Primary Sclerosing Cholangitis as a Premalignant Biliary Tract Disease: Surveillance and Management

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Primary sclerosing cholangitis (PSC) is a premalignant biliary tract disease that confers a significant risk for the development of cholangiocarcinoma (CCA). The chronic biliary tract inflammation of PSC promotes pro-oncogenic processes such as cellular proliferation, induction of DNA damage, alterations of the extracellular matrix, and cholestasis. The diagnosis of malignancy in PSC can be challenging because inflammation-related changes in PSC may produce dominant biliary tract strictures mimicking CCA. Biomarkers such as detection of methylated genes in biliary specimens represent noninvasive techniques that may discriminate malignant biliary ductal changes from PSC strictures. However, conventional cytology and advanced cytologic techniques such as fluorescence in situ hybridization for polysomy remain the practice standard for diagnosing CCA in PSC. Curative treatment options of malignancy arising in PSC are limited. For a subset of patients selected by using stringent criteria, liver transplantation after neoadjuvant chemoradiation is a potential curative therapy. However, most patients have advanced malignancy at the time of diagnosis. Advances directed at identifying high-risk patients, early cancer detection, and development of chemopreventive strategies will be essential to better manage the cancer risk in this premalignant disease. A better understanding of dysplasia definition and especially its natural history is also needed in this disease. Herein, we review recent developments in our understanding of the risk factors, pathogenic mechanisms of PSC associated with CCA, as well as advances in early detection and therapies.

Keywords: Biliary Dysplasia; Chemoprevention; Interleukin-6; Perihilar Cholangiocarcinoma.

Chronic inflammation and cholestasis in primary sclerosing cholangitis (PSC) promote carcinogenesis of the biliary tract by fostering pro-survival signaling pathways and development of genetic aberrations. Inflammatory pathways are not only strongly associated with carcinogenesis but are often retained by cholangiocarcinoma (CCA) cells to facilitate tumor invasion and migration, an observation that is not surprising because inflammation is critical in tissue remodeling and cancer mimics dysregulated tissue remodeling. The risk of CCA among patients with PSC is increased 400-fold when compared with the general population. CCA remains one of the leading causes of liver-related mortality in this population. Perihilar cholangiocarcinoma (pCCA) is the CCA subtype most often seen in the context of PSC. Although there have been reports of a dysplasia-carcinoma sequence in PSC, the characteristics of premalignant lesions and prevalence of biliary dysplasia in PSC are incompletely understood. These facts result in several different questions for the clinician caring for the PSC patient. Is my PSC patient at risk for developing CCA, and if so, what is the surveillance strategy? If I detect dysplasia, what are the therapeutic strategies? If my patient has CCA, what are the treatment options? We review recent advances addressing these pertinent and difficult questions. We also provide our perspectives reflecting on these advances and our vision for research-driven advances to answer these questions. In addition to CCA, patients with PSC remain at risk for other malignancies (Figure 1). PSC associated malignancies have been published elsewhere and will not be discussed in this review.

Epidemiology and Risk Factors

Population-based studies suggest that the annual risk for CCA is approximately 2%, with 10-year and 30-year cumulative incidence of 6%–11% and 20%, respectively. The development of CCA can be the heralding...
event that brings patients with undiagnosed PSC to the attention of clinicians. For example, population-based studies have reported that 27%–37% of biliary cancers are detected within the first year of a diagnosis of PSC. Consequently, it is important to have a high index of suspicion for CCA around the time of the diagnosis of PSC. However, clinicians should maintain vigilance throughout the disease course because the majority of biliary cancers will develop more than a year after the initial PSC diagnosis.

Our knowledge concerning risk factors for CCA in PSC is limited. An older age at the time of PSC diagnosis, history of colorectal cancer or dysplasia, longer duration of inflammatory bowel disease, variceal hemorrhage, smoking, and alcohol consumption have been reported to have a positive association with biliary cancer and PSC. However, the evidence is insufficient to use these factors to risk-stratify PSC.

Figure 1. Malignancies associated with PSC.
patients who are more likely to benefit from a screening program.

There are several PSC subgroups that appear to have a lower risk of CCA. Among longitudinal studies of small duct PSC, biliary cancer has not been reported unless there is progression to large duct PSC. Hence, we do not routinely screen for CCA among asymptomatic patients with small duct PSC. In addition, the risk of CCA among pediatric patients appears to be lower when compared with their adult counterparts. For example, in a case series conducted during a 25-year period at a large tertiary institution that is a referral center for CCA and PSC, there were no cases of biliary cancer among pediatric PSC patients younger than 18 years old. Similarly, among 29 pediatric PSC patients from a population-based study in North America, only 1 person (3%) was diagnosed with CCA before the age of 18 years. Consequently, routine CCA screening of pediatric PSC patients would likely be low-yield. There is emerging evidence to suggest that patients with PSC and a lower alkaline phosphatase level are more likely to have improved outcomes. Although a study found no cases of CCA among PSC patients with a persistent reduction in serum alkaline phosphatase less than 1.5 times the upper limit of normal, this observation was not confirmed in a subsequent study. Therefore, there is insufficient evidence to suggest alkaline phosphatase could be used as a serologic marker to risk-stratify PSC patients who should undergo routine CCA screening.

In our opinion, it is likely that various genetically driven subsets of PSC exist, some of which are susceptible to CCA and some that are not. Hopefully, future studies of PSC patients that use a precision/individualized medicine genetic approach will address this topic.

Mechanisms of Inflammation-induced Biliary Tract Cancer

Although the precise etiology of PSC is ambiguous, recent studies have highlighted an immune-mediated basis. In approximately 25% of cases, PSC is seen in the context of at least one other autoimmune disease outside the gastrointestinal tract. PSC has robust associations within the HLA complex, which provides further credence to the notion that immunity plays a role in its pathogenesis. Haplotypes with well-established associations with PSC include HLA-DRB1*1301-DQB1*0603, HLA-A1-B8-DRB1*0301-DQB1*0201, and HLA-DRB1*1501-DQB1*0602. Overall, there are 16 known genome-wide significant loci in PSC, including the HLA complex on chromosome 6. Of these 16 loci, 12 were recently identified by using the Immunochip, a genotyping array with marker coverage across a number of loci from 12 immune-mediated diseases. A stronger association with PSC than with inflammatory bowel disease is seen with 6 of these 12 loci, which denotes that although there is some overlap, the genetic architecture for these 2 diseases is distinct.

To date, no germline oncogenic genetic mutations have been described in PSC. These findings reinforce the association between PSC and other immune-based diseases, highlight an immune-mediated pathophysiological basis for PSC, and suggest CCA development is a secondary event related to inflammation and not a primary genetic process.

Biliary tract cancer is a prototype of malignancies occurring in the context of inflammation. PSC promotes chronic inflammation of the biliary tree, predisposing to the development of CCA. Chronic inflammation facilitates oncogenesis via induction of DNA damage, promotion of cellular proliferation, and inhibition of apoptosis. For example, inflammatory cytokines activate inducible nitric oxide (iNOS) with excess production of nitric oxide (NO) and consequent nitrosative stress. iNOS is not present in normal biliary epithelia, but its expression has been demonstrated in PSC as well as CCA. Oxidative DNA lesions are the primary mechanism of DNA damage in inflammation, and the most abundant oxidative DNA lesion is 8-oxodeoxyguanine. These lesions are typically excised by DNA repair processes. NO inhibits 8-oxodeoxyguanine base excision DNA repair processes with resultant accumulation of this oxidative lesion in PSC. The failure to repair 8-oxodeoxyguanine is mutagenic and fosters cancer development and progression. Thus, NO has an integral role in mediating DNA damage in biliary tract inflammation and carcinogenesis.

Sublethal proapoptotic signaling has recently been mechanistically linked to the genesis of chromosomal instability, a hallmark of cancer. The proapoptotic death receptor agonist, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), has been implicated in both PSC and cancer development, presumably by this mechanism of inducing chromosomal instability. Thus, TRAIL and sublethal cell injury in the development of PSC-associated CCA merits further study.

Cholestasis occurring in the setting of PSC also confers an enhanced risk of CCA development. Bile acids activate receptor tyrosine kinases such as epidermal growth factor receptor (EGFR). Sustained activation of EGFR in CCA mediates proliferation and induces expression of cyclooxygenase-2 (COX-2) via a mitogen-activated protein kinase (MAPK)-dependent mechanism. COX-2 is also induced by various inflammatory cytokines and contributes to carcinogenesis by promoting proliferation and angiogenesis and inhibiting apoptosis. Oxysterols, oxygenated derivatives of cholesterol, are abundant in the bile of patients with biliary tract inflammation. Stabilization of COX-2 expression by oxysterols has been implicated in the genesis and promotion of CCA. Oxysterols also serve as activators of the hedgehog signaling pathway, a developmental pathway implicated in CCA development.

A recently developed oncogene-driven murine model of CCA highlights the role of inflammatory cytokines in CCA oncogenesis. In this model, biliary transduction of constitutively active Akt and yes-associated protein (YAP)
coupled with lobar bile duct ligation and systemic interleukin (IL)-33 administration resulted in the development of CCA. IL33 promotes downstream activation of IL6 signaling in this model. IL33, an IL1 family member, is a known biliary mitogen that promotes inflammation and fibrosis in the biliary tract. IL6, an inflammatory cytokine, by cholangiocytes. Activation of IL6/signal transducer and activator of transcription (STAT)3 signaling, in turn, promotes growth stimulation of malignant cholangiocytes by activation of p38 or p44/42 MAPK signaling pathways in an autocrine or paracrine manner. A recent article also relates IL6 signaling to YAP activation in mucosal injury of the intestine, and perhaps this pathway is also relevant to the biliary tree, YAP-mediated epithelial regeneration could also be an initiator of carcinogenesis if sustained and unrelenting.

These mechanistic insights linking chronic inflammation to carcinogenesis provide potential therapeutic avenues for chemoprevention in PSC. For example, inhibitors of STAT3 and Janus kinases (JAKs), which activate STAT3 downstream of IL6, are in clinical development. JAK kinase inhibitors are currently being tested for the treatment of inflammatory bowel disease, which coexists in 85% of PSC patients. Thus, the use of JAK inhibitors in these patients may come to fruition, permitting an assessment of their chemopreventive effects in this patient population. In addition to JAK-STAT inhibitors, caspase, iNOS, COX-2, and Hippo pathway antagonists may also be chemopreventive.

**Cholangiocarcinoma Screening and Surveillance**

CCA is divided into pCCA, intrahepatic CCA, and distal subtypes that are based on the anatomic location of the tumor within the biliary tree. pCCA is not only the most common subtype overall but also the subtype primarily seen in the context of PSC. pCCA frequently presents with an obstructive biliary stricture without the presence of a mass on cross-sectional imaging (Figure 2A). Inflammatory/fibrotic obstructive biliary strictures in PSC, so-called dominant strictures, mimic malignant strictures, and hence, distinguishing a benign inflammatory/fibrotic stricture in PSC from a malignant stricture can be quite challenging and is not possible by using noninvasive diagnostic modalities such as magnetic resonance cholangiography (MRC). Endoscopic retrograde cholangiography (ERC) is essential in this setting because it has diagnostic and therapeutic utility.

**Radiologic Imaging**

Imaging plays a central role in the detection of CCA, and abnormalities seen on imaging often trigger additional investigations aimed at establishing a diagnosis of biliary cancer. Ultrasonography, computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI)/MRC have been investigated as diagnostic imaging modalities.

Although inexpensive and noninvasive, ultrasonography may only delineate intrahepatic ductal dilatation without providing further detailed information in the absence of a mass lesion. Although a CT scan can readily characterize mass lesions and investigate invasion into adjacent structures or metastases, its sensitivity and specificity for CCA detection in PSC are 75% and 85%, respectively. A meta-analysis examined 23 studies and the ability of fluorine-18-fluorodeoxyglucose (18F-FDG)-PET or PET/CT to detect CCA. The pooled sensitivity and specificity for CCA among those with and without PSC were 81% and 82%, respectively. Notably, false-positive results can occur as a result of inflammation associated with PSC. A recent study examined the role of 18F-FDG-PET/CT and the 18FDG uptake values, normalized to the background liver, at 180 minutes (standard uptake value [SUV]max/liver) among 70 patients with PSC and a dominant stricture. There were 9 cases of CCA in the cohort, and 55% did not have definitive features of cancer on MRI. An analysis of the 18FDG uptake showed that SUVmax/liver quotient of 3.3 was able to distinguish between CCA and benign strictures with sensitivity and specificity of 89% and 92%, respectively, whereas a quotient of less than 2.4 excluded CCA (sensitivity 100%, specificity 78%). Although these findings warrant confirmation in a larger cohort, these results suggest that the use of 18F-FDG-PET/CT may be helpful in a

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**Figure 2.** Imaging features of pCCA in PSC. (A) ERC image of dominant common hepatic duct stricture (white arrow) in PSC patient with periductal infiltrating pCCA. (B) MRI image of perihilar mass (white arrow) with resultant biliary obstruction.
subset of patients, particularly if there is a persistent concern for CCA despite a negative MRI/MRC and negative biliary brushings. Although $^{18}$F-FDG-PET/CT and the evolving $^{18}$F-FDG-PET/MRI technologies all hold promise for the diagnosis of PSC-associated CCA, many confirmed cases of CCA are $^{18}$F-FDG-PET negative. The ultimate role of this approach for the diagnosis of CCA remains to be clarified.

Of these imaging modalities, MRI/MRC is the diagnostic imaging method of choice. A mass lesion with venous phase enhancement is very specific for CCA (Figure 2B). However, such definitive features are frequently absent. More commonly, CCA infiltrates along the biliary tree, leading to ductal narrowing, thickening, and dilation. When present, CCA typically has maximal enhancement on delayed phases. Such findings can be subtle, and distinguishing benign from malignant strictures in PSC is challenging. Radiographic findings that should raise concern are the development of thickened or nodular bile ducts or a new dominant stricture. Indeed, inflammatory/obstructive strictures have been reported in up to 50% of patients with PSC, which leads to symptoms in approximately 10%–30% of individuals. One-fourth of the so-called dominant strictures are malignant. However, CCA may be detected in individuals without an obstructive stricture, so their absence does not exclude malignancy. The presence of perihilar lymphadenopathy by itself should not raise concern because this is commonly seen in patients with PSC.

**Carbohydrate Antigen 19-9**

The most widely studied serum biomarker for CCA in PSC is carbohydrate antigen 19-9 (CA 19-9). The synthesis and ability to express CA 19-9 are dependent on fucosyltransferase-2 and 3 (FUT-2 and FUT-3) activity, and individuals who lack FUT-3 activity (Lewis antigen negative) are unable to express CA 19-9 (approximately 7% of the population). By using a CA 19-9 cutoff of 129 U/mL, the sensitivity and specificity for CCA detection were 79% and 99%, respectively, and using a threshold of 100 U/mL yielded a similar diagnostic performance. However, only advanced cases of CCA were detected by either cutoff. Furthermore, examining changes in CA 19-9 over time did not add to the diagnostic yield of a single CA 19-9 value. However, other studies have called into question the high specificity of a CA 19-9 value greater than 129 U/mL. For example, 2 studies have reported that one-third of PSC patients with a CA 19-9 greater than 129 U/mL do not have underlying CCA. Recognizing this assay’s limitations, authors have sought to improve the diagnostic performance of CA 19-9 by using FUT2/3 genotype specific CA 19-9 thresholds. This method has been shown to improve the sensitivity and decrease the number of false-positive test results by 43% among a large cohort of PSC patients.

**Biliary Cytology**

Conventional biliary cytology can be classified into 5 categories: nondiagnostic, normal, atypical, suspicious, or positive for adenocarcinoma. The primary advantage of biliary cytology is its high specificity (97%–100%) when adenocarcinoma is detected. However, suspicious cytology also represents a concerning finding. For example, 34%–42% of PSC patients without a mass lesion and suspicious cytology may ultimately be diagnosed with CCA, and suspicious cytology is an independent predictor for the development of biliary cancer.

Atypical cytology is frequently encountered in PSC. This is primarily due to the presence of biliary inflammation and by itself should not raise concern. The chief disadvantage of biliary cytology is its limited sensitivity (43%) and potential for false-negative results. This is a result of the desmoplastic, paucicellular nature of CCA, which can reside in areas that are difficult to access. Therefore, the absence of a positive cytology does not rule out malignancy.

**Overview of Fluorescence In Situ Hybridization**

Because of the limitations of biliary cytology, fluorescence in situ hybridization (FISH) has been used as a second-generation test to enhance clinicians’ ability to risk-stratify PSC patients. The FISH assay examined in patients with PSC uses 3 centromeric probes that target chromosomes 3, 7, and 17 and a locus specific probe to 9p21 from samples obtained by biliary brushings. FISH detects abnormal gains or losses of chromosomes (aneusomy), and the results can be categorized as normal, trisomy (10 or more cells with 3 copies of chromosome 7 and 2 or fewer copies of the other 3 probes), tetrasomy (10 or more cells show 4 copies of all probes), or polysomy (5 or more cells show gains of 2 or more of the 4 probes). FISH polysomy indicating duplication of more than 1 chromosome is a marker for chromosomal instability, a hallmark of cancer.

When compared with a normal FISH result, trisomy/tetrasomy are not independent predictors of CCA. In contrast, polysomy is strongly associated with a diagnosis of CCA. A meta-analysis examined the performance of FISH testing among 690 PSC patients, and the pooled sensitivity, specificity, and positive and negative likelihood ratios for polysomy and CCA were 51%, 93%, 6.8, and 0.6, respectively. This study did not distinguish between other factors that can increase the probability of biliary cancer when polysomy is present, and it is important for FISH results to be interpreted in the clinical context of each individual patient.

Polysomy associated with a dominant stricture or elevated carbohydrate antigen 19-9. Polysomy in the presence of a dominant stricture may increase the probability of cancer. For example, in a study that included 235 PSC patients (with and without a mass
lesion), 55% of patients with polysomy were diagnosed with biliary cancer. However, if a dominant stricture was present in the setting of polysomy, 73% of PSC patients were diagnosed with CCA.66

An elevated CA 19-9 in the setting of polysomy is also concerning for cancer. For example, among PSC patients (without a mass lesion at baseline) suspected of CCA, 100% of patients with CA 19-9 ≥129 U/mL were diagnosed with CCA.65 In a separate study, 71% of patients with polysomy (regardless of cytology) and CA 19-9 ≥129 U/mL who lacked a mass lesion on imaging were diagnosed with CCA compared with 37% of individuals with CA 19-9 <129 U/mL.56 When polysomy was incorporated in the multivariable analyses in both of these studies, CA 19-9 was no longer statistically significant. However, because of the high proportion of individuals with elevated CA 19-9 and polysomy who were diagnosed with cancer, an increased CA 19-9 should raise concern.

Serial or multifocal polysomy. When compared with the PSC population at large, those with polysomy in the absence of definitive features of CCA at the time of the initial assessment (ie, lack a positive cytology or definitive mass lesion) are likely rare (estimated to be less than 5% of total PSC population seen at 1 institution during a 7-year period).56 Although uncommon, this important subgroup of patients represents a diagnostic challenge. Consequently, a study examined the performance of polysomy when it is detected on subsequent examinations (serial polysomy) among those without definitive radiographic features of biliary malignancy. The 3-year cumulative incidence of CCA was 75% among those with serial polysomy and 18% of subjects with non-serial polysomy (polysomy detected only once at the index examination). After 1 year of follow-up the incidence of CCA did not increase among those with non-serial polysomy.67 These results reinforce the importance of repeating an ERC with brushings for cytology and FISH if polysomy is detected in the absence of definitive features of CCA.

The largest study that examined FISH in PSC included 371 patients without a mass lesion on imaging and investigated the natural history of polysomy when it was detected in multiple areas of the biliary tree (multifocal polysomy) when compared with unifocal polysomy, serial polysomy, and other FISH subtypes. In the adjusted analysis, multifocal polysomy was the strongest predictor of a diagnosis of CCA (regardless of whether a dominant stricture or serial polysomy was present), and the 1-year and 3-year cumulative incidence of CCA among those with multifocal polysomy was 65% and 83%, respectively. Among those with polysomy and positive cytology, 71% had polysomy detected at another region of the biliary tree where adenocarcinoma was not detected by routine cytology. These findings reinforce the role of FISH and indirectly support the hypothesis that CCA in PSC may arise from a field defect beyond the primary site of malignancy. It also suggests that it may be important to brush multiple areas of the biliary tree, regardless of whether and where a dominant stricture is located, and place the specimens in separate vials when CCA is suspected. However, individuals with unifocal polysomy should not be ignored because this was also a risk factor for CCA when compared with a normal FISH result.56

Advanced Endoscopic Biliary Imaging Techniques

Because conventional cytology has limited sensitivity and propensity for false-negative results and the availability of FISH is limited, advanced techniques for endoscopic biliary imaging have become a recent research focus. However, these techniques have largely been examined in non-PSC patients. These techniques include Spyglass Spyscope (Boston Scientific, Marlborough, MA), which enables direct endoscopic visualization of bile ducts and directed biopsies of suspicious lesions. In a retrospective analysis, Spyglass Spyscope had 77% accuracy in detection of CCA in patients with diagnostic uncertainty after conventional cytology of endoscopically obtained biliary brushings and endoscopic ultrasound–fine-needle aspiration.69 Intraductal ultrasound (IDUS) of the biliary system provides evaluation of the periductal tissues during endoscopic retrograde cholangiopancreatography (ERCP). In a prospective comparative analysis, ERCP supplemented with IDUS allowed differentiation of benign from malignant biliary strictures in 88% of patients (n = 33).70 Moreover, ERCP plus IDUS had significantly greater accuracy in differentiating between malignant and benign strictures compared with magnetic resonance cholangiopancreatography.70 Another promising diagnostic approach that can supplement conventional ERCP is probe-based confocal laser endomicroscopy. Mucosal imaging was conducted in 14 patients by using a confocal laser scanning miniprobe introduced via the accessory channel of a conventional cholangioscope.71 Subsequently, targeted biopsy specimens were obtained under visual cholangioscopic guidance in all patients.71 By detecting a specific pattern of neovascularization, confocal laser microscopy enabled prediction of CCA with sensitivity of 83% and specificity of 88%.71 In comparison, standard histopathology had sensitivity and specificity of 50% and 100%, respectively.71 Finally, we note that narrow-band imaging in combination with cholangioscopy is disappointing in its ability to identify biliary dysplasia in the setting of PSC.72 These advanced biliary imaging techniques hold promise; however, their utility in PSC patients, a patient population more complex than those with de novo CCA, remains unstudied. Moreover, in our own experience, technical failure with poor visualization of the PSC biliary tract is common
with Spyglass Spyscope technology, diminishing sensitivity and specificity on an “intent-to-diagnose” assessment.

**Approach to Biliary High-grade Dysplasia**

Presence of high-grade dysplasia of the biliary tract may herald CCA development in PSC patients. Liver explants from patients with concomitant PSC and CCA are more likely to harbor high-grade dysplasia compared with PSC explants without CCA. Moreover, cytogenetic assessment of liver explants with biliary dysplasia from patients with PSC has demonstrated that patients with previous or current CCA are more likely to have FISH polysomy in dysplasia than patients without. In this study, FISH polysomy was detected in 58% of the areas with high-grade dysplasia compared with 11% of areas with low-grade dysplasia. Thus, improved cytogenetic techniques can detect high-grade dysplasia before its progression to CCA. The presence of FISH polysomy in the absence of other diagnostic features of CCA (cross-sectional imaging with mass or dominant stricture ± positive cytology) may indicate underlying high-grade dysplasia.

Management of PSC patients with dysplasia remains complex and unclear. On one hand, definitions and stratification into low-grade and high-grade dysplasia are not standardized. The natural history is also unknown. On the other hand, a dysplasia-carcinoma sequence has been reported, and most patients with dysplasia develop CCA (personal observation). Hence, one can argue equally vociferously for careful observation and surveillance or an aggressive approach with liver transplantation. In countries where organ allocation is favorable to these patients, liver transplantation has been suggested as the treatment of choice. In the United States, a conservative approach is the sole option because only patients with overt CCA are eligible for transplantation.

Such patients should be enrolled in intensive surveillance for CCA detection. PSC patients with biliary dysplasia would be ideal candidates for chemopreventive strategies, once potential agents are identified.

**A Rational Approach to Cholangiocarcinoma Screening and Surveillance**

Among adults older than the age of 20 with large duct PSC, it is our practice to obtain liver tests every 3–6 months and an annual MRI/MRC and CA 19-9. If a suspected CCA is detected, a transperitoneal biopsy of the primary mass lesion via endoscopic ultrasound or a percutaneous approach should not be performed because of the high risk of peritoneal seeding, which would make patients ineligible for a liver transplant. Although the cost-effectiveness of this surveillance strategy and its effect on long-term outcome are unclear, patients and physicians desire a surveillance approach because of the disastrous consequences of diagnosing CCA at a time when it is symptomatic. In this regard, noninvasive imaging of the biliary tree and determination of cancer-associated biomarkers are a pragmatic approach until further information is available.

An ERC with brushings for cytology and FISH is typically performed among individuals with suspicious features on imaging (a new dominant stricture or the development of focal bile duct thickening, irregularity, or enhancement), symptoms that suggest biliary obstruction or worsening laboratory tests (including new elevations of CA 19-9 greater than 100 U/mL in the absence of cholangitis). Subsequent follow-up is based on the results of this initial assessment (Figure 3). Currently, there is no evidence to support or refute this practice, and it is unclear whether this strategy improves patient outcomes or is cost-effective. However, because of the prevalence of CCA among PSC patients and the possibility to undergo a curative therapy if detected early, we believe this strategy is rational and pragmatic.

**Future Biomarkers**

Contemporary methods used to establish a diagnosis of CCA are suboptimal. A better understanding of cancer biology, bile acid composition, and key –omics (tumor genomics, epigenomics, transcriptomics, proteomics, and lipidomics) has paved the way for a series of preliminary studies that have examined the role of novel biomarkers in the diagnosis of CCA. Table 1 highlights key studies that have examined unique markers that also included CCA associated with PSC and PSC controls without CCA.

Compared with non-neoplastic tissue, tumor cells often have a higher proportion of aberrant DNA methylation, which can in turn serve as a useful biomarker for cancer detection. Among the studies that have examined new biomarkers in PSC-associated CCA, a 4 methylated gene panel (CD01, CNRP1P1, SEPT9, and VIM) obtained from biliary brushings among patients with and without PSC has the best diagnostic performance reported to date (sensitivity 85% and specificity 98%). However, among the cases of CCA in patients with PSC, 83% had advanced cancers and would have been ineligible for a liver transplant, and the role of this biomarker panel to detect early-stage CCA among patients with PSC who are under a longitudinal surveillance program is unclear but warrants further study.

In addition to DNA methylation, noncoding RNAs have been examined as markers in malignant conditions. Indeed, among patients with CCA (not associated with PSC) and PSC controls, the measurement of U2 small nuclear RNA fragments in bile was able to distinguish CCA from PSC without cancer, area under the curve (AUC) 0.86. A subsequent study examined the role of another group of noncoding RNAs (microRNAs) and found that a panel of biliary vesicle microRNAs among
Figure 3. Screening and surveillance in CCA. Approach to CCA screening and surveillance among adults with large duct primary sclerosing cholangitis. Modified from Eaton et al.\textsuperscript{62}
those with and without PSC had sensitivity of 67% and specificity of 96% for the diagnosis of CCA.\textsuperscript{81}

Volatile organic compounds (VOCs) represent a gas-phase biomarker that has been examined as a potential diagnostic modality in a variety of conditions.\textsuperscript{82} Examining the presence of VOCs in bile or urine to distinguish benign from malignant biliary strictures has also been investigated.\textsuperscript{83} For example, among a dedicated PSC cohort, a model adjusted for age and gender plus VOC levels obtained from bile was able to identify CCA with sensitivity and specificity of 91% and 73%, respectively.\textsuperscript{83} In addition to VOCs, a small pilot study examined the bile lipid profile among those with de novo CCA and benign biliary conditions including PSC and found that a combination of 2 phosphatidylcholines was able to distinguish benign from malignant strictures with sensitivity and specificity of 100% and 83%, respectively.\textsuperscript{84}

To date, much of the published work to improve methods of CCA detection have centered on proteomics obtained from bile, urine, or serum. Indeed, there appears to be a varying protein composition of bile between those with benign and malignant strictures, and a panel of 22 peptides was able to identify 80% of CCA associated with PSC.\textsuperscript{85} Similarly, a panel of 42 urine peptides was able to accurately identify all of the PSC patients with CCA.\textsuperscript{86} A variety of studies have examined protein markers in serum. One such study investigated the performance of circulating angiopoietin-2. This protein was noted to have a better diagnostic accuracy compared with CA 19-9 (AUC, 0.85 versus 0.77) and sensitivity and specificity for the detection of CCA of 74% and 94%, respectively.\textsuperscript{87} An increase in serum cytokeratin 19 fragments was associated with an increase in mortality, and it demonstrated excellent specificity (\textgreater{}95%) even when limited to a PSC only subgroup, but sensitivity remained poor (\textlessthan{}30%) when cutoff of \textgreater{}3 ng/mL was used.\textsuperscript{88} Trypsinogen-2, another serum protein, is increased in CCA, and the use of trypsinogen-2 was able to differentiate between PSC with and without

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**Table 1. Novel Biomarkers for CCA Detection Among Patients With PSC**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Source</th>
<th>Study population</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA methylation\textsuperscript{74}</td>
<td>Biliary brushings</td>
<td>CCA, n = 42 (PSC, n = 24) Controls, n = 50 (PSC, n = 49)</td>
<td>85</td>
<td>98</td>
<td>0.94</td>
</tr>
<tr>
<td>CDO1 CNRIP1 SEPT9 VIM</td>
<td>CCA, n = 46 (PSC, n = 4) Controls, n = 50 (PSC, n = 13)</td>
<td>67</td>
<td>97</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Noncoding RNA\textsuperscript{81}</td>
<td>Bile</td>
<td>CCA, n = 25 (PSC, n = 10) Controls, n = 18 (PSC, n = 18)</td>
<td>84</td>
<td>78</td>
<td>0.87\textsuperscript{b}</td>
</tr>
<tr>
<td>Peptides\textsuperscript{85}</td>
<td>Bile</td>
<td>CCA, n = 11 (PSC, n = 11) Controls, n = 21 (PSC, n = 21)</td>
<td>91</td>
<td>73</td>
<td>0.89</td>
</tr>
<tr>
<td>Peptides\textsuperscript{86}</td>
<td>Urine</td>
<td>CCA, n = 42 (PSC, n = 10) Controls, n = 81 (PSC, n = 45)</td>
<td>83</td>
<td>79</td>
<td>0.87\textsuperscript{c}</td>
</tr>
<tr>
<td>Protein\textsuperscript{87}</td>
<td>Serum</td>
<td>CCA, n = 49 (PSC, n = 7) Controls, n = 48 (PSC, n = 34)</td>
<td>74</td>
<td>94</td>
<td>0.85</td>
</tr>
<tr>
<td>Protein\textsuperscript{88}</td>
<td>Serum</td>
<td>CCA, n = 66 (PSC, n = 6) Controls, n = 58 (PSC, n = 19)</td>
<td>30</td>
<td>97</td>
<td>—\textsuperscript{d}</td>
</tr>
<tr>
<td>Protein\textsuperscript{89}</td>
<td>Serum</td>
<td>CCA, n = 41 (PSC, n = 8) Controls, n = 43 (PSC, n = 43)</td>
<td>—</td>
<td>—</td>
<td>0.80\textsuperscript{e}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Reflects diagnostic performance of entire cohort (ie, patients with and without PSC).
\textsuperscript{b}Data from test cohort (discovery cohort not included). Eighty percent of PSC-CCA correctly identified.
\textsuperscript{c}Data from test cohort (discovery cohort not included). One hundred percent of PSC-CCA correctly identified.
\textsuperscript{d}Among those only with PSC (n = 25), sensitivity and specificity were 17% and 95%, respectively.
\textsuperscript{e}Optimal cutoff for trypsinogen-2 concentration not reported. Among those only with PSC (n = 51), AUC was 0.76.
CCA (AUC, 0.76).89 Last, an additional study combined a variety of serum proteins (CA 19-9, leucine-rich α2-glycoprotein, and IL6) among patients with CCA and conditions associated with benign biliary strictures and reported AUC of 0.98. However, it did not appear that PSC patients with CCA were examined.90 These studies highlight the evolving methods that are being investigated to distinguish benign from malignant strictures among patients with PSC.

Management

Outcomes of surgical resection for pCCA rising in the setting of PSC have been quite disappointing.91,92 Furthermore, curative surgical resection is often not an option for pCCA occurring in the setting of PSC because of the underlying parenchymal liver disease, the field defect, and the predilection for skip lesions.5 These tumors are also usually unresectable because of either local tumor extent or the underlying disease itself.93 Liver transplantation appeared to be an optimal treatment for pCCA in PSC patients because it would address the underlying disease as well as hepatic or vascular invasion.93 However, despite this logical basis the outcomes were subpar, with one study noting a 3-year survival of 30% in PSC patients incidentally diagnosed with CCA.94 Efficacy of palliative radiotherapy led to the development of a protocol combining chemoradiation followed by liver transplantation. Selection criteria for this protocol are quite rigorous and include the following prerequisites in a PSC patient: confirmed diagnosis of pCCA, radial tumor diameter less than 3 cm, and absence of intrahepatic or extrahepatic metastasis (Figure 4).28

According to a retrospective analysis of 191 patients enrolled in neoadjuvant chemoradiotherapy followed by liver transplantation protocol, 16 patients underwent transperitoneal fine-needle aspiration of the primary tumor (13 percutaneous, 3 endoscopic ultrasound).75 A total of 6 patients had biopsies demonstrating malignancy. Five of these patients (83%) were found to have direct metastasis into the peritoneum at operative staging, suggesting peritoneal seeding after transperitoneal biopsy.75 Thus, patients with a prior transperitoneal biopsy of the primary tumor are excluded from this protocol. After judicious selection, patients undergo a combination of radiosensitizing chemotherapy with 5-fluorouracil, external beam radiation therapy, brachytherapy with endoscopically placed iridium-192 beads, and maintenance chemotherapy with capecitabine.95 Before orthotopic liver transplantation, patients undergo a staging laparotomy to assess for the presence of metastasis.95 The pCCA patients who underwent neoadjuvant chemoradiation in anticipation of liver transplantation at 12 U.S. centers had 65% recurrence-free survival at 5 years.95 The overall 10-year survival for PSC patients with pCCA who underwent neoadjuvant chemoradiation in anticipation of liver transplantation at our center is approximately 75% (Figure 5). Approximately 30% of patients drop out of the protocol before liver transplantation, primarily because of cancer progression.96 Small case series of combined orthotopic liver transplantation and en bloc Whipple procedure with chemoradiation alone97 or chemoradiation plus brachytherapy98 have been reported for treatment of hilar cholangiocarcinoma. This is a rational approach for potentially curative therapy of distal cholangiocarcinoma. However, in the setting of hilar cholangiocarcinoma without any involvement of the distal bile duct by malignant or premalignant lesions, this method may be overly aggressive.

For advanced stage pCCA, liver transplantation after neoadjuvant chemoradiation is not an option. In this setting, the combination of gemcitabine and cisplatin remains the pragmatic standard of care.99 Genetic analysis of a tumor can identify potentially actionable events such as mutations that may be candidates for targeted therapy. Recent reports have highlighted evidence of disease regression with chemotherapy directed at the aberrant pathway.100 For instance, patients with fibroblast growth factor receptor 2 (FGFR2) gene fusions had stable disease with ponatinib, an FGFR inhibitor.100 However, FGFR2 gene fusions and other mutations such as isocitrate dehydrogenase 1 (IDH1) and tyrosine kinase domain mutations of FGFR2 may be targets for pharmacologic therapy.

Figure 4. Criteria for liver transplantation in PSC patients with pCCA.

Figure 5. Kaplan-Meier curve for overall survival in PSC patients with pCCA undergoing neoadjuvant chemoradiation followed by liver transplantation.
dehydrogenase 1 and 2 mutations tend to occur more frequently in intrahepatic CCA than pCCA. The genetic aberrations of PSC-associated CCA have yet to be identified. Hopefully, advances in genomic characterization of tumors will allow detection of driver mutations and druggable targets associated with perihilar tumors.

**Future Directions**

The majority of patients with PSC do not develop CCA, and thus it remains unclear which patients have a higher propensity of developing CCA. Recognizing this high-risk subset is imperative and will aid not only in earlier detection but also in selecting patients for intensive screening and potential chemopreventive strategies in the future. Currently, there is a lack of chemopreventive therapies, and further preclinical studies are needed to identify such agents. Detecting early-stage CCA is another challenge in PSC patients. Continued development of tumor biomarkers in biological specimens will be crucial in this regard. Curative nonsurgical treatment of malignancy arising in the context of PSC remains a therapeutic conundrum, partly because of the genetic heterogeneity of these tumors and rapid development of therapeutic resistance with genetic evolution of the tumor. Although significant progress has been made in recognizing oncogenic pathways and mutational changes in CCA, such studies focusing on CCA in PSC remain to be performed. We look forward to these and other advances so that we can prevent and better manage this devastating complication of PSC.

**References**


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1. MRI is the preferred modality for detecting CCA in PSC. Findings that suggest CCA include:
   a. Delayed enhancement of thickened bile duct walls or strictures
   b. Arterial phase enhancing mass lesion
   c. Thickened or nodular bile ducts
   d. Perihilar adenopathy

True or False

2. The most common type of CCA seen in PSC is the intrahepatic type

3. Approximately 50% of dominant strictures will eventually prove to be malignant

4. Up to 1/3 of CCA’s associated with PSC are diagnosed within the first year after initial diagnosis of PSC

5. Standard biliary cytology results are only helpful if they confirm adenocarcinoma

6. Patients with small duct PSC have similar risk of CCA compared to large duct PSC

7. PET-CT is useful for detecting or excluding CCA in PSC, if the PET-CT is negative, it is highly unlikely that CCA is present

8. FISH analysis of biliary fluid cytology enhances the ability to detect CCA, the presence of polysomy is particularly important in detecting CCA

9. If a mass lesion is detected and is suspicious for CCA, a percutaneous biopsy should be obtained for confirmation if the lesion is accessible.

10. Patients with polysomy by FISH analysis of biliary fluid, but no other evidence of CCA should undergo repeat FISH at intervals, persistent positivity raises risk for CCA

11. Recommended surveillance for CCA in PSC includes MRI/MRC and CA 19-9 every 6 months

12. The treatment of choice of asymptomatic CCA is liver transplantation