has become near-routine for the diagnosis of pancreatic cancer, ERCP tissue-sampling methods remain important for more proximal biliary strictures that are less amenable to EUS-FNA diagnosis and in the still-frequent circumstances when EUS is not readily available or is not planned at the initial procedure setting. We review standard tissue sampling techniques, the mainstays of which include brush cytology and intraductal forceps biopsy sampling, along with several newer ancillary techniques that are now in use, including cholangioscopy, pCLE, and FISH, all of which continue to be refined in the quest to improve our diagnostic capabilities.

Obtaining a specimen of adequate cellularity is essential in the evaluation of any potential malignancy. Studies have demonstrated that biliary stricture sampling specimens frequently contain insufficient cellularity, leading to false-negative diagnoses.[3-7] Certain features of a stricture can contribute to obtaining inadequate specimens. For example, hilar strictures, severely narrowed strictures that may not be fully traversed, and tumor location on the medial wall of the duct (as opposed to the lateral wall) all may influence the results of sampling.[4-10] In terms of endobiliary forceps biopsy specimens, it is easier to obtain a biopsy sample from distal tumors. Regardless of a distal or proximal location, forceps biopsy sampling tends to sample only the distal rim of the tumor, which can affect the adequacy of tissue collection.[4-9,11] Additionally, tumor characteristics can have an effect on sampling. Tumor-associated fibrosis or ulceration is a well-known finding in GI mucosal malignancies and can affect the ability to obtain adequate cellularity. Desmoplastic tumors are relatively firm and have lower cellularity, making sampling difficult. Some tumors of the bile duct exhibit submucosal spread, which particularly lowers the yield for brush cytology, the most superficial sampling method. Moreover, and pertaining to all sampling methods, tumors extrinsic to the bile duct such as pancreatic cancers and metastatic tumors are expectedly more difficult to sample from within the duct. Sensitivity for all sampling methods is influenced by the type of tumor that is causing the stricture.[5,6,10,12-31] In most series, brush cytology and forceps biopsy sampling from the bile duct have a higher sensitivity for cholangiocarcinoma (44%-89%) than for pancreatic cancer (30%-65%) (Tables 1 and 2).[5,6,10,12-14,21,26,27,30,31] For malignancies of the major duodenal papilla, forceps biopsy sampling is the best single sampling technique—cancer is detected in 77% to 88% of cases.[10] It does not come as a surprise that cancer detection is higher for primary bile duct and

Abbreviations: DIA, digital image analysis; FISH, fluorescence in situ hybridization; IDUS, intraductal US; miRNA, microRNA; pCLE, probe-based confocal laser endomicroscopy; PDAC, pancreatic ductal adenocarcinoma; PSC, primary sclerosing cholangitis; SOC, single-operator cholangioscopy.

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TABLE 1. Cancer detection rate of brush cytology from the bile duct according to tumor type

Author	Year	Cancer detection rate	
		Cholangiocarcinoma	Pancreatic cancer
Wiersema et al[23]	1992	64% (7/11)	44% (4/9)
Ponchon et al[5]	1995	44% (12/25)	15% (3/20)
Pugliese et al[6]	1995	73% (16/22)	33% (8/24)
Sugiyama et al[21]	1996	59% (10/17)	36% (5/14)
Mansfield et al[12]	1997	63% (10/16)	38% (10/28)
Glasbrenner et al[14]	1999	80% (16/20)	35% (11/31)
Vandervoort et al[13]	1999	60% (6/10)	33% (15/46)
Macken et al[15]	2000	60% (18/30)	65% (13/20)
Farrell et al[24]	2001	50% (2/4)	50% (6/10)
deBellis et al[30]	2003	68% (15/22)	37% (28/75)
Total		63% (112/177)	37% (103/277)

papillary neoplasms that originate from the biliary epithelium, as opposed to processes that compress the bile duct, such as pancreatic or metastatic cancer.[3,9,11,32]

BRUSH CYTOLOGY

This technique remains the first-line and most commonly used method of acquiring tissue at the time of ERCP. It is simple and easy to perform and, given its high specificity, is a very reliable initial method of sampling. Robust data regarding the sensitivity of brush cytology exist. We will focus on a few key studies and then highlight some technical considerations that have been tried over the years in an effort to increase the sensitivity of brush cytology. In addition, later in the review, we compare brush cytology with other techniques such as forceps biopsy sampling and FISH.

Brushing to obtain cytologic material involves advancing a brush with its 6F or 8F catheter sheath through the endoscope into the biliary tree, generally over the guidewire. Several cytology brushes are commercially available (Cytomax II [DLB-21 and DLB-35] and Fusion, Cook Medical, Bloomington, Ind; Rx Brush, Boston Scientific, Marlborough, Mass; Infinity, US Endoscopy, Mentor Ohio; BrushMaster V, Olympus, Shinjuku, Tokyo, Japan). The device is advanced to a point proximal to the stricture, and then the brush is advanced from the catheter, withdrawn slightly, and moved back and forth across the stricture approximately 10 times. The brush is then withdrawn into the catheter, and the device is withdrawn from the endoscope as a unit. Data show that removing the brush and catheter together improves cancer detection compared with pulling the brush through the catheter sheath.[29] The brush can be smeared onto glass slides, cut off from the device and placed into a fixative solution, or both.

The specificity of brush cytology approaches 100%. However, sensitivity is modest, ranging from 30% to 57% (Table 3).[5,6,10,12-15,17,18,25,27,33-41] Nearly all of these studies used a single-brush specimen.

One of the largest published series, by Stewart et al in 2001,[33] reviewed bile duct and pancreatic duct brush cytology samples from 406 patients, for a total of 448 specimens. Of the 246 patients diagnosed with malignancy, 147 were identified cytologically (60% sensitivity). There were 3 false positives, attributed to misinterpretation of cases with relatively scant and/or degenerative atypical epithelial cells (specificity 98%). Interestingly, the sensitivity of brush cytology increased over the 6.5-year course of the study, from 44% in the initial third to 71% in the final third of cases. The authors suggest that greater experience on the part of the pathologists played a large part but that better sampling may have also played a role. Also noteworthy is that the use of repeat brushing may help in diagnosing malignancy, as 10 of 18 patients had positive cytology on a second brush specimen.

A recent meta-analysis reviewed the diagnostic yield of bile duct brush cytology specifically in patients with primary sclerosing cholangitis (PSC).[42] Eleven studies, involving 747 patients, were included. The pooled sensitivity and specificity for a diagnosis of cholangiocarcinoma in PSC patients were 43% and 97%, respectively (includes diagnostic of and suspicious for malignancy), with sensitivity ranging from 7% to 85%. The pooled positive predictive value was 78% and the pooled negative predictive value 87%.

For cases of pancreatic cancer, cytology from pancreatic duct brushing and pancreatic duct juice collection may theoretically increase the diagnostic yield. Overall, the cancer detection rate for pancreatic duct brush cytology in pancreatic cancer ranges from 47% to 85%. Earlier studies of pancreatic duct brush cytology reported higher yields,[43,44] but subsequent studies did not confirm these results.[13,45,46] Moreover, obtaining brush samples from the pancreatic duct can be technically difficult and has been found to be impossible more than 25% of the time because of malignancy-related duct disruption.[10,45] Post-ERCP pancreatitis rates have ranged from 0% to 21.5%, with the risk believed to be higher in cases of benign stricture and relatively low in cases of malignancy.[13,47,48]

Pugliese et al[49] evaluated cancer detection rates for pancreatic juice cytology and pancreatic duct brush cytology in a series of 47 patients with pancreatic duct strictures, 34 of which were confirmed to be malignant. Pancreatic juice was obtained over a 5-minute collection period after intravenous secretin was administered. Cytology from the pancreatic juice had a sensitivity of 58% for pancreatic cancer, in comparison with 74% sensitivity for brush cytology and salvage cytology (a technique in which the brush is immersed in saline solution and

TABLE 2. Cancer detection rate of forceps biopsy sampling from the bile duct according to tumor type

Author	Year	Cancer detection rate		
		Cholangiocarcinoma	Pancreatic cancer	Ampullary cancer
Wiersema et al[23]	1992	86% (6/7)	43% (3/7)	—
Kubota et al[26]	1993	89% (16/18)	50% (10/14)	—
Ponchon et al[5]	1995	44% (7/16)	46% (6/13)	88% (7/8)
Pugliese et al[6]	1995	60% (6/10)	33% (4/12)	89% (8/9)
Sugiyama et al[21]	1996	88% (15/17)	71% (10/14)	—
Jailwala et al[10]	2000	37% (17/46)	37% (11/30)	77% (10/13)
Wright et al[31]	2011	79% (23/29)	74% (49/66)	—
Total		63% (90/143)	60% (93/156)	83% (25/30)

TABLE 3. Biliary brush cytology

Authors	Year	No. of patients	No. of cancers	TP	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Foutch et al[18]	1991	30	17	6	33	100	100	58
Lee et al[34]	1995	149	106	40	37	100	100	39
Ponchon et al[5]	1995	204	127	45	35	97	96	44
Pugliese et al[6]	1995	94	64	35	54	100	100	50
Glasbrenner et al[14]	1999	78	57	32	56	90	94	43
Mansfield et al[12]	1997	43	41	17	42	100	100	8
Jailwala et al[10]	2000	133	104	31	30	100	100	28
Macken et al[15]	2000	106	62	35	57	100	100	62
Stewart et al[33,*]	2001	406	246	147	60	98	98	61
Fogel et al[40]	2006	102	94	28	30	—	—	—
Shieh et al[41]	2014	32	32	25	78	—	—	—
Total		837	578	241	42			

TP, True positive; *PPV*, positive predictive value; *NPV*, negative predictive value.
*Values include an unspecified number of pancreatic duct brush cytology samples.

then the contents of the brush sheath are flushed into the solution). When the results of all techniques were combined (pancreatic juice cytology, brush cytology, and salvage cytology), sensitivity for cancer increased to 76%. The authors concluded that there is minimal added diagnostic yield with pancreatic juice cytology. To enhance the sensitivity of pancreatic duct juice collection, deep cannulation of the pancreatic duct and stimulation of secretion by administration of intravenous secretin can be performed. These techniques may theoretically be useful for evaluation of patients suspected to have small pancreatic cancers without stricture.[50-52] However, this approach is more time consuming, technically challenging, and potentially carries a higher risk of pancreatitis.[50] Thus, pancreatic duct sampling, whether by brush cytology or juice collection, is infrequently used.

Modified brushing techniques

In theory, increased exfoliation of cells, potentially leading to better specimen cellularity and higher diagnostic yield, can be accomplished by manipulating the stricture. This concept was studied by obtaining brush specimens before and after stricture dilation by deBellis in 2003,[30] but cancer detection was not higher after dilation (cancer detection rate 35% predilation vs 31% postdilation). In addition, subgroup analysis showed no statistically significant difference when stricture dilation was performed with a balloon or a Soehendra dilating catheter. However, obtaining the second brush specimen was helpful, increasing the cancer detection rate to 44%. These data confirm a previous study by Rabinowitz et al[53] showing that multiple brush specimens (obtained via percutaneous access) increased yield.

Farrell et al[24] studied the diagnostic effect of combined stricture dilation, 22-gauge endoscopic needle aspiration, and brushing. Brush cytology was positive in 8 of 14 patients (57%) with malignant biliary strictures who underwent brushing alone (Group A) versus 17 of 20 patients (85%) who underwent a combined modality (Group B). The greater sensitivity in Group B was explained by

disruption of the biliary epithelium and subsequent exposure of underlying tumor cells by dilation and needle aspiration. Needle aspiration diagnosed cancer in 9 of 20 patients, or 45%, but none of the cases had a diagnostic needle aspiration with a negative brush cytology. Thus, needle aspiration was considered to be particularly important in exposure of tumor cells.

A more recent publication by Curcio et al[54] introduced the term "intraductal aspiration" to describe a method of scraping the stricture to enhance cellular yield. After standard brushing is performed, the brush tip catheter with the brush removed is used to scrape the stricture at least 10 times, and then a suction catheter is introduced to collect bile and samplings in a specimen trap. Intraductal aspiration increased adequate cellular yield compared with standard brushing (92.8% vs 35.7%) and sensitivity (89% vs 37%). In this study, cytology samples reported as highly suspicious were considered diagnostic of malignancy.

Brush type

The brush type or manufacturer is generally not considered to make a major difference diagnostically. This was illustrated in a 2006 comparative study by Fogel et al[40] involving a new, longer cytology brush, called the Cytolong brush (Cook Endoscopy, Winston-Salem, NC). This brush is 5 cm long, 3 mm in diameter, comes in a 7F sheath, and has stiffer bristles oriented at a 45-degree angle. One hundred two patients with biliary strictures suspicious for neoplasia were randomized to either the Cytolong brush or what is considered a standard brush, the Geenen brush (Cook Endoscopy), which is 3 mm in diameter, 1.5 cm long, is on a 6F sheath, and has bristles oriented at 90 degrees. A repeat brushing sample was then obtained using the other brush. Although there was a modest increase in cellular yield with the Cytolong brush, the cancer detection rate was not better. Of the 94 malignant biliary strictures, in the study, 47% of which were secondary to pancreatic cancer, the cancer detection rate was 25 of 94 (27%) using the Cytolong brush and 28 of 94 (30%) using the standard brush. No patient had positive cytology results with the Cytolong brush and negative cytology results with the standard brush. Insufficient or limited cellularity was seen less frequently with the Cytolong brush (11/98) than with the standard brush (17/98), and the mean cellular yield (scored 0-3, insufficient to excellent) was greater with the Cytolong brush than the standard brush (2.6 vs 2.4).

Preliminary data on a newer brush (Infinity brush; US Endoscopy) found that sensitivity was 85% using the Infinity brush versus 30% using the standard brush (device not specified) when comparing 2 groups of 20 patients with pancreaticobiliary malignancy who underwent sampling with 1 of the 2 brushes.[55] The brush has larger bristles, which are believed to provide more agitation of cells from the stricture.[41] It comes in a 9F catheter, which is slightly larger and may make traversing a stricture somewhat more difficult; indeed, of 20 patients, 1 stricture (a proximal cholangiocarcinoma) could not be traversed with the Infinity brush.

Alternative devices for increasing yield of brush cytology

The use of other devices to disrupt the biliary epithelium has been reported over the years, including a 10F "scraping brush" and a Soehendra stent retriever. However, these were small studies and have not been repeated.[12,56,57]

Adverse events associated with biliary brush cytology

In a series of 223 consecutive biliary strictures sampled by brush cytology, Ponchon et al[5] reported 1 retroperitoneal bile duct perforation related to the brushing. The patient was treated with placement of a biliary stent and remained asymptomatic. These brushings were obtained without the use of a guidewire; when brushing is performed over a guidewire, the likelihood of this adverse event should, in theory, be extremely low.

ENDOBILIARY FORCEPS BIOPSY SAMPLING

This technique is more time consuming and more technically challenging than brushing and thus is less commonly used. It should theoretically provide a sample of bile duct tissue deep to the epithelium that may improve diagnostic yield compared with brushing. Specimens can be obtained by using forceps designed for standard and pediatric upper endoscopes or malleable forceps.[10,50] The biopsy forceps is generally passed into the bile duct after a sphincterotomy has been performed, although some have described use of biopsy forceps with an intact papilla.[21,58] Under fluoroscopic guidance, the forceps is advanced to the level of the stricture, opened, and then closed to grasp a specimen from the distal aspect of the stricture. Usually 1 or 2 specimens are obtained, the forceps are withdrawn, and a few samples are obtained in this manner. The optimum number of biopsy specimens to obtain has not been established.[5] The technical feasibility was described in initial studies.[59,60] Although many studies do not report the number of biopsy samples taken, Ponchon et al[5] and Schoefl et al[61] suggested that at least 3 specimens should be obtained, as did a more recent study by Kimura et al.[62] This study, published in 2013, focused on endobiliary forceps biopsy sampling, specifically in cases of pancreatic cancer. It also suggested that diagnostic yield was higher in patients with bilirubin greater than 10 mg/dL, those with near circumferential involvement of the bile duct, and when biopsy specimens were targeted at the left side wall of

TABLE 4. Endobiliary forceps biopsy sampling

Author	Year	No. of patients	No. of cancers	TP	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Kubota et al[26]	1993	41	32	26	81	100	100	75
Pugliese et al[6]	1994	52	36	19	53	100	100	48
Ponchon et al[5]	1995	128	82	35	43	97	97	41
Sugiyama et al[21]	1996	45	31	25	81	100	100	67
Schoefl et al[61]	1997	103	58	38	65	100	100	69
Jailwala et al[10]	1999	133	104	48	43	90	94	31
Wright et al[31]	2011	133	117	84	72%	100	100	36
Draganov et al[88]	2012	26	17	5	29	100	100	43
Hartman et al[91]	2012	81	39	30	76	100	100	81
Total		742	516	310	60			

TP, True positive; *PPV*, positive predictive value; *NPV*, negative predictive value.

the bile duct. Table 4 summarizes several studies that analyzed the yield of endobiliary forceps biopsy sampling.

In 2011, Wright et al[31] described a method of obtaining immediate cytopathologic diagnosis at ERCP using a cytologic preparation of forceps biopsy sampling and on-site cytopathologist interpretation (Smash protocol). This retrospective study of 133 patients also included results of standard brush cytology and endoscopic intraductal FNA. Freehand forceps biopsy sampling was used to obtain specimens from the lower edge of the stricture. Each biopsy specimen was forcefully smeared between 2 glass slides to attempt to create a cell monolayer, and biopsy sampling was repeated until a positive on-site cytologic result or a minimum of 10 biopsy samples were reviewed. Successful forceps introduction was possible in all 133 cases. Once the Smash protocol biopsy sampling was completed, 2 additional biopsy specimens were obtained for routine histology. Finally, a single-brush cytology specimen and endoscopic FNA specimen were obtained (later in the study, brush cytology was abandoned because it was not adding diagnostic benefit). Of the 117 patients proven to have cancer, an immediate cytologic diagnosis based on evaluation of the forceps biopsy "smash" specimen was made in 84 (72%). The overall sensitivity of the combined techniques (Smash, ERCP-FNA, and final histology) was 76%. Of note, the yield of true positives using this combined approach in cases proven to have cholangiocarcinoma or pancreatic cancer was 77 of 95 (81%). In-room immediate cytopathologic interpretation has been well studied and is widely used in cases of EUS-FNA but has rarely been applied to ERCP tissue sampling. This study emphasized the value of immediate diagnosis on case management, a feature not generally possible with sampling techniques requiring delayed processing. An alternative approach to obtaining an immediate tissue diagnosis when needed to expedite care or ERCP decision-making was analyzed in a study by Lo et al,[63] in which forceps biopsy specimens were placed in saline solution and sent for standard frozen section pathologic interpretation. Thus, immediate tissue analysis, in theory, may improve the sensitivity of ERCP tissues sampling methods.

Adverse events associated with endobiliary forceps biopsy sampling

Adverse events from endobiliary forceps biopsy sampling are uncommon. Minor bleeding has been noted to occur.[5] In the series by Schoefl et al,[61] after biopsy sampling of a Klastskin tumor, major bleeding occurred, requiring transfusion of 4 units of blood and insertion of a nasobiliary catheter. Pugliese et al[6] reported a case of perforation of the common hepatic duct secondary to endobiliary biopsy sampling of a benign stricture. The patient underwent laparotomy with closure of the perforation and t-tube placement. This adverse event was attributed to the size of the forceps, its stiffness, and that multiple specimens were obtained.

ADDITIONAL TECHNIQUES OF STANDARD CYTOLOGIC OR HISTOLOGIC TISSUE ACQUISITION

Two techniques that are affordable, simple, and safe, although with a low diagnostic yield, are bile aspiration and cytopathologic analysis of retrieved biliary stents. Bile aspiration is one of the easiest and oldest methods of acquiring a cytologic specimen from the biliary tree. It is usually performed by placing a catheter at the level of the stricture and aspirating 10 to 50 mL of bile.[17] The optimal volume of bile to collect has not been studied. The sensitivity of bile aspiration for detecting malignancy ranges from 6% to 32%.[12,17,18,20,21,27,64]

Malignant cells may be present on the surface of stents as a result of continuous trauma to the biliary epithelium while the stent is in place. The stent itself, along with the biofilm or sludge that accumulates on its external and internal surfaces, can cause adherence of malignant cells.

In addition, the proximal side of a straight plastic stent can accumulate tissue as it scrapes the tumor during withdrawal. Leung et al[65] demonstrated a cancer detection rate of 79% when analyzing material from occluded biliary stents that were removed from 14 patients with malignant biliary strictures. However, this detection rate is unusually high, and it was not specified whether a diagnosis of cancer had already been made in these patients. Pooled data from several studies support a lower sensitivity, 32%, for this sampling method.[1] Analysis of retrieved stents should be considered for patients undergoing stent exchange for suspected malignant strictures when other sampling methods have failed to provide a diagnosis. However, this technique cannot be considered a first-line approach because sampling must be performed at the initial ERCP and cannot be delayed until stent exchange.

One other sampling device, referenced above in the Smash Protocol study,[31] is endoscopic FNA. It was originally described by Howell et al in 1992[28] and evaluated by Jailwala et al in 2000.[10] The device is a retractable 22-gauge FNA needle in a 7F catheter, and it provided only low-to-moderate sensitivity and was somewhat difficult to use technically. More importantly, the availability and diagnostic accuracy of EUS-FNA, with the possible exception of hilar strictures inaccessible to EUS, has rendered ERCP-based intraductal FNA all but obsolete.

MULTIMODAL TISSUE SAMPLING

Several studies have shown that the use of multiple ERCP tissue sampling techniques in combination can improve the cancer detection rate. Some of these combination studies may have included modalities that have become outdated—for example, combining bile aspiration or endoscopic FNA with brushing[10,39,66]—whereas others that use newer technologies such as FISH are discussed later. However, to demonstrate the utility of combining modalities, we highlight a few studies here.

Ponchon et al[5] published a prospective series of 204 patients who underwent brushing and fluoroscopically guided forceps biopsy sampling. Biopsy and brush cytology had individual sensitivities of 35% and 43%, respectively, but when the 2 techniques were combined, the cancer detection rate improved to 63%. Similar results were obtained by Schoefl et al[61] (sensitivities of 47% and 65% for brush and biopsy sampling separately, respectively, and 70% in combination).

Jailwala et al[10] showed that combination sampling at ERCP has a higher cancer detection rate than that of any single modality. In this study, 133 patients (104 of whom were eventually diagnosed with cancer) underwent sequential brushing, endoscopic FNA, and biopsy sampling, always in that order. The cancer detection rate was 62% (high-grade atypia was considered equivalent to cancer) when the 3 methods were combined. Brush cytology was positive in 30% and forceps biopsy sampling in 43%. Combining forceps biopsy sampling with brush cytology improved the cancer detection rate to 55%. Endoscopic FNA alone was 30% sensitive, but when combined with forceps biopsy sampling, the sensitivity increased to 53%. When comparing the use of either 2 or 3 techniques, there was no significant difference in the cancer detection rate. However, the combination of either brush or FNA with forceps biopsy sampling had a higher diagnostic yield than the combination of brush and FNA alone. Despite the fact that endoscopic FNA has become obsolete, these data, and Jailwala et al's recommendation that at least 2 techniques be used to obtain samples, remain pertinent today and suggest that brush cytology and forceps biopsy sampling should both be obtained.

A recent retrospective study of triple modality sampling in the diagnosis of cholangiocarcinoma also highlights the added yield of a combined approach.[67] The combination of brush cytology, endobiliary forceps biopsy sampling, and FISH (discussed in detail in the next section) was evaluated. A total of 50 patients underwent triple modality sampling, of whom 22 were eventually diagnosed with cholangiocarcinoma. Individual sensitivities were 27% for brush cytology, 50% for endobiliary forceps biopsy sampling, and 59% for FISH. Triple tissue sampling increased the sensitivity to 82% (18/22), with 100% specificity.

MOLECULAR TECHNIQUES

FISH, a cytogenetic technique, uses fluorescently labeled DNA probes that hybridize to complementary specimen DNA to assess for the presence or absence of specific DNA sequences. The use of FISH to detect solid tumor cells in cytologic specimens capitalizes on the fact that most solid tumors are aneuploid, or containing an abnormal number of chromosomes. Such molecular abnormalities have been identified in approximately 80% of biliary and pancreatic cancers.[68] Aneuploidy has been reported in 80% of bile duct specimens from patients with PSC and cholangiocarcinoma.[69]

In 2004, Kipp et al[70] published the first study comparing FISH with routine brush cytology. Bile aspirate and brushings were collected for FISH analysis and brushings were collected for cytology from 131 patients with biliary strictures. Sixty-six of the 131 patients had surgical pathologic and/or clinical evidence of malignancy. The sensitivities of brush cytology and FISH from brushing specimens were 15% and 34%, respectively. FISH sensitivity from bile aspirate was more modest, at only 23%, meaning combined aspirate and brushing sensitivity for FISH was 35%. The specificity of brush cytology and FISH were 98% and 91%, respectively.

Smoczynski et al[71] reported a prospective study of 81 patients with bile duct or pancreatic duct strictures who underwent standard brushing for cytology followed by brushing for FISH. A FISH sample was considered positive if polysomy was identified by any probe or if trisomy of chromosome 3 or 7 was identified in at least 5 cells. Individually, the sensitivities for routine brush cytology and FISH were 35% and 52%, respectively. The specificity of FISH was 89%, whereas the specificity of brush cytology was 100%.

Several other molecular techniques have demonstrated promise at one time or another but have not gained widespread use or have fallen out of favor entirely. An early example of one such technique is flow cytometry, which identifies aneuploidy but requires a sample with a high degree of cellularity and malignant cells.[72] Digital image analysis (DIA) is a technique in which the DNA content of individual cells can be determined. Light transmitted through a glass slide specimen is captured by video, converted into pixels of variable color (white, gray, and black), and then into a digital image of the nucleus and other cellular constituents. DNA content, chromatin distribution, and nuclear morphology can be analyzed for features of malignancy. In a study of 100 biliary strictures, 56 of which were diagnosed as malignant, DIA had a sensitivity and specificity of 39% and 77%, respectively.[73]

Two studies from the Mayo Clinic evaluated FISH in combination with DIA and routine sampling, along with intraductal US (IDUS) in the second study. The first study, published in 2006 by Moreno Luna et al,[74] involved 233 patients with pancreaticobiliary strictures, 86 of whom had PSC. Strictures were brushed and specimens sent for cytology, DIA, and FISH. Results were analyzed separately for proximal and distal biliary strictures. For proximal strictures in the non-PSC group (147 patients), brush cytology was only 9% sensitive (including positive and suspicious for malignancy). DIA was 30% sensitive with 90% specificity. FISH had a sensitivity and specificity of 63% and 100%, respectively. For distal strictures, again in the non-PSC group, cytology, DIA, and FISH each had a sensitivity and specificity of 41% and 96%, 49% and 98%, and 59% and 92%, respectively.

In a prospective study of indeterminate biliary strictures, stratified on the basis of PSC and non-PSC patients, Levy et al[75] evaluated routine cytology, forceps biopsy sampling, IDUS, DIA, and FISH. A total of 86 patients were included, 34 of whom had PSC. When combining both the PSC and non-PSC groups, routine cytology was 32% sensitive for malignancy (when including interpretations of confirmed malignant and suspicious for malignancy) and only 11% sensitive if suspicion for malignancy was not included. Forceps biopsy sampling was 38% sensitive. DIA alone was 38% sensitive and 95% specific. FISH alone was 64% sensitive and 82% specific when including trisomy-7 as indicative of malignancy. Specificity increased to 100% when trisomy-7 was categorized as benign, at a cost of sensitivity decreased to 45%, with the most significant differences being in PSC patients. DIA and FISH in combination increased the sensitivity to 70% (again, trisomy-7 indicative of malignancy), with a specificity of 82%. IDUS provided a sensitivity and specificity of 89% and 64%, respectively. These values are similar to other published reports of IDUS.[76-79] Thus, in both of these multimodality studies, FISH had the highest sensitivity of the tissue sampling techniques, while maintaining a relatively high specificity. DIA and IDUS can be used as adjunctive technologies but have not gained widespread acceptance.

The future of molecular analysis may lie in microRNA (miRNA). These are short, noncoding RNAs consisting of 18 to 25 nucleotides that help regulate biologic processes such as cell proliferation, migration, invasion, survival, and metastases. They have substantial potential as biomarkers because of their biologic stability. A pilot study evaluating the utility of 10 different miRNAs as diagnostic markers for pancreatic ductal adenocarcinoma (PDAC) was published by Cote et al.[80] In it, 77 patients with treatment-naïve PDAC, 67 patients with chronic pancreatitis with bile duct or pancreatic duct pathology, and 71 control subjects with choledocholithiasis were evaluated. These patients had undergone ERCP for the above conditions, at which time they were prospectively enrolled in a biologic repository and plasma, bile, or pancreatic juice (or some combination of those) were collected. Selected miRNAs were identified in plasma and bile as part of a "PDAC Signature Panel," which had 100% sensitivity and specificity in distinguishing PDAC from both chronic pancreatitis and control subjects. Pancreatic juice was available in a limited number of subjects but also performed exceedingly well, correctly distinguishing all individuals with PDAC from those with chronic pancreatitis. Thus, with further validation of accuracy in the future, a plasma panel could serve as a screening tool for high-risk patients such as those with chronic pancreatitis or a family history of PDAC, and bile and/or pancreas juice could improve the diagnostic accuracy of ERCP for indeterminate strictures.

DNA methylation of candidate genes has also been studied as a marker of malignancy, and a 2015 publication by Andresen et al[81] presented data on a panel of 13 candidate genes evaluated in 39 tissue samples of cholangiocarcinoma and 54 nonmalignant controls. They identified the 4 best-performing genes, which detected cholangiocarcinoma with a sensitivity of 85% and a specificity of 98%.

CHOLANGIOSCOPY

Cholangioscopy, or bile duct endoscopy, allows for direct visualization of the lumen of the bile duct. Original

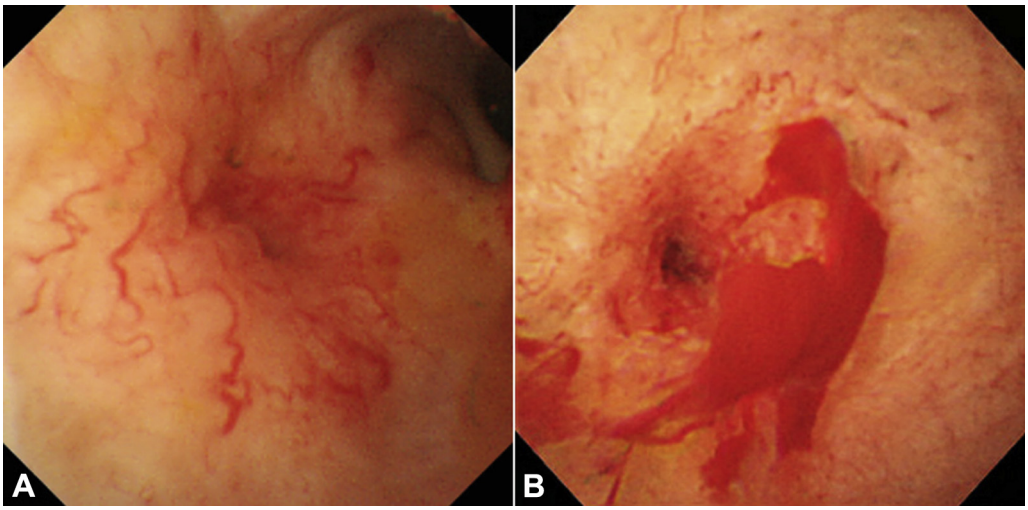

Figure 1. Per-oral cholangioscopy view of a malignant biliary lesion with tumor vessel (**A**) and easy bleeding (**B**). From Nishikawa et al.[85]

iterations of cholangioscopy involved a "mother–baby" scope setup, which required 2 endoscopists and had issues with scope fragility. Another method of visualizing the bile duct lumen is direct per-oral cholangioscopy, using an ultraslim gastroscope, often with the addition of an anchoring balloon.

The SpyGlass direct visualization system (Boston Scientific) allows for single-operator cholangioscopy (SOC) and has replaced the older "mother–baby" 2-operator reusable cholangioscopes. The components of the SOC system include the disposable SpyScope (Boston Scientific), a 10F access, and delivery catheter with a 1.2 mm diameter working channel and 2 dedicated irrigation channels. It is introduced through a duodenoscope with a minimum working channel diameter of 4.2 mm. The catheter is capable of tip deflection of at least 30 degrees in 4 directions. The reusable SpyGlass Fiber Optic Probe (Boston Scientific) provides 6000-pixel images. The disposable SpyBite Biopsy Forceps (Boston Scientific) incorporates jaws at the tip designed to excise and retrieve visually targeted tissue.

The video imaging that can be obtained with per-oral cholangioscopy is the advantage of this method, as compared with the fiberoptic image obtained by SpyGlass. However, a new digital SpyGlass system has recently become commercially available and may make cholangioscopy more accessible and more useful, but additional studies to determine its diagnostic utility are needed. Whether performed with video or fiberoptic imaging, the first advantage of cholangioscopy is that it allows for direct visualization of pathology within the biliary tree, which can provide a diagnostic impression of a biliary stricture and allow for targeted biopsy sampling of suspicious-appearing tissue. In a study of cholangioscopy via percutaneous tract access to the biliary tree published in 2000, Seo et al[82] reported several findings characteristic of biliary tract malignancy, including neovascularization and tumor type (nodular, papillary, or infiltrative). In another study using percutaneous cholangioscopy, also published in 2000, Kim et al[83] showed the presence of "tumor vessels" (Figure 1) to be highly specific for cholangiocarcinoma (specificity 100%, sensitivity 61%). Nodularity is also specific. Cholangioscopy via a percutaneous transhepatic biliary drain tract is rarely performed, but similar visual findings can be seen via direct per-oral cholangioscopy and SpyGlass cholangioscopy.

A series of 97 patients undergoing per-oral cholangioscopy with a "mother–baby" scope system was published by Fukuda et al in 2005.[84] It found endoscopic retrograde cholangiography tissue sampling to have a sensitivity of 58%, whereas the addition of per-oral cholangioscopy visual diagnosis improved the sensitivity to 100%, at a cost of decreasing specificity from 100% to 87%. A similar study was published in 2013 by Nishikawa et al,[85] evaluating 33 patients with the use of per-oral video cholangioscopy and per-oral video cholangioscopy–guided forceps biopsy sampling. The visual findings had a sensitivity and specificity of 100% and 92%, respectively, but forceps biopy sampling had a sensitivity of only 38%.

In a feasibility study of SOC by Chen and Pleskow in 2007[86] that included 22 patients with indeterminate biliary strictures, the sensitivity of SOC biopsy samplings was 71% (other SOC indications were also evaluated in this study, including fragmentation of large bile duct stones, indeterminate filling defects, cystic lesions, and gallbladder stent placement). In another early study of SOC, also published by Chen et al in 2011[87] and also evaluating SOC in terms of workup of biliary tract disease and therapy of large stones, 140 patients underwent SOC-directed biopsy sampling of bile duct lesions. Adequate tissue was secured in 88% of the 140 patients. Although overall sensitivity of SOC visual impression was 78%, it was only 49% for SOC-directed biopsy samples. When stratifying lesions into

intrinsic or extrinsic, the sensitivity of SOC-directed biopsy samples did increase to 66% for intrinsic lesions, versus only 8% for extrinsic lesions. It seems intuitive that sensitivity would be lower for lesions that appear to be extrinsic to the bile duct. However, even for intrinsic lesions, the sensitivity of SOC-directed biopsy sampling is not as high as one might hope for a targeted biopsy sample—this lower sensitivity is likely a reflection of both the fibrotic nature of many of these lesions and the small caliber of the mini-forceps (SpyBite) that are compatible with the working channel of the SpyGlass system.

Draganov et al[88] studied SpyGlass SOC in direct comparison with brush cytology and standard (fluoroscopically guided) forceps biopsy sampling in 26 patients with indeterminate biliary strictures. A diagnosis of cancer was based on positive ERCP sampling, subsequent positive biopsy sampling by an alternative method, or disease progression seen on follow-up imaging. In those patients with negative biopsy specimen results, a diagnosis of benign disease was based on at least 12 months of clinical course and imaging showing no evidence of disease progression. Seventeen of the 26 patients were diagnosed with cancer (15 cholangiocarcinoma, 2 PDAC). ERCP/SOC established the diagnosis in 13 patients. SpyBite biopsy specimens were positive in 77% (13/17), with no false positives (100% specificity). Cytology brushing was only 6% sensitive, and standard biopsy sampling was 29% sensitive (both were also 100% specific).

Another study of SOC for evaluation of indeterminate strictures was published by Ramchandani et al.[89] In it, 36 patients underwent SpyGlass SOC with cholangioscopically guided biopsy sampling. Cholangioscopic visual impression was analyzed, but other modalities of tissue acquisition were not. Features considered suspicious for malignancy included mass, dilated tortuous vessels, papillary or villous projections, and intraductal nodules. A mass with dilated tortuous vessels was categorized as "definite malignant." Benign lesions were characterized by smooth surface mucosa without definite neovascularization and homogeneous granular mucosa without a primary mass. Three to 4 SpyBite biopsy specimens were obtained in 33 cases (in the other 3 the filling defects seen on previous ERCP turned out to be bile duct stones, which were visualized with SOC). Of the 22 patients with a final diagnosis of malignancy, SpyBite specimens yielded malignant histology in 18 (82%). Regarding visual diagnosis, 11 patients were classified as definite malignant, and all these patients had a final diagnosis of malignancy. Thirteen patients were classified as having suspected malignant disease (with findings of villous or papillary projections and nodularity), and 3 of these had a final diagnosis of benign disease. Two patients with possible malignant strictures by cholangioscopy visual impression and inadequate biopsy specimens underwent surgery and were found to have benign disease. Overall, sensitivity and specificity for visual impression were 95% (21/22) and 79% (11/14), respectively. For SpyBite biopsy sampling, sensitivity and specificity were both 82%. In a similar but retrospective analysis of 30 patients with indeterminate strictures eventually diagnosed as malignancy, SOC-directed biopsy samples were diagnostic of malignancy in 77% (23/30 patients, with 7 biopsy samples labeled as highly suspicious included as confirmatory diagnoses).[90]

Fluoroscopic-guided biopsy sampling was compared with SOC-guided biopsy sampling in a retrospective study by Hartman et al.[91] The authors concluded that the former was more sensitive than the latter (76% vs 57%, respectively). Each method of biopsy sampling had 100% specificity. They evaluated size of the tissue samples and concluded that the higher sensitivity is likely related to the larger amount of tissue obtained by fluoroscopic-guided biopsy sampling. In addition, they proposed their method of handling the small SpyBite samples in a way that optimized diagnostic yield. Their description is noteworthy because of the small size of the biopsy specimens that are obtained. They submit the fragments in formalin-filled containers, as one normally would do. In the pathology laboratory, if fragments appear smaller than 1 mm, the fluid is centrifuged and a cell block is created.

Finally, a systematic review of SpyGlass SOC studies was published in *Gastrointestinal Endoscopy* by Navaneethan et al in 2015.[92] Included in the review were 10 studies involving 456 patients. The pooled sensitivity and specificity of SOC-guided biopsy sampling in the diagnosis of malignancy were 60% and 98%, respectively. Four studies included patients who had previous negative brushings and/or intraductal biopsy sampling, and in these studies SOC-guided biopsy sampling had a pooled sensitivity and specificity of 75% and 93%, respectively.

CONFOCAL LASER ENDOMICROSCOPY

A newer diagnostic modality, CLE, has recently become available. CLE allows real-time imaging of the mucosal cellular characteristics. It initially showed promise in the evaluation of mucosal dysplastic changes in the GI lumen (ie, Barrett's esophagus), and in addition to its use in pancreaticobiliary strictures, it has begun to be studied in the evaluation of pancreatic cystic lesions. For biliary strictures, a pCLE device has been designed (Cholangioflex, Cellvizio; Mauna Kea Technologies, Paris, France). pCLE was initially evaluated in a series of 14 patients with biliary strictures, 6 of which were diagnosed as malignant.[93] A specific pattern was found predominately in patients with cancers and was characterized by a dark-gray background without identification of mucosal structures but large white streaks resembling fluorescein-filled tortuous, dilated, and saccular vessels with inconsistent branching. In the video mode, erythrocytes easily could be identified rushing through these streaks, showing that indeed vascular structures could be identified. The second pattern comprised a

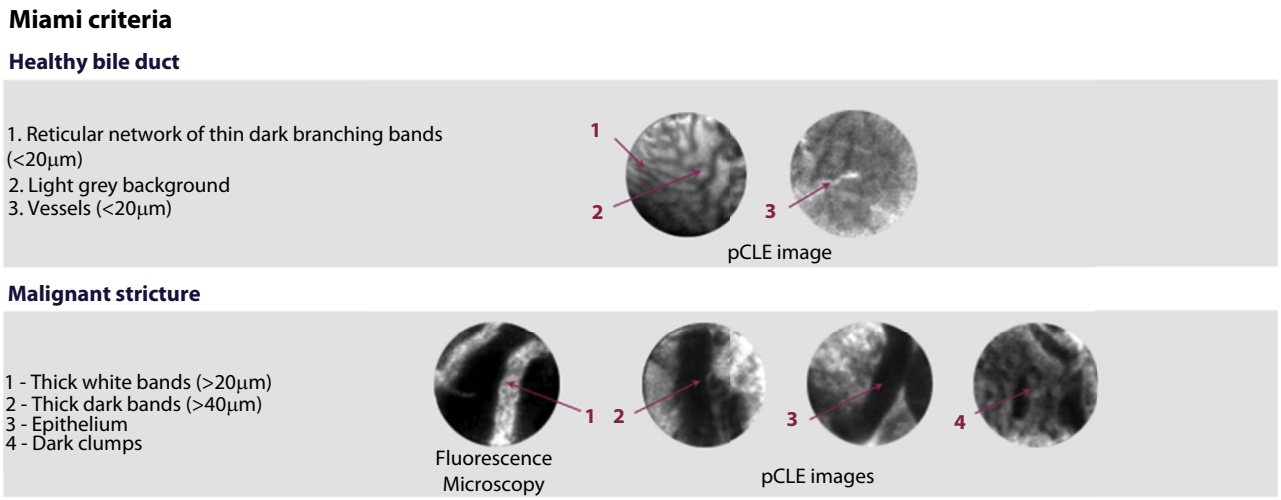

Figure 2. Miami criteria for the interpretation of biliary/pancreatic pCLE. From Slivka et al.[96]

reticular pattern of different gray scales or small dark-gray villous structures but no white streaks. The sensitivity and specific for malignancy, applying these features, were 83% (5/6) and 88% (7/8). Biopsy specimens had a lower sensitivity of 50% (3/6) and, as expected, a specificity of 100%. Surgical resection of tumors was performed in 3 patients. Here, histopathology revealed invasive adenocarcinomas in 2 patients. Interestingly, preoperative biopsy specimens revealed reactive changes of the epithelium with no intraepithelial neoplasia or invasive carcinoma, whereas laser microscopy revealed malignancy in these cases.

These preliminary data led to a multicenter registry study of 102 patients to design a classification scheme for the interpretation of pancreaticobiliary pCLE, which would be designated the Miami Classification (published as interim analysis in 2010 and as a full publication in 2012, shown in Figure 2).[94] Data from this same registry of patients were published in 2011, showing that the overall sensitivity of pCLE for detecting malignancy was 98%, with a specificity of 67%.[95] Index pathology in these cases had a sensitivity and specificity of 45% and 100%, respectively. Of the 89 assessable patients (81 with biliary strictures, 8 with pancreatic strictures), 40 were eventually proven to have cancer.

A prospective multicenter international study of pCLE evaluated 112 patients with indeterminate biliary strictures.[96] Prior tissue sampling had been performed and deemed negative in 39 of 54 patients (72%) who had already undergone ERCP. The investigators performed pCLE evaluation, followed by brushing for cytology and forceps biopsy sampling for histology. They made an assessment of malignancy based on their impression of the cholangiogram findings alone, which had a sensitivity and specificity of 84% and 76%, respectively. By using the pCLE information during the ERCP procedure, the investigators suspected cholangiocarcinoma in 75 patients (67%) and suspected a benign lesion in 37 cases (33%). These impressions had a sensitivity and specificity of 89% and 71%, respectively. The sensitivity of brush cytology and biopsy sampling was 56%, but when pCLE was included with pathology results, the sensitivity of overall diagnostic testing was 89%, with a specificity of 88%.

EUS-FNA IN CONJUNCTION WITH ERCP AND IN COMPARISON WITH ERCP

A review of pancreaticobiliary tissue sampling would not be complete without discussing EUS. Although not intended to be a thorough review of EUS, we would like to include in our discussion some studies comparing ERCP and EUS tissue sampling methods. As a general rule, EUS is an excellent diagnostic modality for pancreas masses but remains less useful for proximal biliary strictures.

EUS-FNA is a very safe procedure, with extremely low rates of adverse events such as pancreatitis, hemorrhage, infection, bile peritonitis, perforation, or malignant seeding.[97] Of particular concern, however, is the risk of malignant tumor seeding in situations when liver transplantation may be a consideration. The Mayo Clinic protocol for neoadjuvant chemotherapy and liver transplantation for otherwise unresectable cholangiocarcinoma lists transperitoneal biopsy sampling (including percutaneous and EUS-guided FNA) as an absolute contraindication to transplantation. A 2011 retrospective study reviewed 191 patients who underwent neoadjuvant chemoradiotherapy followed by liver transplantation for hilar cholangiocarcinoma.[98] A total of 16 patients underwent transperitoneal FNA biopsy sampling, with 6 of those biopsy samples positive for adenocarcinoma. Of the 6, 5 (83%) had peritoneal metastases identified at operative staging, compared with

only 14 of 175 (8%) who did not undergo transperitoneal biopsy sampling. The potential for future liver transplantation and this contraindication must be taken into consideration anytime EUS-FNA is considered in the workup of suspected hilar cholangiocarcinoma.

The year 2004 brought us several studies related to EUS-guided bile duct tissue sampling, with sensitivity of EUS-FNA ranging from 47% to 89%. Lee et al[99] aimed to characterize the diagnostic accuracy of EUS visual impression in addition to that of EUS-FNA. Bile duct wall thickness greater than or equal to 3 mm had 79% sensitivity for malignancy. Rösch et al[100] compared same-session ERCP and EUS in 50 consecutive patients with obstructive jaundice, 28 of whom were diagnosed with malignancy. During ERCP, all strictures were sampled by forceps biopsy and 2 brush cytology specimens, with very modest sensitivities of 36% and 46%, respectively. EUS-FNA sensitivity was better overall, at 75%, but this value included pancreas masses. For the subgroup of biliary tumors, ERCP was more sensitive compared with EUS (75% vs 25%, respectively). Fritscher-Ravens et al[101] studied 44 consecutive patients with suspected hilar cholangiocarcinoma and found EUS-FNA to be 89% sensitive in diagnosing malignancy.

DeWitt et al[102] published an analysis of 24 consecutive patients undergoing EUS-FNA for proximal biliary strictures (defined as located at, or proximal to, the upper one third of the extra hepatic bile duct, characterized by cholangiography findings). All of them had brush cytology at previous ERCP that were negative or unable to be performed. EUS-FNA correctly diagnosed malignancy in 17 of 22 patients (77% sensitivity). A series of 81 patients with cholangiocarcinoma who underwent EUS-FNA was published by Mohamadnejad et al in 2011.[103] The overall sensitivity of EUS-FNA for the diagnosis of cholangiocarcinoma was 73% and was significantly higher in distal compared with proximal cholangiocarcinoma (81% vs 59%, respectively). This study also assessed the role of EUS in assessing resectability and found it to be very specific in this regard.

A few comparative studies have involved ERCP and EUS-based sampling methods. One such study by Nguyen et al[104] aimed to evaluate the next best step in the workup of an indeterminate biliary stricture—specifically, looking at EUS versus SOC. All patients had previously undergone ERCP with negative brush cytology results. EUS identified the abnormality responsible for the biliary stricture in 39 of 40 cases (98%) (bile duct wall thickening was defined as a wall thickness > 3 mm). However, FNA of the primary lesion was not deemed safe in all patients and thus was performed in 25. A tissue diagnosis was established in 23 of 25 cases (92%) in which FNA of the primary lesion was performed. If EUS did not establish a diagnosis, then SOC was performed within 1 week of the EUS. SOC was performed in 19 patients (the 17 in which EUS did not provide a tissue diagnosis and 2 in which EUS diagnosed autoimmune pancreatitis). In 1 case, SOC was attempted but was not successful because of a severely strictured extrahepatic bile duct in a patient with PSC. SOC-guided biopsy sampling was performed in all 18 successful SOC procedures, and the correct diagnostic yield was reported as 89% (16/18). There were 2 cases in which biopsy specimens were reported as nonspecific inflammation but subsequent surgical specimen revealed malignancy. All patients were followed for at least 6 months. The authors concluded that EUS-FNA may reduce the need, and associated costs and risk of adverse events, of SOC in 60% of cases.

In 1 of the only studies directly comparing EUS and ERCP tissue sampling, Weilert et al[105] evaluated 51 patients with malignant biliary obstruction in a prospective, single-blind study. Patients underwent same-session EUS followed by ERCP, with a second endoscopist performing the ERCP, blinded to the results of the EUS-FNA. Nine patients had undergone previous ERCP at another institution, 8 of whom had negative sampling results and 1 of whom had a failed attempt at ERCP. During EUS, lymph nodes or liver masses could also be targeted for FNA, in addition to the primary lesions, and lesions that would confer a higher cancer staging were targeted first. ERCP failed in 7 cases (14%), all of which had duodenal deformity because of pancreatic cancer. These cases were considered to have "nondiagnostic" results in the analysis. A diagnosis of malignancy was made if the pathologist reported results "malignant" or "suspicious for malignancy." The sensitivity of EUS-FNA was 100% for pancreas masses, 79% for biliary masses, and 80% for biliary strictures. The sensitivity of ERCP sampling, which included 1 cytology brushing and 2 to 3 bites with an endobiliary biopsy forceps, was 38% for pancreas masses, 79% for biliary masses, and 67% for strictures.

Overall, EUS-FNA is a tremendously powerful diagnostic tool, and as its use becomes more and more widespread, we will rely less on ERCP tissue sampling. However, EUS-FNA is less sensitive for more proximal bile duct strictures and lesions, as compared with its excellent sensitivity for pancreas masses and distal bile duct lesions. In addition, even with widespread availability, the decision to perform EUS at the same setting as the initial ERCP is not universal and not necessarily indicated in all situations. Thus, accurate methods of ERCP tissue sampling remain important, especially in cases of proximal bile duct lesions and to potentially make a diagnosis on the first procedure.

CONCLUSIONS

The ideal sampling method is very sensitive and specific while also being relatively simple technically and cost-effective. When evaluating a patient with a potentially malignant pancreaticobiliary stricture, the resectability of the tumor and the patient's candidacy for surgery must be established. In this regard, if a patient is clearly a suitable surgical candidate, then tissue sampling to establish a definitive

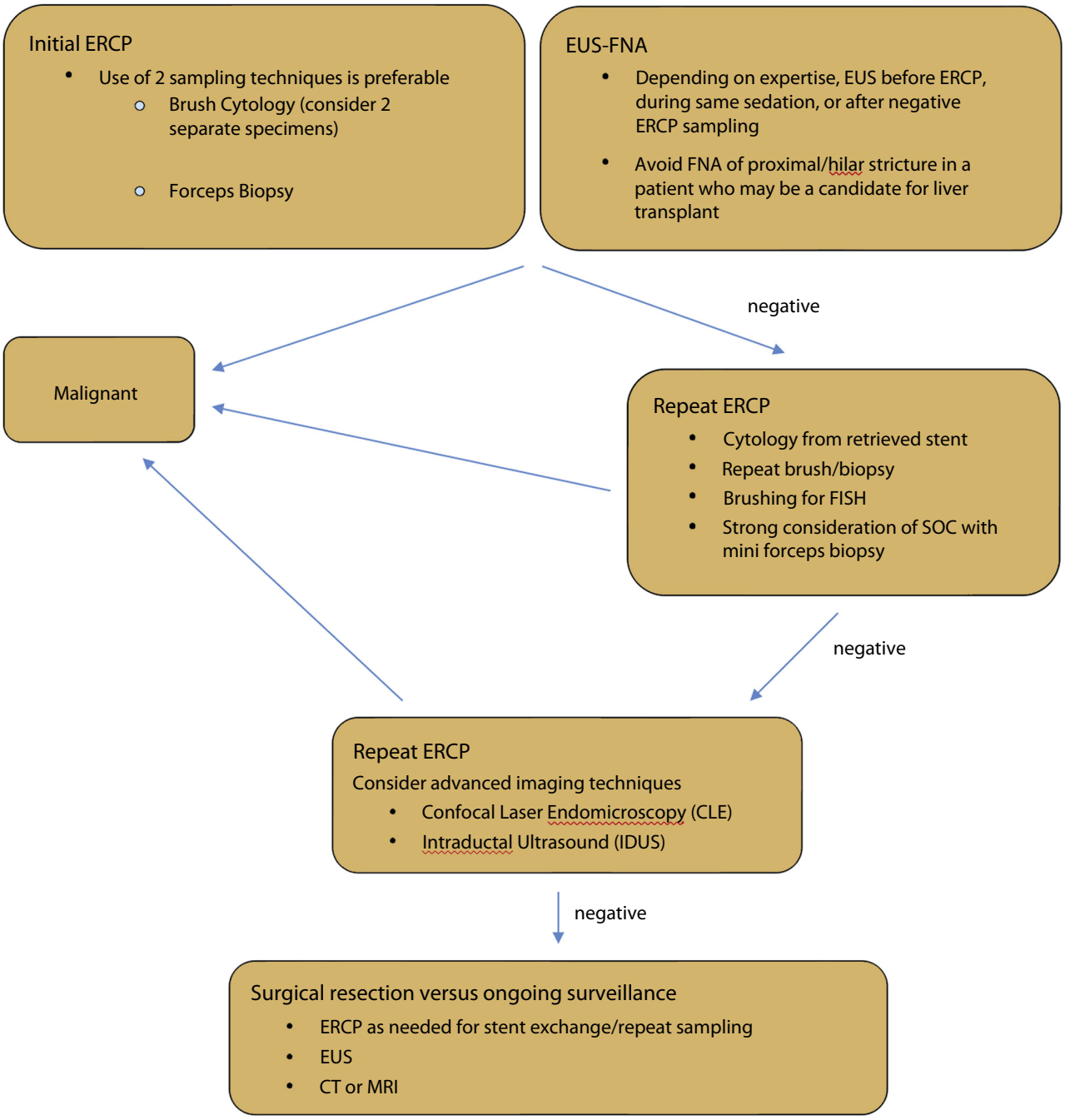

Figure 3. Diagnostic algorithm for the workup of biliary structures. *FISH*, fluorescence in situ hybridization; *SOC*, single-operator cholangioscopy.

diagnosis preoperatively may not be necessary. However, in most circumstances and at most institutions, a tissue diagnosis is desired before subjecting patients to extensive surgical resection, particularly if neoadjuvant chemotherapy and/or radiation therapy are planned. In addition, many patients present with unresectable disease or are not suitable operative candidates because of comorbid conditions.

In either case, a timely diagnosis of malignancy or a test that can reliably exclude malignancy are of utmost importance. Which ERCP-based techniques should be used to achieve this goal? Figure 3 provides a suggested diagnostic algorithm. Although ERCP brush cytology will never achieve adequate sensitivity on its own, its simplicity, low cost, and specificity make it the best minimum first step. To enhance diagnostic yield, obtaining 2 separate brush cytology specimens can be considered. Manipulation of the stricture by dilation to expose more cells to the brush has not proven to be of benefit, but scraping of the stricture with the brush catheter was helpful in 1 study and may warrant further

investigation. Endobiliary forceps biopsy sampling, under fluoroscopic guidance, seems to be more sensitive than brushing and has been shown to increase sensitivity when combined with brushing. Thus, when expertise is available, this method should be considered, with a goal to obtain a minimum of 3 specimens.

Some of the newer (and potentially more costly, time consuming, and technically challenging) techniques are often reserved for indeterminate strictures that have already had negative sampling at least once. Examples of these include cholangioscopy, pCLE, and molecular sampling. SOC is more commonly used at tertiary centers, but SOC-guided biopsy sampling remains relatively insensitive. It remains to be determined whether the new SpyGlass system, which uses video imaging (as opposed to fiberoptic) and has a larger working channel, will make SOC more effective. pCLE has shown promise but needs to be refined and is in use only at select centers. Of the molecular markers, FISH has gained acceptance because it improves sensitivity while only mildly decreasing specificity. However, its results need to be interpreted cautiously, and it is only commonly used at referral centers at this point. As all of medicine becomes more personalized to each individual's and each tumor's genetic code, it is no longer unfathomable to envision a day when any form of biopsy sampling will no longer be necessary and a serum test can detect the presence of malignancy and determine the type of cancer if present. However, that future is still a long way off, and we must continue to refine and expand our ERCP-based tissue sampling methods.

REFERENCES

1. deBellis M, Sherman S, Fogel EL. Tissue sampling at ERCP in suspected malignant biliary strictures (Part 1). Gastrointest Endosc 2002;56:552-61.
2. deBellis M, Sherman S, Fogel EL. Tissue sampling at ERCP in suspected malignant biliary strictures (Part 2). Gastrointest Endosc 2002;56:720-30.
3. Layfield LJ, Wax TD, Lee JG, et al. Accuracy and morphologic aspects of pancreatic and biliary ducts brushings. Acta Cytol 1995;39:11-8.
4. Kocjan G, Smith AN. Bile duct brush cytology: potential pitfalls in diagnosis. Diagn Cytopathol 1997;16:358-63.
5. Ponchon T, Gagnon F, Berger F. Value of endobiliary brush cytology and biopsies for the diagnosis of malignant bile duct stenosis: results of a prospective study. Gastrointest Endosc 1995;42:565-72.
6. Pugliese V, Conio M, Nicolò G. Endoscopic retrograde forceps biopsy and brush cytology of biliary strictures: a prospective study. Gastrointest Endosc 1995;42:520-6.
7. Jin YH, Kim SH, Park CK. Diagnostic criteria for malignancy in bile cytology and its usefulness. J Korean Med Sci 1999;15:643-7.
8. Renshaw AA, Madge R, Jiroutek M, et al. Bile duct brushing cytology. Am J Clin Pathol 1998;110:635-40.
9. Bardales RH, Stanley MW, Simpson DD. Diagnostic value of brush cytology in the diagnosis of duodenal, biliary and ampullary neoplasms. Am J Clin Pathol 1998;109:540-8.
10. Jailwala J, Fogel EL, Sherman S. Triple tissue sampling at ERCP in malignant biliary obstruction. Gastrointest Endosc 2000;51:383-90.
11. Ryan ME. Cytologic brushings of ductal lesion during ERCP. Gastrointest Endosc 1991;37:139-42.
12. Mansfield JC, Griffin SM, Wadebra V. A prospective evaluation of cytology from biliary strictures. Gut 1997;49:671-7.
13. Vandervoort J, Soetikno RM, Montes H. Accuracy and complication rates of brush cytology from bile duct versus pancreatic duct. Gastrointest Endosc 1999;49:322-7.
14. Glasbrenner B, Ardan M, Boeck W. Prospective evaluation of brush cytology of biliary strictures during endoscopic retrograde cholangiopancreatography. Endoscopy 1999;31:712-7.
15. Macken E, Drijkoningen M, Van Aken E. Brush cytology of ductal strictures during ERCP. Acta Gastroenterol Belg 2000;63:254-9.
16. Foutch PG. Diagnosis of cancer by cytologic methods performed during ERCP. Gastrointest Endosc 1994;40:249-52.
17. Kurzawinski T, Deery A, Davidson BR. Diagnostic value of cytology for biliary strictures. Br J Surg 1993;80:414-21.
18. Foutch PG, Kerr DM, Harlan JR, et al. A prospective, controlled analysis of endoscopic cytotechniques for diagnosis of malignant biliary strictures. Am J Gastroenterol 1991;86:577-80.
19. Trent V, Khurana KK, Pisharodi LR. Diagnostic accuracy and clinical utility of endoscopic bile duct brushing in the evaluation of biliary strictures. Arch Pathol Lab Med 1999;123:712-5.
20. Davidson B, Varsamidakis N, Dooley J. Value of exfoliative cytology for investigating bile duct strictures. Gut 1992;33:1408-11.
21. Sugiyama M, Atomi N, Wada N. Endoscopic transpapillary bile duct biopsy without sphincterotomy for diagnosing biliary strictures: a prospective comparative study with bile and brush cytology. Am J Gastroenterol 1996;91:465-7.
22. Pescatore P, Heubner C, Heine M. The value of histological analysis of occluded biliary endoprostheses. Endoscopy 1995;27:597-600.
23. Wiersema M, Lehman G, Hawes R. Improvement of diagnostic yield of brush cytology in malignant strictures by the use of supplemental tissue sampling technique [abstract]. Gastrointest Endosc 1992;35:265A.
24. Farrell RJ, Jain AK, Brandwein SL, et al. The combination of stricture dilation, endoscopic needle aspiration and biliary brushings significantly improves diagnostic yield from malignant bile duct strictures. Gastrointest Endosc 2001;54:587-94.
25. O'Donohue JW, Stewart CJR, Mutch AF. Brush cytology in the investigation of biliary strictures: a prospective study [abstract]. Gut 1997;40:68A.
26. Kubota Y, Takaoka M, Tani K. Endoscopic transpapillary biopsy for diagnosis of patients with pancreaticobiliary ductal strictures. Am J Gastroenterol 1993;88:1700-4.
27. Kurzawinski TR, Deery A, Dooley JS. A prospective study of biliary cytology in 100 patients with bile duct strictures. Hepatology 1993;18:1399-403.
28. Howell DA, Beveridge RP, Bosco J, et al. Endoscopic needle aspiration biopsy at ERCP in the diagnosis of biliary strictures. Gastrointest Endosc 1992;38:531-5.
29. Baron TH, Lee JG, Wax TD. An in vitro randomized prospective study to maximize cellular yield during bile duct brush cytology. Gastrointest Endosc 1994;40:146-50.
30. deBellis M, Fogel EL, Sherman S. Influence of stricture dilation and repeat brushing on the cancer detection rate of brush cytology in the evaluation of malignant biliary obstruction. Gastrointest Endosc 2003;58:176-82.
31. Wright E, Bakis G, Srinivasan R. Intraprocedural tissue diagnosis during ERCP employing a new cytology preparation of forceps biopsy (Smash Protocol). Am J Gastroenterol 2011;106:294-9.
32. Logrono R, Kurtycz DF, Molina CP. Analysis of false-negative diagnoses on endoscopic brush cytology of biliary and pancreatic duct strictures. The experience at 2 University Hospitals. Arch Pathol Lab Med 2000;124:387-92.
33. Stewart CJR, Mills PR, Garter R. Brush cytology in the assessment of pancreatico-biliary strictures: a review of 406 cases. J Clin Pathol 2001;54:449-55.

34. Lee JG, Leung JW, Baillie J, et al. Benign, dysplastic, or malignant—making sense of endoscopic bile duct brush cytology: results in 149 consecutive patients. Am J Gastroenterol 1995;90:722-6.
35. Foutch PG, Kerr DM, Harlan JR, et al. Endoscopic retrograde wire-guided brush cytology for diagnosis of patients with malignant obstruction of the bile duct. Am J Gastroenterol 1990;85:791-5.
36. Venu RP, Geenen JE, Kini M. Endoscopic retrograde brush cytology. A new technique. Gastroenterology 1990;99:1475-9.
37. Scudera PL, Koizumi J, Jacobson IM. Brush cytology evaluation of lesions encountered during ERCP. Gastrointest Endosc 1990;36:281-4.
38. de Peralta-Venturina MN, Wong DK, Purslow MJ, et al. Biliary tract cytology in specimens obtained by direct cholangiographic procedures. A study of 74 cases. Diagn Cytopathol 1996;14:334-48.
39. Cunningham JT, Lewin D, Tarnasky P, et al. A prospective study of multiple sampling methods in the assessment of biliary strictures [abstract]. Gastrointest Endosc 1997;45:AB128.
40. Fogel EL, deBellis M, McHenry L. Effectiveness of a new long cytology brush in the evaluation of malignant biliary obstruction: a prospective study. Gastrointest Endosc 2006;63:71-7.
41. Shieh F, Luong-Player A, Khara H, et al. Improved endoscopic retrograde cholangiopancreatography brush increases diagnostic yield of malignant biliary strictures. World J Gastrointest Endosc 2014;6:312-7.
42. Trikudanathan G, Navaneethan U, Njei B. Diagnostic yield of bile duct brushings for cholangiocarcinoma in primary sclerosing cholangitis: a systematic review and meta-analysis. Gastrointest Endosc 2014;79:783-9.
43. Osnes M, Serck-Hansenn A, Kristensen O, et al. Endoscopic retrograde brush cytology in patients with primary and secondary malignancies of the pancreas. Gut 1979;20:279-84.
44. Sawada Y, Gonda H, Hayashida Y. Combined use of brush cytology and endoscopic retrograde pancreatography for the early detection of pancreatic cancer. Acta Cytol 1989;33:870-4.
45. McGuire DE, Venu RP, Brown R, et al. Brush cytology for pancreatic carcinoma: an analysis of factors influencing results. Gastrointest Endosc 1996;44:300-4.
46. Ferrari AP Jr, Lichtenstein DR, Slivka A, et al. Brush cytology during ERCP for the diagnosis of biliary and pancreatic malignancies. Gastrointest Endosc 1994;40:140-5.
47. Lee JG, Schutz SM, Layfield L, et al. A prospective study of endoscopic duct cytology in 34 consecutive patients [abstract]. Gastrointest Endosc 1994;40:AB116.
48. Lehman GA. Sampling methods for bile duct strictures—which is the best? ASGE postgraduate course 1996: integrating visual, technical and cognitive skills in endoscopy. Manchester (MA): American Society for Gastrointestinal Endoscopy; 1996:147-9.
49. Pugliese V, Pujic N, Saccomanno S, et al. Pancreatic intraductal sampling during ERCP in patients with chronic pancreatitis and pancreatic cancer: cytologic studies and k-ras-2 codon 12 molecular analysis in 47 cases. Gastrointest Endosc 2001;54:595-9.
50. Lee JG, Leung JW. Tissue sampling at endoscopic retrograde cholangiopancreatography in suspected pancreatic cancer. Gastrointest Endosc Clin North Am 1998;8:221-35.
51. Nakaizumi A, Tatsuta M, Uehara H. Cytologic examination of pure pancreatic juice in the diagnosis of pancreatic carcinoma: the endoscopic retrograde intraductal catheter aspiration cytologic technique. Cancer 1992;70:2610-4.
52. Nakaizumi A, Tatsuta M, Uehara H. Effectiveness of cytologic examination of pure pancreatic juice in the diagnosis of early neoplasia of the pancreas. Cancer 1995;76:750-7.
53. Rabinowitz M, Zajko AB, Hassanein T. Diagnostic value of brush cytology in the diagnosis of bile duct carcinoma: a study in 65 patients with bile duct strictures. Hepatology 1990;12:747-52.
54. Curcio G, Traina M, Mocciaro F. Intraductal aspiration: a promising new tissue-sampling technique for the diagnosis of suspected malignant biliary strictures. Gastrointest Endosc 2012;75:798-804.
55. Barrioz T, Wangermez M, Levillain P, et al. The Infinity brush improves the results of brush cytology of malignant biliary strictures. Societe Nationale Francaise de Gastro-Enterlogie (SNFGE). 25-4-2013 10:13:01.
56. Parasher VK, Huibregtse K. Endoscopic retrograde wire- guided cytology of malignant biliary strictures using a novel scraping brush. Gastrointest Endosc 1998;48:288-90.
57. Brand B, Thonke F, Obytz S. Stent retriever for dilation of pancreatic and bile strictures. Endoscopy 1999;31:142-5.
58. Vandervoort J, Soetinko RM, Montes H, et al. Use of a new angled forceps to biopsy pancreatic and biliary strictures [abstract]. Gastrointest Endosc 1997;45:AB41.
59. Aabakken L, Kåresen R, Serck-Hanssen A, et al. Transpapillary biopsies and brush cytology from the common bile duct. Endoscopy 1986;18:49-51.
60. Rustgi AK, Kelsey PB, Guelrud M, et al. Malignant tumors of the bile ducts: diagnosis by biopsy during endoscopic cannulation. Gastrointest Endosc 1989;3:248-51.
61. Schoefl R, Haefner M, Wrba F. Forceps biopsy and brush cytology during endoscopic retrograde cholangiopancreatography for the diagnosis of biliary stenoses. Scand J Gastroenterol 1997;32:363-8.
62. Kimura H, Matsubayashi H, Sasaki K. Factors affecting the yield of endoscopic transpapillary bile duct biopsy for the diagnosis of pancreatic head cancer. Pancreatology 2013;13:524-9.
63. Lo DY, Howell DA, Wright E. Frozen section of ERCP forceps biopsy specimens permits efficient intraprocedural diagnosis [abstract]. Gastrointest Endosc 2011;73:AB263.
64. Desa LA, Akosa AB, Lazzara S, et al. Cytodiagnosis in the management of extrahepatic biliary stricture. Gut 1991;32:1188-91.
65. Leung JWC, Sung JY, Chung SCS, et al. Endoscopic scraping biopsy of malignant biliary strictures. Gastrointest Endosc 1989;35:65-6.
66. Lee SJ, Lee YS, Lee MG. Triple-tissue sampling during endoscopic retrograde cholangiopancreatography increases the overall diagnostic sensitivity for cholangiocarcinoma. Gut Liver 2014;8:669-73.
67. Nanda A, Brown JM, Berger SH. Triple modality testing by endoscopic retrograde cholangiopancreatography for the diagnosis of cholangiocarcinoma. Ther Adv Gastroenterol 2015;2:56-65.
68. Barr Fritcher EG, Kipp BR, Halling KC, et al. FISHing for pancreatobiliary tract malignancy in endoscopic brushings enhances the sensitivity of routine cytology. Cytopathology 2014;25:288-301.
69. Bergquist A, Tribukait B, Glaumann H, et al. Can DNA cytometry be used for evaluation of malignancy and premalignancy in bile duct strictures in primary sclerosing cholangitis? J Hepatol 2000;33:873-7.
70. Kipp BR, Stadheim LM, Halling SA. A comparison of routine cytology and fluorescence *in situ* hybridization for the detection of malignant bile duct strictures. Am J Gastroenterol 2004;99:1675-81.
71. Smoczynski M, Jablonska A, Matyskiel A. Routine brush cytology and fluorescence in situ hybridization for assessment of pancreatobiliary strictures. Gastrointest Endosc 2012;75:65-73.
72. Ryan ME, Baldauf MC. Comparison of flow cytometry for DNA content and brush cytology for detection of malignancy in pancreatobiliary strictures. Gastrointest Endosc 1994;40:133-9.
73. Baron TH, Harewood GC, Rumalla A. A prospective comparison of digital image analysis and routine cytology for the identification of malignancy in biliary tract strictures. Clin Gastroenterol Hepatol 2004;2:214-9.
74. Moreno Luna LE, Kipp B, Halling KC. Advanced cytologic techniques for the detection of malignant pancreatobiliary strictures. Gastroenterology 2006;131:1064-72.
75. Levy MJ, Baron TH, Calyton AC. prospective evaluation of advanced molecular markers and imaging techniques in patients with indeterminate bile duct strictures. Am J Gastroenterol 2008;103:1263-73.
76. Farrell RJ, Agarwal B, Brandwein SL. Intraductal US is a useful adjunct to ERCP for distinguishing malignant from benign biliary strictures. Gastrointest Endosc 2002;56:681-7.

77. Vazquez-Sequeiros E, Baron TH, Clain JE. Evaluation of indeterminate bile duct strictures by intraductal US. Gastrointest Endosc 2002; 56:372-9.
78. Stavropoulos S, Larghi A, Verna E. Intraductal ultrasound for the evaluation of patients with biliary strictures and no abdominal mass on computed tomography. Endoscopy 2005;37:715-21.
79. Krishna NB, Saripalli S, Safdar R. Intraductal US in evaluation of biliary strictures without a mass lesion on CT scan or magnetic resonance imaging: significance of focal wall thickening and extrinsic compression at the stricture site. Gastrointest Endosc 2007;66:90-6.
80. Cote GA, Gore AJ, McElyea SD. A pilot study to develop a diagnostic test for pancreatic ductal adenocarcinoma based on differential expression of select miRNA in plasma and bile. Am J Gastroenterol 2014;109:1942-52.
81. Andresen K, Boberg KM, Vedeld HM. Four DNA methylation biomarkers in biliary brush samples accurately identify the presence of cholangiocarcinoma. Hepatology 2015;61:1651-9.
82. Seo DW, Lee SK, Yoo KS. Cholangioscopic findings in bile duct tumors. Gastrointest Endosc 2000;52:630-4.
83. Kim HJ, Kim MH, Lee SK. Tumor vessel: a valuable cholangioscopic clue of malignant biliary stricture. Gastrointest Endosc 2000;52:635-8.
84. Fukuda Y, Tsuyuguchi T, Sakai Y. Diagnostic utility of peroral cholangioscopy for various bile-duct lesions. Gastrointest Endosc 2005; 62:374-82.
85. Nishikawa T, Tsuyuguchi T, Sakai Y. Comparison of the diagnostic accuracy of peroral video cholangioscopic visual findings and cholangioscopy-guided forceps biopsy findings for indeterminate biliary lesions: a prospective study. Gastrointest Endosc 2013;77:219-26.
86. Chen YK, Pleskow DK. SpyGlass single-operator peroral cholangiopancreatoscopy system for the diagnosis and therapy of bile-duct disorders: a clinical feasibility study (with video). Gastrointest Endosc 2007; 65:832-41.
87. Chen YK, Parsi MA, Binmoeller KF. Single-operator cholangioscopy in patients requiring evaluation of bile duct disease or therapy of biliary stones (with videos). Gastrointest Endosc 2011;74:805-14.
88. Draganov PV, Chauhan S, Wagh MS. Diagnostic accuracy of conventional and cholangioscopy-guided sampling of indeterminate biliary lesions at the time of ERCP: a prospective, long-term follow-up study. Gastrointest Endosc 2012;75:347-53.
89. Ramchandani M, Reddy DN, Gupta R. Role of single-operator peroral cholangioscopy in the diagnosis of indeterminate biliary lesions: a single-center, prospective study. Gastrointest Endosc 2011;74:511-9.
90. Siddiqui AA, Mehendiratta V, Jackson W. Identification of cholangiocarcinoma by using the SpyGlass SpyScope system for peroral cholangioscopy and biopsy collection. Clin Gastroenterol Hepatol 2012; 10:466-71.
91. Hartman DJ, Slivka A, Giusto DA. Tissue yield and diagnostic efficacy of fluoroscopic and cholangioscopic techniques to assess indeterminate biliary strictures. Clin Gastroenterol Hepatol 2012;10:1042-6.
92. Navaneethan U, Hasan MK, Lourdusamy V. Single-operator cholangioscopy and targeted biopsies in the diagnosis of indeterminate biliary strictures: a systematic review. Gastrointest Endosc 2015;82: 608-14.
93. Meining A, Frimberger E, Becker V. Detection of cholangiocarcinoma in vivo using miniprobe-based confocal fluorescence microscopy. Clin Gastroenterol Hepatol 2008;6:1057-60.
94. Meining A, Shah RJ, Slivka A. Classification of probe-based confocal laser endomicroscopy findings in pancreaticobiliary strictures. Endoscopy 2012;44:251-7.
95. Meining A, Chen YK, Pleskow D. Direct visualization of indeterminate pancreaticobiliary strictures with probe based confocal laser endomicroscopy: a multicenter experience. Gastrointest Endosc 2011;74: 961-8.
96. Slivka A, Gan I, Jamidar P. Validation of the diagnostic accuracy of probe-based confocal laser endomicroscopy for the characterization of indeterminate biliary strictures: results of a prospective multicenter international study. Gastrointest Endosc 2015;81:282-90.
97. ASGE Standards of Practice Committee; Early DS, Acosta RD, Chandrasekhara V. ASGE guideline: adverse events associated with EUS and EUS with FNA. Gastrointest Endosc 2013;77:839-43.
98. Heimbach JK, Sanchez W, Rosen CB. Trans-peritoneal fine needle aspiration biopsy of hilar cholangiocarcinoma is associated with disease dissemination. HPB 2011;13:356-60.
99. Lee JH, Salem R, Aslanian H. Endoscopic ultrasound and fine-needle aspiration of unexplained bile duct strictures. Am J Gastroenterol 2004;99:1069-73.
100. Rösch T, Hofrichter K, Frimberger E. ERCP or EUS for tissue diagnosis of biliary strictures? A prospective comparative study. Gastrointest Endosc 2004;60:390-6.
101. Fritscher-Ravens A, Broering DC, Knoefel WT. EUS-guided fine-needle aspiration of suspected hilar cholangiocarcinoma in potentially operable patients with negative brush cytology. Am J Gastroenterol 2004;99:45-51.
102. DeWitt J, Misra VL, LeBlanc JK. EUS-guided FNA of proximal biliary strictures after negative ERCP brush cytology results. Gastrointest Endosc 2006;64:325-33.
103. Mohamadnejad M, DeWitt J, Sherman S. Role of EUS for preoperative evaluation of cholangiocarcinoma: a large single-center experience. Gastrointest Endosc 2011;73:71-8.
104. Nguyen NQ, Schoeman MN, Ruszkiewicz A. Clinical utility of EUS before cholangioscopy in the evaluation of difficult biliary strictures. Gastrointest Endosc 2013;78:868-74.
105. Weilert F, Bhat YM, Binmoeller KF. EUS-FNA is superior to ERCP-based tissue sampling in suspected malignant biliary obstruction: results of a prospective, single-blind, comparative study. Gastrointest Endosc 2014;80:97-104.

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