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Current Diagnostic and Management Options in Perihilar Cholangiocarcinoma

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Abstract

Cholangiocarcinomas (CCA) are heterogeneous biliary tract tumors with dismal prognosis. Perihilar cholangiocarcinoma (pCCA) involves the large bile ducts of the hepatic hilum, and is the most common type of CCA. Primary sclerosing cholangitis (PSC) is an established risk factor for pCCA. Although the diagnosis of pCCA is challenging, recent advances have been made including cytologic techniques such as fluorescence in situ hybridization. Endoscopic ultrasound with sampling of regional lymph nodes is emerging as a valuable diagnostic modality in the diagnosis and staging of pCCA. Curative treatment options are limited to early stage disease, and include surgical resection and liver transplantation after neoadjuvant therapy. This underscores the importance of early detection, and the need for development of innovative diagnostic tools such as biomarkers. A dense desmoplastic tumor stroma plays an integral role in pCCA progression. The tumor stroma represents an additional target for development of new therapies. Herein, we discuss these advances in the diagnosis and treatment of pCCA.

Keywords

Fluorescence in situ hybridization; liver transplantation; perihilar cholangiocarcinoma; primary sclerosing cholangitis; tumor microenvironment

Introduction

Cholangiocarcinomas (CCAs) are heterogeneous tumors arising from the biliary tree with features of biliary epithelial differentiation [1]. Perihilar cholangiocarcinoma (pCCA) involves the large bile ducts in the hepatic hilum, and is anatomically separated proximally from intrahepatic cholangiocarcinoma (iCCA) by the second-order biliary ducts and distally from distal cholangiocarcinoma (dCCA) by the cystic duct insertion [2]. pCCA is the most common type of cholangiocarcinoma, accounting for 50% of CCA cases with dCCA and iCCA representing approximately 40% and less than 10% of cases, respectively [3]. Epidemiologic registries have indicated a global rise in the incidence and mortality rates from iCCA and concomitant fall in pCCA + dCCA rates over the past 3 decades [4]. These

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trends may partly reflect a misclassification of perihilar tumors as iCCAs under the second edition of the International Classification of Diseases for Oncology [4,5].

pCCAs are classified morphologically based on primary growth patterns. These include the intraductal, periductal infiltrating, and mass-forming subtypes (Figure 1) [2,6]. The periductal infiltrating subtype is the most common type of pCCA, and results in development of bile duct stricturing and obstruction. Initially, these tumors display a tropism for bile with a periductal longitudinal growth pattern. With tumor progression, radial growth away from the bile duct and resultant mass-forming lesions occur [2]. Histologically, pCCAs are typically highly desmoplastic, mucin-producing adenocarcinomas with an abundant fibrous stroma. Intraductal (polypoid) pCCAs are often well-differentiated neoplasms with a characteristic papillary growth pattern and mucin production. Intraductal tubular neoplasms, a recently described intraductal subtype, have predominantly a tubular architecture and scant mucin production [7]. These two intraductal subtypes have a more favorable prognosis than nodular or periductal infiltrating tumors.

In an effort to standardize pCCA reporting, a surgical staging system was recently introduced. This staging system expands on the traditional Bismuth-Corlette classification and takes into consideration bile duct involvement (common bile duct, hepatic duct confluence, right or left hepatic duct, both hepatic ducts), tumor size (<1 cm, 1–3 cm, ≥3 cm), tumor form (sclerosing, mass, mixed or both types, and polypoid), portal vein involvement, hepatic artery involvement, liver remnant volume, underlying liver disease (e.g. fibrosis, primary sclerosing cholangitis), lymph node involvement (none, hilar and/or along the hepatic artery, periaortic), and presence of metastases [8].

Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) with associated chronic inflammation is a well-established risk factor for CCA, particularly pCCA. The lifetime risk of CCA development among PSC patients is 5–10% [9,10]. Approximately 50% of patients with CCA in the setting of PSC are diagnosed within 1–2 years of initial PSC diagnosis [11,12]. Thereafter, the risk is lower with CCA development in 9% of patients 10 and 20 years after PSC diagnosis [9]. A number of risk factors have been reported to confer a higher risk of CCA in PSC patients. Among these are smoking and alcohol use, older age at PSC diagnosis, long-standing associated inflammatory bowel disease, concomitant colorectal cancer/dysplasia in the setting of ulcerative colitis, proctocolectomy, variceal bleeding, hyperbilirubinemia, cholelithiasis, and NKG2D gene polymorphisms [10]. However, definitive data are lacking and further studies are needed to confirm these associations.

Clinical Features and Diagnostic Criteria

Clinical Features

pCCA involvement of the large bile ducts and ensuing cholestasis results in an earlier presentation compared to iCCA. Painless jaundice is the most frequent presentation, occurring in 90% of patients. pCCA manifests as bacterial cholangitis in 10% of patients [13]. Nonspecific symptoms such as weight loss, malaise, cachexia, and abdominal pain also

occur. Vascular encasement by pCCA can lead to atrophy of the affected lobe with compensatory hypertrophy of the contralateral, unaffected lobe. This “atrophy-hypertrophy” complex can present as a palpable lobe on physical examination [13].

Laboratory and Imaging Studies

Laboratory analyses are typically not specific in pCCA as elevations of alkaline phosphatase, bilirubin, and the tumor marker carbohydrate antigen 19-9 (CA 19-9) may be a reflection of the tumor itself or the resultant obstructive cholestasis [2]. In patients with PSC, when using a cutoff value of 129 U/mL for CA 19-9, the sensitivity and specificity for CCA diagnosis is 79% and 98%, respectively [14]. Also, only two-thirds of patients with a sustained elevated CA 19-9 > 129 u/L have pCCA – one-third do not appear to develop this cancer [10]. The sensitivity is 76% in patients without PSC when using a CA 19-9 cutoff value of 100 U/mL [2]. It is important to note that 7% of the general population will have undetectable CA 19-9 levels due to absence of the Lewis antigen [10].

Imaging modalities utilized for pCCA diagnosis include computed tomography (CT) and magnetic resonance imaging (MRI) (Figure 2). The diagnostic accuracy of CT in evaluating biliary extent of tumor is 86% [15]. Additionally, CT has adequate accuracy for assessment of portal vein and hepatic artery involvement but is subpar in determining nodal involvement [15]. MRI, on the other hand, has a suboptimal accuracy (67–73%) in ascertaining vascular encasement [16,17]. In PSC patients, the combination of MRI and magnetic resonance cholangiopancreatography (MRCP) has a sensitivity of 89% and accuracy of 76% in detecting CCA, which is comparable to endoscopic retrograde cholangiography (ERC) with a sensitivity and accuracy 91% and 69%, respectively (Figure 2C) [18]. Positron emission tomography (PET) has insufficient diagnostic utility in pCCA; in patients with pCCA or dCCA PET scanning had a sensitivity of 55% and specificity of 33% [19]. Clinically, we evaluate all pCCA patients with an MRI/MRCP.

Endoscopic ultrasound

Endoscopic ultrasound (EUS) is emerging as a valuable diagnostic modality for the diagnosis and staging of cholangiocarcinoma, particularly in the evaluation of unresectable perihilar cholangiocarcinoma for liver transplantation [20]. EUS has a tumor detection rate of 94% in CCA patients, albeit it has a significantly higher detection rate for distal tumors (100%) compared to proximal tumors (83%) [21]. Fine-needle aspiration (FNA) of the primary tumor has a diagnostic sensitivity of 73% when the presence of malignancy is indicated by ‘positive’ as well as ‘suspicious’ cytologic results [21]. EUS-FNA sensitivity is greater in dCCA compared to pCCA (81% vs. 59%) [21]. EUS-FNA does carry a significant risk of tumor seeding. This risk was assessed in a study of 191 patients with locally unresectable CCA being evaluated for liver transplantation [22]. Of these, 16 patients underwent transperitoneal FNA, and malignancy was confirmed in six. Peritoneal metastasis was detected in five of these six patients (83%) compared to zero of nine patients without malignancy (0%) [22]. Furthermore, peritoneal metastases were noted in only 8% (14 of 175) of patients who did not undergo transperitoneal FNA. Although obtaining a tissue diagnosis remains challenging, the risk of tumor seeding is significant and should deter from performing EUS-FNA of primary tumor. At our center, this is an absolute contraindication

to liver transplantation [20]. Lymph node involvement by CCA portends a poor outcome, as locoregional nodal metastasis precludes liver transplantation and distant nodal involvement is a contraindication for both liver transplantation and curative resection [20]. Accordingly, assessment of nodal metastasis is essential in CCA staging. The value of EUS in staging was highlighted in a study of 47 potential liver transplant candidates with unresectable perihilar CCA. Regional lymph nodes were identified in all patients and EUS guided fine-needle aspiration (FNA) detected the presence of malignant lymph nodes in eight patients. In contrast, CT and/or MRI identified malignant lymph nodes in only two of these eight patients. Furthermore, staging exploratory laparotomy confirmed that 91% of lymph nodes identified as benign by EUS-FNA were in fact benign. The study also found that EUS imaging features of lymph nodes had a poor predictive value for distinguishing benign from malignant nodes. This underscores the utility of sampling visualized lymph nodes regardless of their morphological features [23].

ERC and Conventional Cytology

ERC is essential in the evaluation and management of pCCA as it delineates the biliary anatomy, allows for acquisition of biliary brushings for subsequent cytologic evaluation, and serves as a therapeutic tool for dilation and stenting of biliary strictures. ERC detection of a dominant stricture with or without associated proximal biliary ductal dilatation, or the presence of a polypoid lesion is indicative of the presence of pCCA. These findings should prompt further cytological evaluation via biliary brushings. Cytological assessment includes conventional cytology and fluorescence in situ hybridization (FISH). The potential findings on conventional cytology include negative, atypical, suspicious, or diagnostic of malignancy. Conventional cytology is fraught with sampling errors, paucicellular or acellular specimens, and pathologic misinterpretations secondary to subtle differences between benign and malignant cells. Moreover, the presence of inflammation secondary to PSC or associated infection can pose a dilemma on cytologic evaluation as reactive cells can mimic cancer cells [24]. Consequently, conventional cytology has a low sensitivity; 15% when only positive for malignancy results are used and 38% if positive and suspicious for malignancy results are used [24].

Fluorescence in situ hybridization

Chromosomal instability, a hallmark of cancer, results in structural chromosomal abnormalities and aneuploidy or abnormalities in the number of chromosomes within a cell. FISH employs fluorescently labeled DNA probes to identify chromosomal aberrations in cells. FISH utilizing directly labeled DNA probes to the pericentromeric regions of chromosome 3, 7, 17, and the chromosomal locus 9p21 has an increased sensitivity (34% to 58%) and preserved specificity for detection of FISH polysomy (chromosomal amplification) compared to routine cytology [25–27]. FISH analyses which include probes to detect deletion of 9p21 increased diagnostic yield to 93% [25]. FISH results can be categorized as follows: negative or disomy, trisomy 7 (10 cells with 3 copies of chromosome 7 and two or fewer copies of the other 3 probes), polysomy (5 cells with 3 signals in 2 or more of the 4 probes (Figure 3) [27]. High mitotic rates during the M phase of mitosis can yield tetrasomy and thus this finding should be interpreted with caution [2]. Trisomy 7 is frequently detected in the setting of inflammation of the biliary tree. Patient

with FISH trisomy or tetrasomy have outcomes similar to those with negative FISH analysis [28]. FISH polysomy in the setting of a dominant stricture is strongly suggestive of the presence of pCCA [1]. The occurrence of FISH polysomy in the absence of other corroborative evidence of malignancy poses a diagnostic challenge. PSC patients with FISH polysomy in the absence of other diagnostic features had a significantly higher risk of CCA development if polysomy was confirmed on subsequent FISH analysis compared to patients with non-serial polysomy (69% vs. 18%) [29]. In the majority of individuals with serial FISH polysomy, a diagnosis of CCA was attained within 6 months of the initial detection of polysomy, although in 4 patients CCA was not diagnosed until 1–2.7 years after initial polysomy result. Hence, patients with polysomy should have close and prolonged surveillance.

In the absence of imaging criteria for pCCA, equivocal (atypical or suspicious) cytology is another challenging clinical scenario. In a study of 102 PSC patients with equivocal cytology and no baseline imaging features of CCA, 42% of patients with suspicious cytology were ultimately diagnosed with CCA compared to 23% with atypical cytology [30]. Moreover, FISH polysomy in conjunction with equivocal cytology had a positive predictive value of 76%. Interestingly, all patients with FISH polysomy and CA 19-9 levels ≥ 129 U/mL had eventual CCA development (90% within the first 2 years).

In summary, conventional cytology positive for malignancy is diagnostic of pCCA. Likewise, the following scenarios are highly indicative of a pCCA diagnosis: presence of FISH polysomy with a CA 19-9 ≥ 129 U/mL, FISH polysomy in conjunction with suspicious cytology, or FISH polysomy with a dominant stricture on imaging. In contrast, pCCA is unlikely in the setting of FISH trisomy 7 or tetrasomy (Figure 4).

Treatment

Surgical Resection

Curative resection for pCCA remains an arduous task with five year survival rates of 11–41% [31]. The surgery is complex and entails lobar or extended lobar hepatic and bile duct resection, regional lymphadenectomy, and Roux-en-Y hepaticojejunostomy. Exclusion criteria for resection of pCCA includes bilateral involvement of the second order bile ducts, bilateral or contralateral hepatic artery or portal vein encasement, intra- or extra-hepatic metastasis, and distant lymph node metastasis. Regional lymphadenopathy (cystic, portal, hepatic arterial, pericholedochal, and posterior pancreaticoduodenal nodes) does not necessarily preclude resection, albeit patient outcomes are less favorable in this scenario [31]. pCCA occurring in the setting of PSC is also deemed unresectable owing to the propensity for skip lesions, the field defect, and underlying parenchymal liver disease [1]. Portal vein embolization is employed in instances when a tumor is potentially resectable but the remnant lobe has limited volume. In this technique, embolization of the portal vein in the affected lobe is carried out, and this stimulates hypertrophy of the contralateral unaffected lobe [31]. Approximately one-third of patients undergoing portal vein embolization may not have adequate hypertrophy of the remnant lobe and thus are unable to undergo a resection. Recently, the associating liver partition and portal vein ligation for staged hepatectomy or ALPPS procedure has been described as another manner of inducing increase in the remnant

lobe volume [32]. Portal vein ligation is combined with parenchymal transection along the falciform ligament in this procedure. Isolated parenchymal transection following failed portal vein embolization has also been reported [33].

Neoadjuvant Chemoradiation and Liver Transplantation

Orthotopic liver transplantation alone as a potentially curative treatment for CCA, albeit a promising endeavor, has been a futile practice. Reported five-year survival rates for liver transplantation for this indication have been 20–30%, with recurrence rates of 53–84% [34,35]. With the high recurrence rates, pCCA became a contraindication to orthotopic liver transplantation.

Reports of palliative efficacy of radiotherapy provided grounds for the University of Nebraska transplant team to pioneer an approach utilizing high-dose neoadjuvant brachytherapy and 5-fluorouracil (5-FU) followed by liver transplantation [35]. Subsequently, the Mayo Clinic developed a protocol combining radiosensitizing chemotherapy with 5-FU, external beam radiation therapy, brachytherapy with endoscopically placed iridium-192 beads, maintenance chemotherapy with capecitabine, staging laparotomy to assess for presence of metastasis, followed by orthotopic liver transplantation [34]. This protocol employs rigorous selection of early stage pCCA patients, with the following inclusion criteria: confirmed diagnosis of pCCA, radial tumor diameter less than 3 cm, absence of intra- or extrahepatic metastasis, unresectability in non-PSC patient, and pCCA in a PSC patient [1]. Exclusion criteria include transperitoneal tumor biopsy, prior radiation or attempted resection with disruption of the bile ducts, and uncontrolled infection [36]. With reports of survival rates approaching 70%, the United Network of Organ Sharing approved prioritization of pCCA by allocation of a model of end-stage liver disease (MELD) exception score in 2009 [34]. This exception score increases every 3 months, reflecting the 10% expected waitlist mortality. To test the appropriateness of this allocation, a large study analyzing data from 12 high-volume transplant centers was conducted [34]. pCCA patients treated with neoadjuvant therapy followed by liver transplantation had intent-to-treat two-year and five-year survival rates of 68% and 53%, respectively. Similarly, two-year and five-year recurrence free survival rates were 78% and 65%, respectively. Dropout rate prior to liver transplantation was 11.5% after 3.5 months of therapy [34]. Outcomes were significantly worse in patients with prior transperitoneal tumor biopsy, metastatic disease, and tumor mass > 3 cm. This study confirmed not only the excellent clinical outcomes for neoadjuvant chemoradiation followed by liver transplantation and appropriateness of the MELD allocation system for pCCA but also validated the stringent selection criteria for this protocol.

Predictors of pretransplant dropout and posttransplant recurrence were assessed in a recent study of 199 patients enrolled in the neoadjuvant chemoradiation protocol in anticipation of liver transplantation [37]. The pretransplant dropout rate was 31%, and predictors included CA 19-9 levels > 500 U/mL, radial diameter of tumor mass > 3 cm, brushing or biopsy result positive for malignancy, and MELD score > 20. Five-year posttransplant recurrence free survival was 68%, and predictors of recurrence included elevated CA 19-9, portal vein encasement, and residual tumor on explant, with the latter being the most strongly associated

(hazard ratio 9.8). Pretreatment pathological confirmation of pCCA by conventional cytology or endoscopic biopsy is not a requisite as it does not result in lower rates of residual CCA in explants or lower recurrence after transplantation [38]. Despite its rigor, patients who underwent the neoadjuvant chemoradiation followed by liver transplantation protocol for pCCA reported excellent quality of life, at par or better than those who underwent liver transplantation for other indications [39].

Treatment of Advanced pCCA

Systemic chemotherapy is the mainstay of treatment for patients who are not candidates for curative resection or neoadjuvant therapy followed by liver transplantation. The combination of gemcitabine and cisplatin is the preferred regimen in this setting. Compared to gemcitabine alone, the addition of cisplatin confers a survival advantage with median progression free survival of eight months without a significant increase in toxicity [40]. Relief of biliary obstruction of jaundice is advocated to enhance patients' tolerability to chemotherapy. An optimal bilirubin level following endoscopic intervention with stenting is 2 mg/dL. In patients who fail to reach this level following stenting, repeat biliary intervention should be considered in 6 weeks if their pre-stent bilirubin level was 10 mg/dL or 3 weeks if the present bilirubin level was < 10 mg/dL [41]. Metal stents are a more durable and cost-effective option as they have a superior patency to plastic stents (5.56 months versus 1.86 months) with patients requiring less frequent procedures [42]. Accordingly, if a patient is not a candidate for curative surgical treatment and has a life expectancy of at least 4–6 months, then metal stents should be utilized [42].

Future Directions

Despite recent advances, diagnosis of pCCA at a stage early enough to warrant eligibility for curative treatment options remains a significant challenge. Survival rates for this highly lethal malignancy remain low, as treatment options are limited, particularly for inoperable tumors. The only effective treatment options are surgical resection and combined neoadjuvant therapy with liver transplantation for the subset of patients with early stage pCCA. Advances in diagnosis enabling early detection of pCCA are needed. Utilization of a stool assay for methylated genes and mutant Kirsten rat sarcoma viral oncogene homolog (*KRAS*) to diagnose pancreatic cancer was recently reported [43]. pCCA is known to have aberrant *KRAS* expression [44]. Hence, one can envision detection of CCA genetic signatures, such as mutant *KRAS*, in biological specimens including bile, serum, or stool.

Emerging evidence has highlighted the critical role of the tumor microenvironment in tumor progression, metastases, and therapeutic resistance [45]. An intense tumoral desmoplastic stroma with a striking accumulation of α -smooth muscle actin expressing cancer-associated fibroblasts (CAF) is a characteristic feature of pCCA. To date, therapeutic efforts have been directed at the cancer cell with limited consideration of the tumor stroma. Stromal depletion via selective targeting of CAF utilizing a BH3 mimetic has been reported recently [46]. Combining cancer cell directed therapy with stromal CAF deletion represents a novel therapeutic approach in pCCA (Figure 5).

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Abbreviations

5-FU	5-fluorouracil
CA 19-9	carbohydrate antigen 19-9
CAF	cancer-associated fibroblast
CCA	cholangiocarcinoma
CT	computed tomography
dCCA	distal cholangiocarcinoma
ERC	endoscopic retrograde cholangiography
EUS	endoscopic ultrasound
FISH	fluorescence in situ hybridization
FNA	fine-needle aspiration
KRAS	Kirsten rat sarcoma viral oncogene homolog
MELD	model of end-stage liver disease
MRCP	magnetic resonance cholangiopancreatography
MRI	magnetic resonance imaging
pCCA	perihilar cholangiocarcinoma
PSC	primary sclerosing cholangitis

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Figure 1. Perihilar cholangiocarcinoma (pCCA) growth patterns. The primary growth patterns in pCCA are intraductal, periductal infiltrating, and mass-forming subtypes.

Figure 2. Fluorescence in situ hybridization (FISH) analysis of biliary brushings. Each color signal denotes one chromosome. Normal or disomic cells have two signals of the same color. Cells with polysomy have three signals of the same color.

Figure 3.
Approach to management of abnormal FISH and cytology results.

Figure 4.

MRI images of pCCA mass with (A) Occlusion of the right anterior portal vein (black arrow) and narrowing of right posterior portal vein (white arrow), (B) right hepatic artery encasement, (C) separation of the right anterior and right posterior bile ducts (black arrow).

Figure 5.

Therapeutic Deletion of CAF in CCA. In the course of tumorigenesis, stromal fibroblasts acquire a modified phenotype and become activated or primed for apoptosis. Navitoclax, a BH-3 mimetic, causes apoptotic cell death in activated CAFs [46]. This targeted deletion of CAFs leads to secondary cancer cell apoptosis and reduction in tumor size.