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Review Article

Comprehensive management of cholangiocarcinoma: Part I. Diagnosis

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A B S T R A C T

Cholangiocarcinoma is the second most common primary hepatic malignancy and its incidence is increasing worldwide. Classification and staging of intrahepatic, perihilar, and distal cholangiocarcinomas provide useful prognostic information and further guide in their management. Establishing diagnosis is frequently challenging and may require a multi-modality approach that includes advanced radiological imaging studies and procedures for tissue acquisition; the endoscopic procedures that have been utilized in the management of cholangiocarcinoma comprise endoscopic retrograde cholangiopancreatography with brushing and biopsy, endoscopic ultrasound-guided fine needle aspiration, cholangioscopy with targeted biopsy, and intraductal confocal endomicroscopy. In this review, we will examine the strengths and limitations of each diagnostic tool and assess the serum and bile tumor markers.

Keywords: Cholangiocarcinoma; Cholestasis; Endoscopic ultrasonography; *In situ* hybridization-fluorescence; Positron-emission tomography

Introduction

Cholangiocarcinoma (CC) is a rare malignancy of the biliary tree, with often insidious presentation, elusive diagnosis, limited therapeutic armamentarium, and dismal prognosis. It is the second in incident hepatic cancer after hepatocellular carcinoma (HCC) and accounts for approximately 3% of all gastrointestinal and 10% to 25% of all hepatobiliary cancers.[1,2] CC is categorized anatomically into intrahepatic (ICC), perihilar (PCC), and distal (DCC). The ICC extends proximally up to the second order bile ducts (right anterior, right posterior, left medial, and left lateral hepatic ducts), the PCC extends between the cystic duct and the first order ducts (common hepatic, right and left hepatic ducts) and the DCC extends between the cystic duct and the ampulla of Vater.[3] This classification, though strongly encouraged by many authors, is subject to variability due to the variable origin of the cystic duct.[4] Also, it is difficult to classify tumors that extend from the ampulla of Vater to the perihilar region as either PCC or DCC. The most prevalent type of CC is PCC (50%) with ICC being the least common (less than 10% of the cases). Bi-phenotyic HCC-CC has been recently acknowledged as a distinct type representing around 1% of all hepatic malignancies, but this entity will be outside the topics of this review.[5] The etiology of CC is unclear, with multiple factors being implicated in its development, such as liver fluke infestation (common in southeast Asia), primary sclerosing cholangitis (PSC), hepatolithiasis, Caroli's disease, choledochal cysts, exposure to Thorotrast, cirrhosis, hepatitis B and C infections, inflammatory bowel disease, choledocholithiasis, cholangitis, obesity, diabetes mellitus, alcohol, smoking, and various genetic polymorphisms among others.[2,3,6] The incidence of CC is rising globally and is particularly high in Northeastern Thailand (85 cases per 100,000 people), with up to 97% of cases being PCC.[7] Conversely, the incidence is about 10 times lower in China, Japan, and Korea (even lower in Europe and North America).[8] Age-specific mortality rate of ICC has been universally rising with the notable exception of Denmark.[9,10] Although the classification of CC to ICC and extrahepatic CC (ECC) is no longer recommended, multiple studies in the literature use this nomenclature.[11] A recent study encompassing 75% of the United States population from 33 States, calculated the overall incidence of ICC and ECC at 0.88

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Table 1 Incidence Rates of Intrahepatic and Extrahepatic Cholangiocarcinoma in the United States[12]

Race	Intrahepatic cholangiocarcinoma incidence*	Extrahepatic cholangiocarcinoma incidence
Asians/Pacific islanders	1.28	1.10
Hispanics	1.16	0.92
American Indians/Alaskan Natives	0.98	0.72
Whites	0.84	0.69
Blacks	0.83	0.64

*Per 100,000 people.

and 0.72 per 100,000 people respectively. The highest incidence was observed in Asians/Pacific Islanders and the lowest in blacks (Table 1).[12]

However, in the United States, higher ICC and ECC incidence rates were observed in the northeast, upper Midwest, Southwest, Hawaii, and Alaska. Both types were more common in people older than 75 years with almost equal incidence in men and women (1.5 to 1).[12] In this comprehensive review we will discuss current CC classification staging schemes, diagnostic modalities, and management options for resectable and unresectable disease.

Classification and Staging

Intrahepatic cholangiocarcinoma

The Liver Cancer Study Group of Japan[13] in 1997 suggested the macroscopic classification of ICC based on the tumor manifestation as mass forming (MF), periductal infiltrating (PI), or intraductal growth (IG). The MF type is the most common subcategory of ICC.[14] In its early stage, ICC does not invade the portal triad. The PI type does not invade the bile duct but extends parallel to it and causes stenosis and dilation of the proximal ducts, whereas the IG type extends into the duct and may radiographically present as thickened duct wall. An unclassified form of ICC has also been described.[13,15] The IG type signifies an earlier stage of ICC with better prognosis, whereas the mixed MF-PI type is considered more advanced and therefore associated with worse outcomes.[16,17] Lymph node (LN) metastases ranges between 50% and 73% in the mixed MF-PI type and are subsequently associated with low 5-year survival (0%–19%).[18] The study by Aishima and Oda[19] suggested a classification of ICC into large duct (second order ducts) tumors and small duct tumors corresponding to perihilar type and peripheral type respectively, with more perineural and intraductal involvement in the former type. ICC demonstrates usually well to moderate differentiation with desmoplastic elements. The peripheral type is thought to arise on the ground of cirrhosis and chronic hepatitis, possibly stemming from the canals of Hering and the interlobular ducts. In contrast, history of PSC, hepatolithiasis and choledochal cysts seem to favor the development of perihilar, large-duct ICC.[19] More recently, a new and more detailed classification based on gross and histological features was proposed. This classified the ICC into conventional (small bile duct-peripheral and large-perihilar types with well, moderate and poor differentiation), bile ductular, intraductal types, and rare variants.[20] Two groups from Japan have proposed staging systems for ICC. The study by Okabayashi et al[21] utilized 14 clinical and 12 postoperative factors without including tumor size in the staging. The Liver Cancer Study Group of Japan in 2003 published guidelines for ICC staging following a different staging approach.[15] This staging system considered the differentiation between regional and distant LN involvement as a prognostic key factor, also including the size of the tumor (≤ 2 cm), portal, hepatic vein and serous membrane invasion as important elements of prognosis.[15] In 2010, the 7th edition of the American Joint Committee on Cancer (AJCC 7th) issued a new staging system for ICC based on data from the Surveillance, Epidemiology and End Results (SEER) program on almost 600 patients, who had undergone surgical resection.[14,22] Analysis included 15-year resection data out of 12 tertiary hepatobiliary centers with 168 R0 (no residual tumor) patients. Analysis of data demonstrated that N0M0 patients had 1-year and 5-year median survival rate of almost 90% and 44% respectively. The patients with stage I disease (T1N0M0) had 5-year survival rate of 64%. All patients with stage III and IV (T3N1M0 or any T with N1, and M1) died within 18 months after resection. The AJCC 7th does not incorporate tumor size in the staging, but focuses on multiplicity of tumors, vascular invasion, and LN involvement. However, some limitations in the study raised concerns. Although the T2b stage includes multiple tumors with or without vascular invasion, it remains difficult to differentiate among patients with multifocal tumors or a single tumor with intrahepatic metastases.[11] Also, that study excluded almost 70% of the original cohort who were R1 (microscopic residual tumor) or R2 (macroscopic residual tumor) after resection. Additionally, LN resection for ICC was not performed consistently in the SEER cohort, therefore only 17% of M0 patients were positive for LN involvement and the rest were considered N0 instead of Nx. Given the reported incidence of LN involvement from 35% to 45%, the AJCC 7th staging system may have understaged the disease in some of the subjects.[23]

Perihilar cholangiocarcinoma

PCC most commonly manifests as a tumor with PI (also known as sclerosing or scirrhous, > 70% of the cases) or nodular characteristics (20%), or IG tumor. Most of the PCC are adenocarcinomas and often demonstrate lymphatic spread and perineural invasion.[4,24] The intraductal papillary tumors are usually well differentiated and demonstrate favorable prognosis.[3] Proper staging is paramount to determine the appropriate surgical approach. The Bismouth-Corlette (BC) classification is commonly used to describe the location and extend of PCC: 1) Type I: below the confluence of the hepatic ducts within 2 cm of the hilum, 2) Type II: at the confluence of the hepatic ducts, 3) Types IIIa and IIIb: involving the right and left hepatic ducts respectively and obstructing the common hepatic duct, and 4) Type IV: multi-centric or bilateral intrahepatic involvement or involvement of both hepatic ducts. The BC system does not provide satisfactory staging information because neither accounts for vascular involvement, lobar atrophy and distant metastases, nor correlates with survival.[25,26] To address the issue of proper staging and unresectability for PCC, the group from Memorial Sloan Kettering Cancer Center (MSKCC) proposed the T staging system based on data from 87 patients.

The system did not account for LN involvement or distant metastases. The median survival of patients with T1 stage was 21 ± 3 months, compared to 10 ± 2 months for T3. Twenty percent of patients with T1 stage survived at 5 years after resection compared to 16% with stage T3 and 0% with stage T4 disease.[27] The TNM staging system for PCC has been proposed by the AJCC 7th, which includes tumor invasion of the vasculature, regional or distant LN metastases, and residual tumor after resection. However, it has been criticized that it failed to indicate the local tumor resectability. A new system has been proposed recently that was designed to assist with more accurate pre- or postoperative staging of the PCC.[28] This staging system, besides ductal involvement, incorporates more parameters in PCC staging: 1) tumor size (< 1 to > 3 cm in size), 2) tumor type (sclerosing, MF, mixed and polypoid or IG), 3) involvement of portal vein (PV) or hepatic artery (HA), 4) involvement of regional and distant LN, 5) remnant liver volume after resection, and 6) underlying liver disease such as fibrosis and PSC. More recently, the BC, MSKCC and TNM systems were compared in patients with PCC and it was found that TNM and MSKCC systems, tumor differentiation, LN status, distant metastases and R status were significantly correlated with survival, whereas BC classification, age, gender, jaundice, and upper abdominal pain were not. The authors proposed a modified staging system for PCC based on the MSKCC system, and used scores to predict survival: 1) score 0, stage I (MSKCC T1, high/moderate differentiation without distant metastases or R0), 2) score 1, stage II (MSKCC T2, poor differentiation or R1), and 3) score 2–6, stage III (MSKCC T3, TNM IV or R2). The survival was >3, 1-3, and ≤1 years in stages I, II, and III respectively. The accuracy of this system for predicting survival rate was almost 70%.[29]

Distal cholangiocarcinoma

DCC involves the area between the cystic duct (variable location) and the ampulla of Vater (without inclusion of the ampulla). Due to its anatomic location, it is difficult to differentiate from pancreatic carcinoma.[30,31] DCC are well to moderately differentiated adenocarcinomas and are classified into intraductal papillary and biliary intraepithelial neoplasms. MF tumors are uncommon[4,32] The DCC has similar clinical presentation to the PCC, with painless jaundice, pruritus, weight loss, decreased appetite, and abdominal pain, but less incidence of fever.[33] Contrary to the other two types of CC, there is only one widely accepted staging system for DCC. The AJCC 7th staging system incorporates features, such as depth of tumor invasion into and beyond the bile duct wall and into adjacent organs, presence of celiac axis or superior mesenteric artery involvement, and regional LN metastases. The median survival of patients with DCC after resection was 18 months, with 5-year survival rate of 23% in one series of 239 patients. Positive LN is more common in DCC than ICC or PCC (60% compared to 29% and 28% respectively, considering though that LN sampling in ICC is not a consistent practice).[34] Tumor depth < 5 mm is associated with significantly better survival compared to depths of 5 to 12 mm (survival of 29 months) and > 12 mm (13 months). Perineural and vascular invasion are other important prognostic factors.[35] Tumor size < 2 cm, high degree of tumor differentiation, R0 resection and negative LN involvement have been shown as markers of favorable survival. The study of 56 patients with equally distributed well-, moderate and poorly differentiated DCC demonstrated 5-year survival rate of 43% (median, 24.5 months). The statistically significant favorable parameters were higher degree of differentiation, a more favorable primary tumor factor by TNM staging, absence of LN metastases and R0 surgical margins.[36]

Diagnosis of Cholangiocarcinoma

Radiological diagnosis

Imaging, along with pathology, has always been the cornerstone of CC diagnosis, and owing to rapid technological developments its utilization continues to expand. The cheapest and most accessible imaging modality for diagnosis of CC is transabdominal ultrasound (US).[37] Although the utility of US in the diagnosis of DCC is limited, it is helpful in ruling out common bile duct stones. With the addition of color Doppler, US can identify PV, hepatic vein and HA invasion with good sensitivity and specificity.[38,39] For detection of CC in patients with PSC, US has good specificity and negative predictive value (90%) but low sensitivity and positive predictive value.[26]

Magnetic resonance imaging (MRI) with magnetic resonance cholangiopancreatography (MRCP) after 3 to 6 hours of fasting, is probably the best modality to image the biliary tree, identify MF tumors, ductal or vascular invasion, and strictures. It is also preferred by many because it does not require iodinated contrast and does not carry the radiation exposure risk of computed tomography (CT). MRCP can accurately stage CC (81%–96%), with tendency to underestimate ductal involvement.[40] Common imaging characteristics of ICC and PCC are shown in Table 2.[41–45]

MRI/MRCP is also useful in the diagnosis of DCC. These tumors may present as strictures or polypoid masses and demonstrate low intensity signaling in T1-weighted fat-suppressed images.[46,47] The distal duct tumor usually present with obstruction and may be difficult to distinguish from adjacent pancreatic mass. However, presence of a mass in the distal common bile duct that does not invade the bile duct or does not cause thickening of the duct wall strongly favors pancreatic cancer.[48] In cases of MRI suboptimal special resolution or motion artifacts, multi-detector CT (MDCT) has a negative predictive value between 85% and 100% and may be useful as a secondary imaging modality. CT should include arterial and portal venous phase and should include the pelvis and the thorax if tumor staging is warranted.[44] Prior metal or plastic stents may reduce the MRI and CT quality. Thus, it is recommended to image the liver sufficiently prior to stenting.

The study of 32 patients with PCC showed that high-resolution CT has high accuracy in predicting unresectability with sensitivity of 94% and specificity of 79%. Radiologic factors associated with unresectability were bilateral HA or main PV involvement, distant metastases, liver metastases, lobar atrophy and portal lymphadenopathy.[49] MDCT reaches the optimal sensitivity and accuracy when a delayed (> 6 minutes) phase is applied. The accuracy of MDCT with direct cholangiography is equal to that of MRI-MRCP.[50] MDCT accuracy in PCC was also depicted to be 86% for PV and 93% for HA invasion, and 84% for extent of ductal invasion and LN metastases. The overall accuracy of multiphasic CT in combination with percutaneous cholangiography (PTC) was about 75%.[51]

ICC is a maligancy that demonstrates high uptake of ^{18}F-fluorodeoxyglucose (^{18}F-FDG). Positron emission tomography (PET)-CT is a whole-body imaging modality that is seeking to establish a more definitive role in the staging of CC. So far, use of PET and PET-CT does not have pivotal role in diagnosing CC, but may be a useful adjunct to the more established CT and MRI-MRCP.[52] PET-CT has been shown to change the management of CC in 24% of the cases in one study, mainly by avoiding operation in patients deemed to be resectable by CT or MRI.[53] It is subjected; however,

Table 2 Imaging Characteristics of ICC and PCC[41–45]

	Ultrasound	MDCT-MRI/MRCP
ICC	Lack of hypoechoic halo (differentiation from HCC) Tumors < 3 cm tend to be hypoechoic and > 3 cm hyperechoic Hyper-enhancement (rim-like) or hypo-enhancement in the arterial phase Hypo-enhancement or rarely iso-enhancement in the portal phase Hypo-enhancement in the delayed phase Peripheral duct dilation	Peripheral arterial enhancement Progressive central enhancement Diffusion restriction Capsular retraction Hypo or iso-intense on T1-weighted imaging Variably hyper-intense in T2 (scirrhous has lower intensity compared to well differentiated ICC) Lack of fibrous capsule Typically hypo or iso dense in non-contrast CT Hypo-dense in the arterial and portal phases (the periphery of the tumor enhances rapidly with contrast infusion and becomes hypodense in portal phase) Hyper-dense in the delayed phase
PCC (PI)	Segmental dilation and non-union of the right and left ducts Dilated intrahepatic but normal extrahepatic duct Isoechoic irregular duct wall thickening Tumor "shouldering"	Long strictures with thick wall, with arterial or portal phase enhancement Fat infiltration LN involvement Tumor "shouldering"
PCC (MF)	Hypoechoic rim Hypoechoic or hyperechoic tumors	Rim enhancement Central enhancement in delayed phase Portal vein thrombi Iso- or hypointense on T2-weighted images
PCC (IG)	Polypoid mass without acoustic shadow Smooth duct wall	Presence or absence of intraductal masses with focal of diffuse biliary dilation Cast-like appearance

ICC, intrahepatic cholangiocarcinoma; PCC, perihilar cholangiocarcinoma; MDCT, multi-detector computed tomography; MRI, magnetic resonance imaging; MRCP, magnetic retrograde cholangiopancreatography; HCC, hepatocellular carcinoma; CT, computed tomography; PI, periductal infiltrating; LN, lymph node; MF, mass forming; IG, Intraductal growth.

to false positive and negative results and low detection rates of small tumors.[54–56] The sensitivity of CT, MRI-MRCP and PET-CT, as demonstrated in the study of 123 potentially resectable ICC patients, was 95%, 100%, and 95% respectively. The high sensitivity of these modalities was also demonstrated in the diagnosis of ECC (89% for CT, 95% for MRI-MRCP, and 81% for PET-CT). The specificity of these modalities for ICC was 100% for CT, 87% for MRI, and 80% for PET-CT whereas for ECC the specificity was lower at 57%, 79%, and 79%, respectively. ^{18}F-FDG PET-CT provides a useful alternative to CT and MRI for detection of metastases in the LN. The overall sensitivity and specificity of PET-CT has been shown to be 84% and 79%, respectively. PET-CT has 76% accuracy in detecting regional LN metastases compared to 61% of the CT, and 88% accuracy in detecting distant metastases compared to 79% of the CT.[57] The meta-analysis of 23 studies and over 1,200 patients assessed the accuracy of ^{18}F-FDG-PET and PET-CT in the diagnosis of CC. It demonstrated that ^{18}F-FDG-PET and PET-CT had a pooled sensitivity and specificity of 81% and 82% respectively. The sensitivity and specificity of these modalities for diagnosis of ICC, hilar CC and ECC were 95%/83%, 84%/95%, and 76%/74%, respectively. However, the lack of uniform anatomic nomenclature of CC in the analyzed studies may have confounded the results.[58] The accuracy, sensitivity and specificity of US, CT, MRI, and PET were assessed in the meta-analysis of 448 patients with PCC. CT demonstrated pooled accuracy for the detection of ductal invasion of 86%, a sensitivity and specificity of 89% and 92% in detecting PV invasion, 84% and 93% for HA invasion, and 61% and 88% for nodal involvement. The number of studies, however, was insufficient to appropriately compare all imaging modalities.[59] CT and MRI have been studied in distinguishing between HCC and ICC in patients with cirrhosis.[60,61] These modalities have also been studied retrospectively in differentiating intraductal papillary neoplasms from CC with intraductal invasion and were found to be accurate.[62]

A common diagnostic problem is the proper differentiation of malignant from benign strictures. In the recent retrospective study of 51 patients with malignant and benign biliary strictures, the use of three-dimensional dynamic contrast-enhanced MRI with MRCP was shown to provide effective differentiation of these strictures. Increased thickness of the stricture and hyper-enhancement in portal venous and equilibrium phases were significantly associated with malignancy. Similar stricture characteristics can be found with the use of multiphasic helical CT.[63,64] MRCP was compared to endoscopic retrograde cholangiopancreatography (ERCP) for distinguishing between benign and malignant extrahepatic biliary structures and was found that MRCP has similar sensitivity, specificity and accuracy compared to ERCP (81%, 70%, 76% and 74%, 70%, 72%, respectively).[65] Dual-time point PET-CT has been shown to be useful in differentiating malignant from benign extrahepatic disease.[66]

Endoscopic ultrasound

Endoscopic ultrasound (EUS) is an invasive procedure with an important role in the diagnosis of CC, particularly in the setting of other non-diagnostic, non-invasive tests (MRI/MRCP/CT).[67] The meta-analysis of EUS-fine needle aspiration (FNA) for the diagnosis of CC in almost 200 patients demonstrated overall pooled sensitivity and specificity of 66% and 100%, respectively. For those studies that had negative brush cytology, the sensitivity and specificity of EUS-FNA were 59% and 100%, respectively.[68] The retrospective study of 81 patients with PCC and DCC demonstrated the superiority of EUS in diagnosing CC compared to MRI or multiphase CT (tumor detected in 94% of the cases with EUS vs 42% and 30% with MRI and CT, respectively). The sensitivity of EUS was shown to be significantly higher in distal than proximal CC (81% and 59%, respectively).[69] Furthermore, the more recent study of EUS-FNA and fine needle biopsy in 39 patients with sus-

pected perihilar malignant strictures demonstrated EUS sensitivity of 79% and accuracy of 82%.[70] Other older studies that mainly investigated distal strictures demonstrated lower EUS-FNA sensitivity for malignancy (25% and 47%).[71,72] The combination of EUS with MRCP has been shown to yield higher sensitivity and specificity (90% and 98%, respectively) compared to MRCP alone (80% and 90%, respectively).[73]

It is suggested that EUS should not be used without FNA for the diagnosis of malignant LN, since it has been shown that LN metastasis in CC do not follow the typical imaging prognostic features of malignancy and EUS without FNA is associated with low sensitivity for LN metastases. EUS-FNA may detect malignant LN despite negative CT or MRI and alter surgical management and potential for liver transplantation (LT).[69,74] We could not find reports on ICC tumor seeding after percutaneous biopsy. However, data from the meta-analysis have reported the risk for peritoneal seeding after percutaneous biopsy of HCC to be 2.7%.[75] EUS- or percutaneous FNA of PCC is associated with significant risk of tumor seeding (83% vs 8% in patients without FNA) and therefore should be avoided in cases of potentially curative resection or LT. The risk of seeding seems to be higher in transperitoneal FNA than after PTC or FNA of HCC. The reason for this may be related to the distance of perihilar tumors from the abdominal wall, which allows more space for seeding during biopsy.[76] A recent prospective study compared EUS-FNA and ERCP with biopsy and demonstrated that both modalities have a similar sensitivity and accuracy in evaluating proximal or distal malignant strictures (79% and 80%) and sensitivity (80% and 67%, respectively) for proximal and distal indeterminate strictures.[77]

ERCP, PTC, biopsy and brush cytology

ERCP is a commonly performed invasive procedure for the evaluation of malignant biliary strictures and diagnosis of PCC or DCC. It has the diagnostic advantage over non-invasive modalities such as MRCP by providing an opportunity for tissue acquisition. PTC is also performed; however, as second line diagnostic intervention, in cases when ERCP is unsuccessful or when there is need for imaging the biliary tree proximal to the stricture, or in cases where endoscopic biliary decompression is not feasible. There is no clear evidence that PTC should be favored over ERCP based on the level of biliary obstruction.[24,26] ERCP has been the mainstay in PCC or DCC where a dominant stricture, a polypoid intraductal mass or significant dilation of the proximal duct may be seen.[31] In differentiating benign from malignant biliary strictures, ERCP has been shown to have similar sensitivity, specificity and accuracy to MRCP (74% vs 70%, 76% vs 74%, and 70% vs 73%).[65]

Due to the highly desmoplastic nature of CC resulting in the presence of fibrous tissue and the relatively small number of available malignant cells, endoscopic sampling is subject to errors and false negative results.[78] Multiple studies have assessed the diagnostic yield of bile duct brushings during ERCP, but in general, despite the excellent specificity (close to 100%), the sensitivity has been disappointing and ranges widely between 18% (in the study of 131 subjects) and 80% (in 86 subjects).[79,80] Overall, the sensitivity of brush cytology as a single modality of diagnosis is around 55% based on published data.[81,82] Similar findings were reported in the meta-analysis of brush cytology. In the study with 747 patients with PSC-related strictures, it demonstrated sensitivity and specificity of 43% and 97%, respectively.[83] Transpapillary biopsies during ERCP have specificity that approaches 100% and have a better sensitivity than that of brush cytology. However, they are more operator dependent and technically challenging. When biopsies are used as a single modality, the sensitivity ranges between 30% (study of 30 subjects) and 100% (study of 22 subjects).[84,85] The overall sensitivity and specificity for transpapillary biopsy as a single approach is around 65%.[81,83] The unacceptable sensitivity of brush cytology and the relatively low sensitivity of biopsy as a single approach to the diagnosis of CC have led many physicians to use a combination of these two techniques. With the dual approach, the sensitivity improves ranging between 47% and 86%.[84,86] To improve the yield of cytology and biopsy, fluorescence in situ hybridization (FISH) has been added to the diagnostic armamentarium.

FISH

FISH is a cytogenetic technique that uses fluorescent DNA probes to assess for specific DNA sequences under a fluorescent microscope. The combination of polysomy, the most common chromosomal amplification on FISH, and presence of a dominant biliary stricture are considered to be diagnostic criteria for CC.[4] The sensitivity of FISH is considered to be higher than that of cytology. The sequential approach with brush cytology and FISH in the recent study of 36 patients with biliary strictures due to CC demonstrated that the sensitivity of FISH was 80% compared to only 47% of cytology.[87] The recent meta-analysis in 828 patients with PSC yielded pooled sensitivity of 68% and specificity of 70% for CC detection.[88] To increase the sensitivity even further, the first study of tri-modal approach was recently published. Sixty-one patients who underwent brush cytology alone were compared with 50 patients who were managed with brushings, biopsy, and FISH. The sensitivity of brushings alone was 42% compared with 82% of the triple approach. The sensitivity of FISH alone in this cohort was 59%. Specificity was 100% in all groups.[82]

Per-oral cholangioscopy

Per-oral cholangioscopy (POCS) enables biopsies of the target tissue under direct visualization and aims to facilitate the diagnosis of biliary strictures. Dilated and tortuous vessels in the wall of the stenotic bile duct and intraductal nodules or masses are indicative of malignancy. However, lesions that do not appear in the mucosa or are just causing extrinsic compression cannot be visualized by POCS.[89,90] POCS shows sampling sensitivity of 82% to 97%.[91] A prospective study of 26 patients with indeterminate biliary strictures compared the usual tissue acquisition techniques of cytology brushings and standard forcep biopsies with POCS-guided mini forcep biopsies and demonstrated a significantly better sensitivity of the latter compared to the other two modalities (76.5% compared to 6% for brushing and 29% for standard biopsies). Overall, the diagnostic accuracy, sensitivity and specificity of POCS-guided biopsies has been shown to be 72%–85%, 49%–82%, and almost 100%, respectively.[92–94] POCS can increase the yield of ERCP in the assessment of the longitudinal extension of PCC and DCC. The prospective study of 43 patients with CC showed that POCS had accuracy of 82% and 92% for diagnosing longitudinal extension of CC on the hepatic side (to the second order bile ducts) and papillary side (intrapancreatic common bile duct) respectively. POCS improved the overall accuracy of ERCP in assessing longitudinal extension of tumor by 20% on the hepatic side and 12% on the papillary side.[95] To enhance the diagnostic yield of POCS, chromo-cholangioscopy with methylene blue staining of the duct wall and POCS with narrow band imaging have been reported. It is known that CC cells do not stain or

stain minimally for methylene blue.[96] Intraductal administration of 0.1% methyene blue reveals dark blue areas associated with inflammation and dysplasia. However, there is paucity of data on this approach and therefore no clinically applicable conclusions can be reached.[97] The use of narrow band imaging has been shown to increase the quality of visualization of the majority of intraductal lesions compared to those seen with white light.[98] It is of paramount importance to use carbon dioxide instead of air with minimal water irrigation when performing POCS.

Intraductal ultrasound

Intraductal US (IDUS) is used in order to increase the sensitivity of intraductal biopsies during ERCP. It has higher resolution than EUS and can be used to image the proximal biliary system.[99] The prospective study of 62 patients showed that IDUS followed by biopsy has sensitivity of 92% for intraductal sessile lesions, 80% for polypoid lesions, 53% for sessile tumors outside of the duct, and 50% for localized ductal wall thickening. Tumors larger than 1 cm, sessile and interrupted wall structures are considered to be independent variables of malignancy.[100] IDUS has been studied in the assessment of the longitudinal extent of CC towards the hepatic or papillary side. The sensitivity, specificity, and accuracy of IDUS were 82%, 70%, 78% for the hepatic and 85%, 43%, 70% for the papillary side respectively. There was improvement in these statistical parameters when IDUS was combined with transpapillary biopsy on the hepatic and papillary side (88%, 80%, 85% and 77%, 86%, 80%, respectively).[101] In another study of 60 patients (17 with biliary cancers), the accuracy was increased from 83% with the use of IDUS alone to 98% when IDUS was combined with transpapillary biopsy.[102] According to Tamada et al,[81] the accuracy of IDUS through the transpapillary or percutaneous route for the assessment of longitudinal extension of malignancy has been reported in the literature to be between 68% and 92%. The presence of stents in the bile ducts alters the imaging characteristics of IDUS and in such patients IDUS use has not been advocated by some authors. Additionally, EUS has the advantage over IDUS of assessing LN and performing FNA for cytology.[7]

Probe-based confocal endomicroscopy

Probe-based confocal laser endomicroscopy (pCLE) is a new technique that provides *in vivo* real time images of the tissue under examination. Its utility in the bile ducts was assessed in the prospective multicenter study of 112 patients with biliary strictures. By using the Paris classification for biliary strictures, it was demonstrated that ERCP with cytology, biopsy, and pCLE had overall accuracy of 88%, sensitivity of 89%, and specificity of 88% in detecting CC. ERCP with cytology and biopsies but without pCLE showed accuracy of 79%, sensitivity of 85%, and specificity of 69%. About a half of these patients had undergone mean of 2.5 ERCPs prior to the study enrollment. Therefore, pCLE may decrease the number of ERCP needed for diagnosis.[103] Another pCLE study that used the older Miami classification showed accuracy of 81%, sensitivity 98%, and specificity 67% compared to accuracy 75%, sensitivity 45%, and specificity 100% of histopathology.[104] The Paris classification was recently validated for the characterization of indeterminate biliary strictures with increase in specificity, no impact in accuracy and fair interobserver variation (Table 3).[103–107]

pCLE was also studied in 15 PSC patients with dominant strictures, some of whom had biliary stents in place, and demonstrated a sufficient stricture visualization of 95%, sensitivity 100%, and specificity 61% in detecting neoplasia.[108]

Serum and bile tumor markers

Serum and bile tumor markers are not specific for any type of CC. Carbohydrate antigen 19-9 (CA 19-9) is a tumor marker with utilization in CC, pancreatic cancer, and other gastrointestinal malignancies. However, despite its widespread use, it is hindered by a poor sensitivity and specificity. An overall sensitivity of 40% to 70% and specificity of 50% to 80% have been reported, based on different cut-off values.[26,109,110] In patients with PSC, the CA 19-9 level of 129 U/mL is specific for ICC (almost 100%), but not sensitive (13%–79%). However, other studies found that 32%–37% of patients with CA 19-9 level above 129 U/mL did not have CC after long term follow up. In many circumstances, the reason of elevated CA 19-9 remains obscure.[111,112] CA 19-9 cut-off values of 129 U/mL or higher are associated with diagnosis of CC at a more advanced stage, compared to a cut-off of 20 U/mL.[113,114] Despite its poor sensitivity for CC diagnosis, CA 19-9 may have a utility as a preoperative marker in patients with PCC. According to the recent study of 168 patients with PCC who underwent radical resection, preoperative CA 19-9 levels of < 150 U/mL were associated with doubled survival postoperatively (44 months vs 22 months for CA 19-9 > 150 U/mL).[115] In order to improve the discrimination power of serum markers for the diagnosis of CC, the group from Thailand used a computational model to study 8 tumor markers in 85 patients with CC and 82 controls. No single

Table 3 Miami and Paris Classification for Biliary Strictures by Probe Based Confocal Endomicroscopy[103–107]

Type of stricture	Miami	Paris
Benign stricture	Thin, dark-branching bands (< 20 μm) Thin white bands Light grey background Vessels < 20 μm	Thin, dark-branching bands (< 20 μm) Thin white bands Light grey background Vessels < 20 μm
Inflammatory stricture		Vascular congestion Thickened reticular structure Increased inter-glandular space Multiple white bands Dark granular pattern
Malignant stricture	Thick, dark bands (> 40 μm) Thick, white bands (> 20 μm) Villi, glands Fluorescein leak Dark clumps	Thick, dark bands (> 40 μm) Thick, white bands (> 20 μm) Villi, glands Fluorescein leak Dark clumps

marker reached more than 90% sensitivity and specificity. Nevertheless, CC-associated CA (CCA-CA), a newly discovered marker, reached sensitivity, specificity and accuracy of 91%, 84%, and 87%, respectively. The commonly used markers CA 19-9 and carcinoembryonic antigen (CEA) achieved much lower sensitivity and accuracy (66%/77% for CA 19-9 and 74%/60% for CEA, respectively). The combined marker of alkaline phosphatase and CCA-CA reached a sensitivity of 96%, specificity of 95%, and accuracy of 95%.[116] A novel marker, serum mucin 5AC (MUC5AC), that is found in epithelial cancers, was studied in 88 patients. It was shown that the serum level of MUC5AC was higher in biliary malignancies compared to benign biliary disease. A MUC5AC cut-off value ≥ 14 ng/mL was associated with LN metastasis and stage IVb disease. In postoperative patients, serum levels of MUC5AC ≥ 14 ng/mL were associated with worse prognosis compared to lower levels (3 year survival rate about 21% vs 59%).[117] Another marker, serum matrix metaloproteinase (MMP)-7, was shown to be significantly elevated in patients with CC. The study of 187 patients with obstructive jaundice compared the MMP-7 (cut-off value 5.5 ng/mL) with CA 19-9 (cut-off value 100 U/mL) and found that MMP-7 was more accurate and more sensitive in the diagnosis of CC (sensitivity/specificity 75%/78% for MMP-7 and 68%/87% for CA 19-9).[118] The overall sensitivity and specificity of MMP-7 across different cut-off values are estimated to be between 53%–76% and 47%–92%, respectively. Multiple other markers have been reported but will require further study before their potential application in clinical practice.[119]

Bile biomarkers found in the supernatant of bile have shown promise in the diagnosis of malignant biliary strictures. Although it is still early to reach practical conclusions, two bile markers have shown promise. The neutrophil gelatinase-associated lipocalin was studied in patients with biliary strictures and demonstrated sensitivity/specificity of 94%/55% and 73%/72% with cut-off values of 570 ng/mL and 459 ng/mL, respectively.[120,121] The biomarker carcinoembryonic cell adhesion molecule 6 seems also promising. It delivered a 93% sensitivity and 83% specificity in the diagnosis of malignant strictures with cut-off value of 69.7 ng/mL.[122,123]

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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