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Review

Cholangiocarcinoma

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Summary
Liver cancer represents the third leading cause of cancer-related death worldwide. Cholangiocarcinoma (CCA) is the second most common type of liver cancer after hepatocellular carcinoma, accounting for 10-15% of all primary liver malignancies. Both the incidence and mortality of CCA have been steadily increasing during the last decade. Moreover, most CCAs are diagnosed at an advanced stage, when therapeutic options are very limited.
CCA may arise from any tract of the biliary system and it is classified into intrahepatic, perihilar, and distal CCA, according to the anatomical site of origin. This topographical classification also reflects distinct genetic and histological features, risk factors, and clinical outcomes. This review focuses on histopathology of CCA, its differential diagnoses, and its diagnostic pitfalls.

Key words: biliary, neoplasia, malignant, cholangiocarcinoma, subtypes

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Introduction

Cholangiocarcinoma (CCA) is a heterogeneous group of aggressive malignancies arising from different locations within the biliary tree. Depending on their anatomical site of origin, CCAs are classified into *intrahepatic* (iCCA), *perihilar* (pCCA), and *distal* CCA (dCCA), that differ for etiology, risk factors, prognosis, and clinical and therapeutic management. Gallbladder cancer and tumors arising in the ampulla of Vater are not included in this group. iCCA and pCCA taken together represent more than 90% of all CCAs worldwide [1-5].
CCA is the second most common primary hepatic malignancy after hepatocellular carcinoma (HCC), comprising about 15% of all primary liver tumors and 3% of all gastrointestinal cancers. CCA is a rare cancer, but its incidence and mortality rates are constantly increasing worldwide during the past decades. Globally, CCA has an incidence rate of 0.3-6/100,000 inhabitants per year, with a mortality rate of 1-6/100,000 inhabitants per year. Specific regions, such as South Korea, China, and Thailand, show a particularly high incidence rate, with more than 6 cases/100,000 habitants per year. The peak of incidence of CCA is between the fifth and the seventh decades of life, with a slight male predominance for iCCA. Some risk factors are shared by all CCA subtypes, while others are more specific for one subtype or for specific geographical regions [1-5] (Tab. I).
Regrettably, CCAs are often diagnosed at advanced stages, when therapeutic options are very limited. Margin-negative resection is the most

Table I. Main risk factors for intrahepatic and extrahepatic cholangiocarcinoma.

Intrahepatic CCA	Extrahepatic CCA (pCCA plus dCCA)
Cirrhosis	PSC
Chronic pancreatitis	Choledocal cysts
HBV and HCV	Caroli disease
Alcohol consumption	Choledocholitiasis and cholelithiasis
NAFLD	Liver fluke infection (*South-eastern Asia*)
Hepatolithiasis	IBD
Haemochromatosis	Diabetes and obesity
Diabetes and obesity	Chronic pancreatitis
Smoking	Gout
Congenital hepatic fibrosis	Smoking
Chemical exposure (i.e. thorotrast)	Chemical exposure (i.e.1,2-dichloropropane)

CCA: cholangiocarcinoma; pCCA: perihilar cholangiocarcinoma; dCCA: distal cholangiocarcinoma; HBV: hepatitis B virus; HCV: hepatitis C virus; NAFLD: non-alcoholic fatty liver disease; PSC: primary sclerosing cholangitis; IBD: inflammatory bowel disease.

important critical factor influencing prognosis, and is related to a better survival both in iCCA and pCCA/dCCAs. However, a significant proportion of patients presents with locally advanced and unresectable disease. iCCAs arising in non-cirrhotic livers show the worst prognosis [3,6-8].

Prognosis has not significantly improved in recent years, despite a deeper understanding of CCA pathogenesis thanks to advanced technologies, such as DNA and/or RNA sequencing. About 40% of CCA patients show targetable genetic alterations; however, rapid translation into clinical trials is limited, mainly due to the low number of patients [9-15].

This review focuses on histopathology of CCA, including its subtypes, differential diagnoses, and diagnostic pitfalls.

Intrahepatic cholangiocarcinoma

The WHO classification defines iCCA as a malignant intrahepatic epithelial neoplasm with biliary differentiation. It represents nearly 10-20% of all CCAs and arises from bile ductules to the second-order bile ducts (i.e. segmental bile ducts), proximal to the left and right hepatic ducts [3].

Many risk factors for iCCA are closely related to a chronic inflammation of the biliary epithelium and bile stasis (Tab. I). The prevalence of some of these risk factors, especially alcohol consumption and non-alcoholic fatty liver disease, is increasing worldwide, thus contributing to iCCA incidence rise. However, most of iCCAs occur in the absence of known risk factors and represent an incidental finding in around 20-25% of cases during imaging studies performed for other reasons [1,2,16].

In 60-70% of patients, iCCA is mostly identified as a single mass. Radiologic criteria can only suggest a possible iCCA diagnosis, and a definitive diagnosis can only be based on histology. In particular, histological confirmation of iCCA on liver biopsy is mandatory in cases of unresectable disease, to determine subsequent patient management [1,2,17].

iCCAs are usually asymptomatic in early stages. Jaundice is not frequent and generally associated with an advanced disease. Other non-specific symptoms, typically seen in advanced disease, include fatigue, abdominal pain, malaise, nausea, anorexia, and weight loss. CA19-9 is characteristically elevated [1,2,4,5,17].

Pathology

Macroscopically, iCCA may show 3 different growth patterns, named *mass forming* (MF type), *periductal infiltrating* (PI type), and *intraductal growing* (IG type), with the MF type being the most common one [2,3,18]. The IG type is not a recognized growth pattern of CCA by AJCC/UICC [1,2,4,5]. MF type iCCAs look like nodular mass lesions in the hepatic parenchyma; iCCAs with PI type grow longitudinally along the bile duct, as periductal nodular and sclerosing lesions, determining biliary strictures or obliterations, and eventually liver parenchymal invasion; IG type iCCAs display papillary growth towards the duct lumen, representing, in a majority of cases, the malignant progression of an intraductal papillary neoplasm of the bile duct (IPNB). MF type iCCA originates from peripheral small bile ducts while PI and IG type iCCAs arise from large intrahepatic bile ducts [18]. A few studies suggested that the PI type may be associated with a poor prognosis, but the prognostic significance of growth patterns remains controversial [19].

Histologically, iCCAs are usually well to moderately differentiated adenocarcinomas, with a ductal, tubular or cord-like pattern, and with variable, and often abundant, fibrous stroma. Two histological subtypes of iCCA are recognized: the *large duct type*, arising in the large intrahepatic bile ducts near the hepatic hilus, and the *small duct type*, which mainly occurs in the

hepatic periphery [3,20]. It is worth noting that the histological subtype reflects the high molecular heterogeneity of iCCAs and can be ascribed to different cells of origin and pathogenesis. Hepatic stem or progenitor cells and cuboidal cholangiocytes are the putative cells of origin of small duct type iCCAs, while large duct iCCAs seem to derive from columnar mucous cholangiocytes or peribiliary glands [1].

Large duct iCCAs histologically resemble pCCA or dCCA. They are composed of large, irregular, dilated glands, embedded in an often abundant fibrous stroma, characterized by dense connective tissue with loose spindle cells, hyalinized or sclerotic collagen fibers, and disorganized blood vessels. Cancer cells are cuboidal or columnar, with atypical hyperchromatic nuclei, and frequent mucus secretion (Fig. 1). Typical features of large duct iCCA include extensive portal infiltration, perineural and lymphatic invasion (Fig. 2), papillary structures, and features of intraductal dysplasia. Lymph node metastases are also common. Large duct iCCAs often evolve from pre-invasive lesions, including biliary intraepithelial neoplasia (BiIIN) and IPNB. Differently from small duct iCCA, tumor cells show S100P and trefoil factor 1 expression [3,18,20] (Fig. 3).

As already mentioned, small duct iCCAs show a MF type growth pattern, appearing as whitish or grey nodular lesions in the peripheral hepatic parenchyma. They are composed of small, cuboidal cells with uniform round nuclei, arranged in small sized tubular or acinar structures, with no mucin production (Fig. 4). Less differentiated areas display solid, cord-like, or cribriform patterns. Advanced lesions may show highly sclerotic and hypovascular central areas, with a more solid growth at the periphery. No defined precursor lesions have been reported for small duct iCCAs [3,18,20-22]. All iCCAs are graded as well-, moderately, or poorly differentiated adenocarcinomas, according to their cell morphology [3].

Histological subtypes of small-duct iCCA include *cholangiolocarcinoma* (CLC) (formerly considered a subtype of combined hepatocellular-cholangiocarcinoma) and *iCCA with ductal plate malformation pattern*. CLC closely resembles the ductular reaction seen

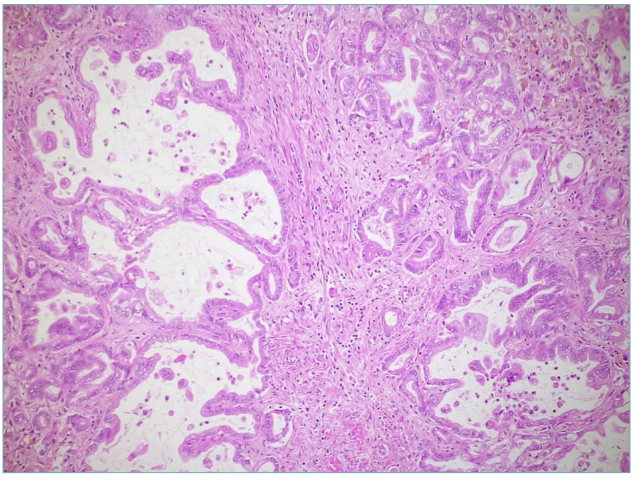

Figure 1. Large duct intrahepatic cholangiocarcinoma is composed of large irregularly dilated glands, embedded in abundant fibrous stroma. Note neoplastic cell mucus secretion (hematoxylin-eosin; original magnification 10x).

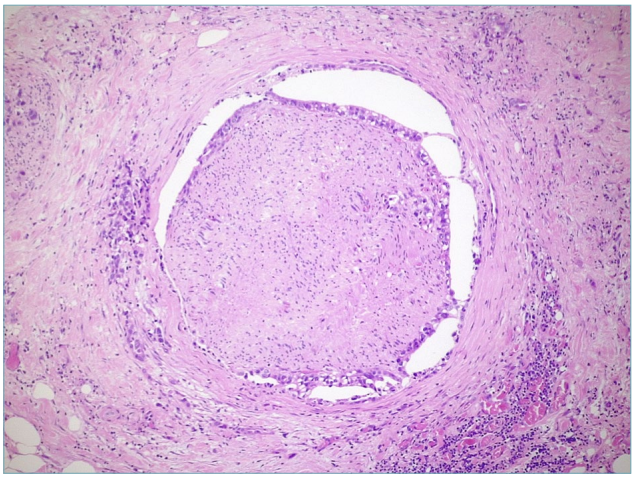

Figure 2. An example of perineural invasion in a large duct intrahepatic cholangiocarcinoma (hematoxylin-eosin; original magnification 10x).

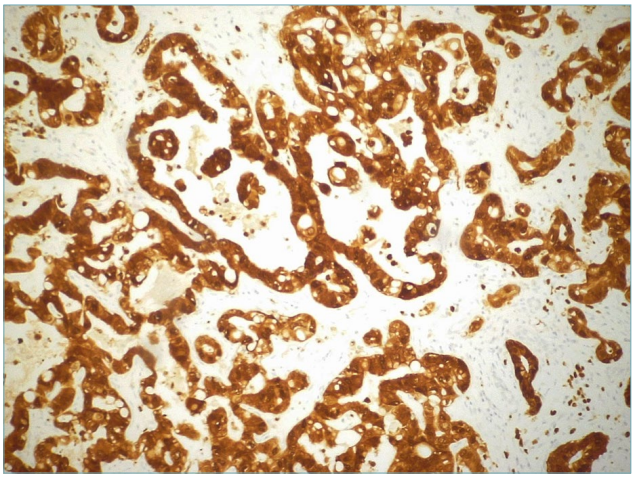

Figure 3. A diffuse S100P expression in a large duct intrahepatic cholangiocarcinoma (S100P immunostain; original magnification 20x).

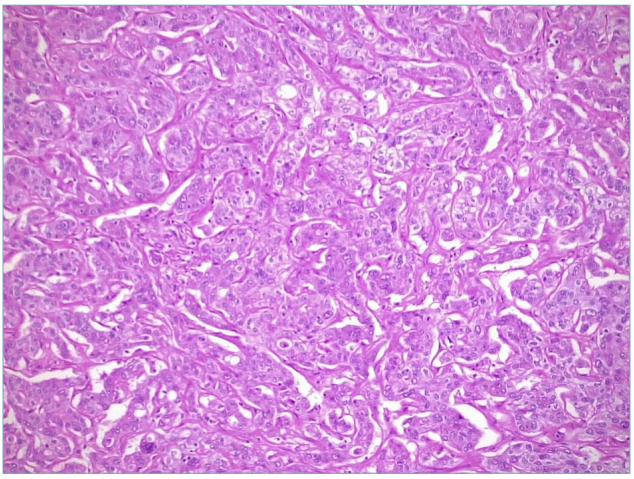

Figure 4. Small duct intrahepatic cholangiocarcinoma is composed of cuboidal cells arranged in small sized tubular or acinar structures, with areas of solid growth pattern, and with no mucin production (hematoxylin-eosin; original magnification 10x).

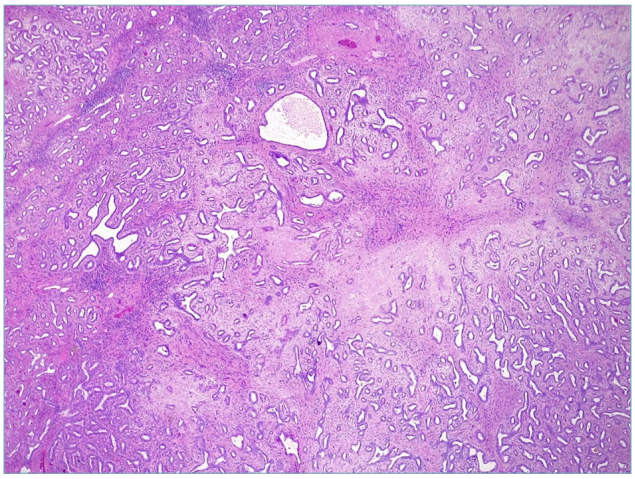

Figure 6. An intrahepatic cholangiocarcinoma with ductal plate malformation pattern, with tumor structures that look like ductal plate malformation, within a dense fibrotic stroma (hematoxylin-eosin; original magnification 2.5x).

in chronic cholangiopathies, and is diagnosed when more than 80% of ductular configuration is present. In this subtype, malignant ductular-like structures seem to radiate from a portal tract or surround it, in a tubular, cord-like, anastomosing "antler-like" pattern, within a dense and hyalinized fibrotic stroma (Fig. 5). Tumor cells are smaller and cuboidal, with round to oval nuclei and scant cytoplasm. They often show immunohistochemical expression of CD56 (NCAM) and EMA [3,23,24]. iCCA with ductal plate malformation pattern is composed of tumor structures that look like ductal plate malformation, with common presence of inspissated bile, in a dense fibrotic stroma (Fig. 6). Neoplastic cells are benign-looking, resembling biliary epithelial cells. Like CLC, they may stain positive for CD56 (NCAM) and EMA [3,25].

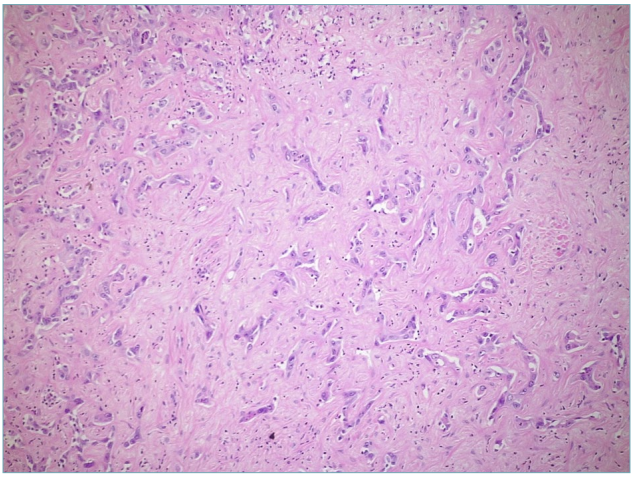

Figure 5. Cholangiolocarcinoma is made of malignant ductular-like structures, arranged in tubular, cord-like, anastomosing "antler-like" pattern, in a dense hyalinized fibrotic stroma (hematoxylin-eosin; original magnification 10x).

iCCA VARIANTS

Rare variants of iCCA include *squamous* or *adenosquamous* carcinoma, *lymphoepithelioma-like* carcinoma related to Epstein-Barr virus infection, and *sarcomatous* carcinoma. The latter demonstrate areas of mesenchymal morphology, such as spindle or rhabdoid cell, in association with glandular areas, and are more aggressive than conventional CCAs [3,26] (Fig. 7). A putative novel variant of iCCA, mimicking a neuroendocrine tumor, has been recently described and named *cholangioblastic cholangiocarcinoma*, due to the presence of blastemal-like areas within the tumor. Histologically, it shows a trabecular and solid/hepatoid growth pattern, with immunohistochemical expression of cytokeratin (CK) 7 and CK19, chromogranin A and/or synaptophysin, and a strong and diffuse expression of inhibin A (Fig. 8). Only a few cases have been described so far, all with an aggressive clinical course, with recurrence and metastasis to the peritoneum, liver, and lungs. The molecular profile of these tumors showed alterations in the *TGF*β and *WNT* signaling pathways, known to regulate ductal plate development [27].

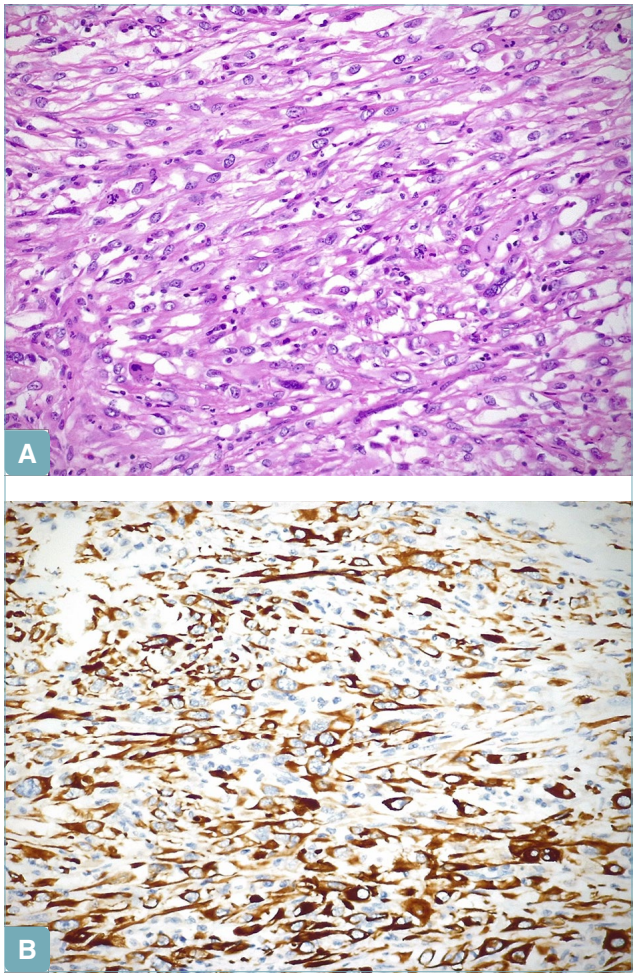

Figure 7. A sarcomatoid intrahepatic cholangiocarcinoma, with diffuse spindle cell morphology. Numerous mitotic figures are present (A). As conventional intrahepatic cholangiocarcinoma, neoplastic cells diffusely express cytokeratin 7 (B) (A: hematoxylin-eosin; original magnification 20x; B: cytokeratin 7 immunostain; original magnification 20x).

iCCA DIFFERENTIAL DIAGNOSES

iCCA diagnosis can be challenging due to several different mimickers, including benign and malignant lesions [3,22]. Therefore, clinical and histological features should be accurately considered dealing with an intrahepatic mass. Several immunohistochemical stains are available, and may help in leading to a definite diagnosis in most cases. However, none is accurate in differentiating benign from malignant lesions, and the expression of most of these markers, even the lineage-specific ones, frequently overlap among different lesions. Thus, it is highly recommended to always use a panel of multiple immunohistochemical markers in clinical practice.

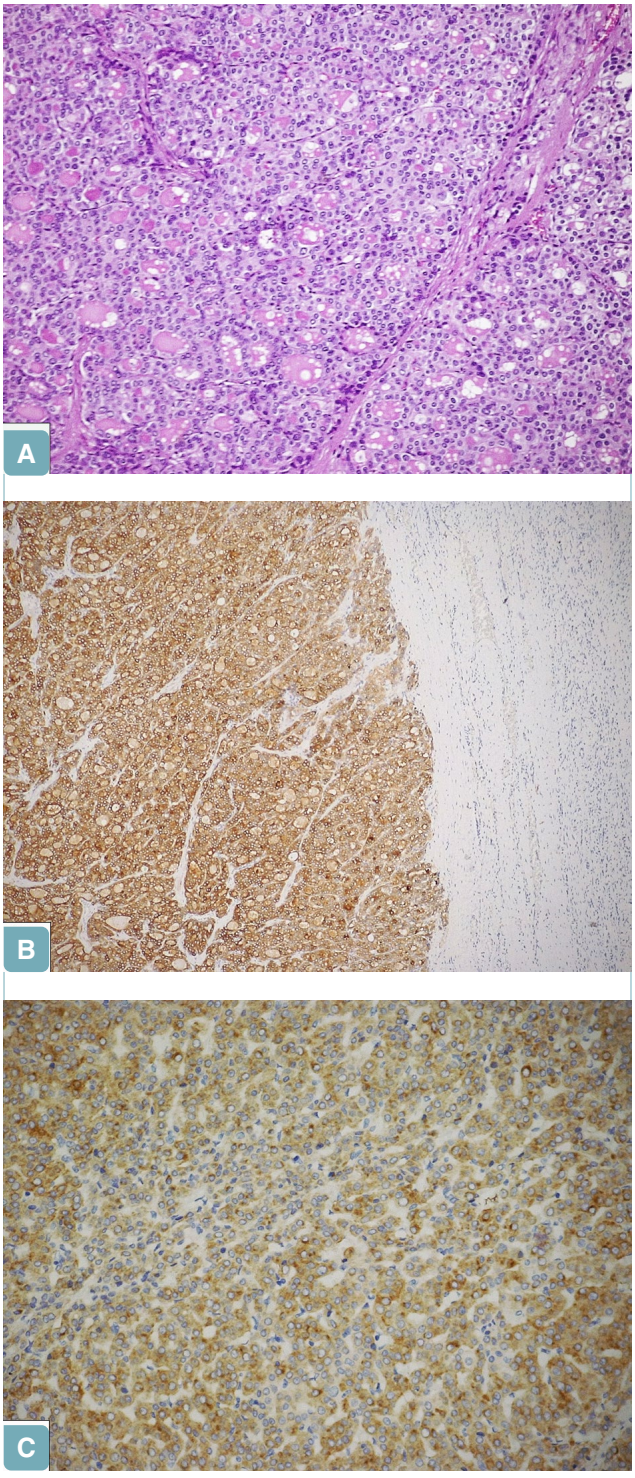

Figure 8. Cholangioblastic variant of intrahepatic cholangiocarcinoma (A). Tumor cells diffusely express inhibin A (B) and show a granular cytoplasmic positivity for chromogranin A (C) (A: hematoxylin-eosin; original magnification 10x; B: inhibin A immunostain; original magnification 5x; C: chromogranin A immunostain; original magnification 20x).

iCCA versus benign bile duct lesions

Morphology and clinical history is usually helpful in differentiating iCCA from benign lesions [17]. However, the distinction between a well-differentiated iCCA and its benign mimics can be challenging, particularly in small biopsies with scant cellularity. All biliary lesions share the CK immunohistochemical profile, since they all stain positive for CK7 and CK19, with variable expression of CK20. A high proliferative index favors iCCA; in fact Tsokos et al. found an average Ki-67 expression of 23% in iCCA versus 1.4% in all biliary benign lesions [28]. However, a low Ki-67 expression does not always exclude iCCA. p53 and p16 can be used in combination to distinguish iCCA from benign lesions, particularly bile duct adenomas (BDAs). p53 usually shows a strong and diffuse expression in malignant lesions (Fig. 9A), even if no general consensus exists regarding the interpretation of staining results. p16 is constantly expressed in BDAs, but not in iCCAs. Therefore, a negative p16 staining supports iCCA diagnosis [29] (Fig. 9B). Promising results have also been recently obtained by using DNA flow cytometry on formalin-fixed and paraffin-embedded tissue from bile duct biopsies. In this study, a high rate of aneuploidy (70%) was observed in malignant cases, while a normal DNA content was found in all benign lesions [30].

iCCA versus metastatic adenocarcinoma

The main purpose of liver biopsy in the setting of malignancies arising in a non-cirrhotic liver is the differential diagnosis between iCCA and metastatic tumor (Tab. II). Indeed, secondary liver cancers are much more frequent than iCCA. The most common neoplasms that metastasize to the liver are colorectal carcinoma, breast carcinoma, neuroendocrine tumors, lung carcinoma, and gastric carcinoma. Clinical information is fundamental to guide the diagnostic approach. In case of an adenocarcinoma with unclear histological features in a patient with unknown extrahepatic primary tumors, the performance of different immunohistochemical panels is recommended.

Colorectal adenocarcinoma (CRC) typically shows a CK20- and CDX2-positive and CK7-negative immunophenotype. CDX2 is a highly sensitive and specific marker of intestinal differentiation. It may be expressed by iCCA, but never as diffuse and strong as in CRC. Be aware that CDX2 might be negative in some poorly differentiated CRCs. Special AT-rich sequence-binding protein 2 (SATB2) is another specific marker for intestinal and appendiceal adenocarcinoma, with a higher expression in well-to-moderately than in poorly differentiated CRC [31,32].

Gastric adenocarcinoma may be difficult to differentiate from iCCA, mostly of the large duct type. CK7-positivity and CK20-negativity represent the most common immunoprofile of gastric carcinoma, although some cases may stain positive for CK20. Both gastric carcinoma and iCCA show CK19 expression. CDX2 may be of help, since it is strongly expressed in about 60% of gastric adenocarcinoma [33].

Breast cancer is usually CK7-positive and CK20-negative. Estrogen/progesterone receptors, gross cystic disease fluid protein-15 (GCDFP-15) and mammaglobin are useful markers to exclude breast cancer liver metastasis. However, GCDFP-15 and mammaglobin have a high specificity but low sensitivity [34]. Nuclear expression of GATA3 has been recently reported as a sensitive marker for breast cancer [35].

As reported in the 5th edition of the WHO blue book,

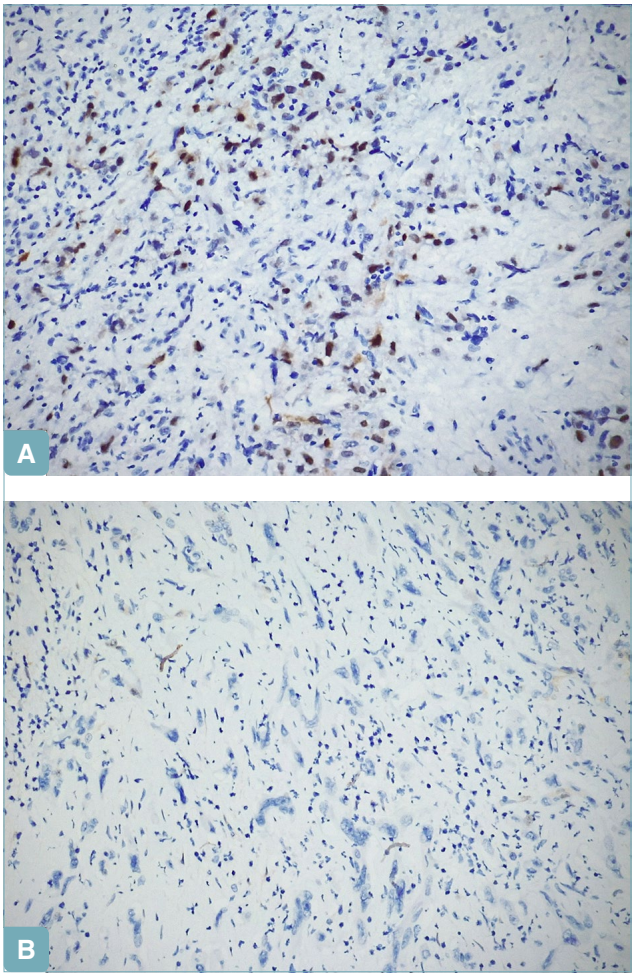

Figure 9. Differently from benign lesions, intrahepatic cholangiocarcinoma shows a strong and diffuse nuclear expression of p53 (A), and a complete absence of p16 (B) (A: p53 immunostain; original magnification 20x; B: p16 immunostain; original magnification 20x).

Table II. Immunohistochemical markers useful in the differential diagnosis between intrahepatic cholangiocarcinoma and metastatic tumors.

Markers	iCCA	CRC	GA	BC	NEN	LA	PDA
CK7	+	-	+	+	+/-	+	+
CK20	-/+	+	-/+	-	+/-	-	-/+
CDX2	-/+	+	+/-	-	(+[&])	-	-/+
SATB2	-	+	-	-	-	-	-
CK19	+	-	+	-	-	-	+
Breast markers*	-	-	-	+	-	-	-
Neuroendocrine markers[#]	-	-[§]	-	-	+	-	-
TTF1	-	-	-	-	(+[&])	+	-
Napsin A	-	-	-	-	-	+	-

*: Estrogen/progesterone receptors, gross cystic disease fluid protein-15, mammaglobin, GATA3;
[#]: chromogranin A, synaptophysin, CD56; [§]: in non-neuroendocrine carcinoma; [&]: in metastatic neuroendocrine neoplasms, depending on their organ of origin (large bowel/lungs).
iCCA: intrahepatic cholangiocarcinoma; CRC: colorectal carcinoma; GA: gastric adenocarcinoma; BC: breast carcinoma; NEN: neuroendocrine neoplasms; LA: lung adenocarcinoma; PDA: pancreatic ductal adenocarcinoma; CK: cytokeratin; SATB2: special AT-rich sequence-binding protein 2; TTF1: thyroid transcription factor 1.

neuroendocrine neoplasms (NENs) may occur as primary liver tumors, even if extremely rare and much less common than the metastatic ones [3]. Therefore, to define a liver neuroendocrine tumor as primary, metastasis from other organs must be thoroughly excluded. The differential diagnosis between metastatic NEN and iCCA is based on tissue expression of neuroendocrine markers, such as chromogranin A, synaptophysin, and CD56, which are usually absent or only focally expressed in iCCA. Primary and metastatic NENs are histologically and immunohistochemically undistinguishable, although lineage-specific markers, such as CDX2 or thyroid transcription factor 1 (TTF1), may be of help in defining the organ of origin in well-differentiated tumors.

Lung adenocarcinoma typically shows immunoreactivity for CK7, TTF-1, and napsin A. TTF1 is less frequently expressed in invasive mucinous adenocarcinomas and in adenocarcinomas with solid pattern, but it is always absent in iCCA.

Pancreatic ductal adenocarcinoma metastatic to the liver is impossible to distinguish from large duct iCCA, both by morphology and immunohistochemical profile [3,36,37]. Clinical history and imaging must be considered.

iCCA versus hepatocellular carcinoma

Differential diagnosis between iCCA and HCC is usually straightforward. However, in poorly differentiated lesions, when conventional histology does not allow a definite differential, a panel of immunohistochemical stains can be of help, and it should include hepatocyte markers (Arginase-1, HepPar-1), biliary cytokeratins (CK7 and CK19), polyclonal carcinoembryonic antigen (p-CEA), CD10, and Glypican-3 [3,21]. A mucin stain may be also useful, but it is important to remind that not all iCCA produce mucin. Arginase-1 is the most sensitive (> 90%) and highly specific marker for HCC, including poorly differentiated and scirrhous HCC [38,39]. However, it may rarely be observed in other tumors, including some poorly differentiated cholangiocarcinoma. Again, HepPar1 has a low sensitivity in poorly differentiated HCC, as well as p-CEA and CD10, whose canalicular pattern of staining is classically considered a specific marker of hepatocellular differentiation [40]. Glypican-3 is an oncofetal protein expressed in most HCC, with higher sensitivity for poorly differentiated tumors. Nevertheless, it is not a lineage marker and several tumor types may express it. Luckily, Glypican-3 expression is uncommon in iCCA, therefore it is a useful tool in the differential diagnosis with HCC. iCCA typically stains with CK7 and CK19. However, while a CK7- and CK19-negative tumor is unlikely to be an iCCA, their expression does not necessarily point towards a biliary differentiation [39].

iCCA versus epithelioid emangioendothelioma

Epithelioid hemangioendothelioma (EHE) is a rare malignant vascular neoplasia, which may occur in the liver. It is composed of epithelioid cells within a myxoid-hyaline or fibrous stroma [3]. Neoplastic cells may show intracytoplasmic vacuoles mimicking mucin vacuoles of an adenocarcinoma, and the presence of a dense fibrous stroma may lead to an incorrect diagnosis of poorly differentiated iCCA. However, differently from iCCA, EHE neoplastic cells are consistently positive for one or more endothelial markers, including ERG, CD31, CD34 and FLI1. The main pitfall in this differential is represented by the aberrant expression of cytokeratins in many EHEs [41,42]. In a recent study,

CK7- and panCK AE1/AE3-positivity was reported in 5/9 (56%) and 6/9 (67%) of hepatic EHEs, respectively [43]. Thus, keeping this in mind is fundamental for preventing misdiagnosis of EHEs as iCCAs.

Perihilar and distal cholangiocarcinoma

pCCA is the most common CCA (50-60% of cases) and develops from the extrahepatic biliary tree, proximally to the origin of cystic duct (right and/or left hepatic duct and/or at their junction). dCCA (20-30% of all CCAs) involves the extrahepatic bile ducts, distally to the insertion of cystic duct (common bile duct, i.e. choledochus) [1-3,44]. Recent guidelines recommend to avoid the use of the terms extrahepatic-CCA for dCCA or Klatskin tumor for pCCA [1-3,44].

All available studies on CCA epidemiology are based on the old CCA classification, which divided CCA into intra- and extra-hepatic; thus, data on pCCA-specific incidence and risk factors are still too scant. The most frequent symptom in pCCA and dCCA is jaundice due to biliary tract obstruction. CA19-9 is typically elevated [1,2]. In these tumors, the association of contrast-enhanced magnetic resonance and magnetic resonance-cholangiopancreatography is the first diagnostic tool, due to its accuracy in discriminating between benign and malignant obstruction, as well as in assessing the degree of biliary extension. However, like iCCA, a definitive diagnosis can only be based on pathologic confirmation. In particular, recent guidelines suggest endoscopic ultrasound-fine needle aspiration or biopsy (EUS-FNA/B) as the first approach, followed by endoscopic retrograde cholangiopancreatography with brushing and/or biopsy and/or cholangioscopy-guided biopsy of a target lesion, when EUS-FNA/B is inconclusive, since they have been considered the most accurate techniques to obtain a final diagnosis of pCCA and dCCA [1-3,45].

Pathology

Tumors near the hepatic hilum and dCCA are usually small, since they cause early obstructive jaundice. Frequently, the macroscopic boundaries of pCCA and dCCA are blurred and difficult to determine. Grossly, both pCCA and dCCA may present as flat or poorly-defined nodular sclerosing masses or, less frequently, as intraductal papillary tumors [2,3].

The vast majority of pCCA and dCCA are mucin- secreting adenocarcinomas characterized by widely spaced, well-formed irregular glands and small cell clusters, within a desmoplastic sclerotic stroma (Fig. 10). They often show perineural and lymphovascular invasion. Most of them are of pancreatobiliary-type, but other histological patterns include the intestinal-type, the foveolar-type, the mucinous, the signet ring cell, the clear cell, the pyloric gland, the hepatoid, and the invasive micropapillary ones [2,3,46-48]. pCCA and dCCA are graded as well-, moderately, or poorly differentiated adenocarcinomas, according to their cell morphology and gland formation [3]. Rare subtypes include squamous, adenosquamous, and sarcomatoid carcinoma. As for large duct iCCAs, pCCAs and dCCAs are often preceded by pre-invasive lesions, including BilIN and IPNB [3,21,22].

In bile and brush cytology, the presence of epithelial cells with prominent nucleoli, thickening and irregularity of nuclear membrane, and increased chromatin is

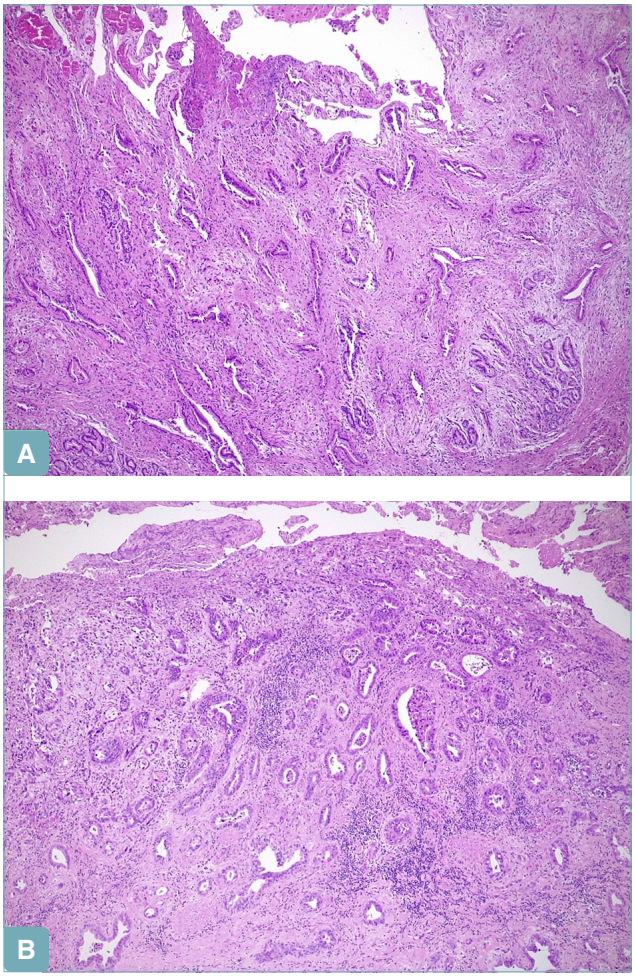

Figure 10. Perihilar (A) and distal (B) cholangiocarcinomas share similar morphological features, being characterized by well-formed irregular neoplastic glands in a desmoplastic stroma (A: hematoxylin-eosin; original magnification 5x; B: hematoxylin-eosin; original magnification 5x).

diagnostic for malignancy. Tumor cells may show different degree of pleomorphism, mitotic activity, and loss of nuclear polarity. The distinction between invasive and in situ carcinoma is not possible on cytological smears [3,49].

The distinction between pCCAs/dCCAs and reactive periductal glands is the main differential issue, since it is not always straightforward on morphology alone. Clinical history and imaging must always be considered. Involvement of extrahepatic bile ducts by pancreatic duct adenocarcinoma is indistinguishable from CCA, both by morphology and immunohistochemical profile [3,36,37].

Molecular background of cholangiocarcinoma

Many molecular alterations have been recently described in CCAs, but full molecular profiling or gene mutation analyses are not yet routinely recommended, since they do not currently result in any improvement in patient management [11,12,14,15,22].

Small duct type iCCA show frequent *IDH1* and *IDH2* mutations (10-20%), associated with poor prognosis, and *FGFR2* fusions (8-14%), associated with a better prognosis, both representing possible therapeutic targets. On the contrary, *KRAS* and *TP53* have been demonstrated in large duct iCCAs, pCCAs, and dCCAs. *TP53* mutations are present in about 50% of pCCAs and dCCAs and are a late pathogenic event, while *KRAS* mutations occur early in 20-30% of dCCAs. dCCAs may show *MDM2* amplification in 12% of cases [50,51]. Other genes frequently mutated in CCAs are those involved in chromatin remodelling, such as *ARID1* in pCCA and dCCA, and *BAP1* in iCCA [3,11,12,14,52]. A molecular classification of CCA has been recently proposed, with different subclasses showing different features and prognosis [10,53] (Tab. III). Lately, a multi-platform molecular characterization of extrahepatic CCAs (pCCAs plus dCCAs) has been performed in a cohort of 189 patients, revealing four novel transcriptome-based molecular classes and identifying about 25% of tumors with actionable genomic alterations, with potential prognostic and therapeutic implications [54].

Cholangiocarcinoma staging

Staging for iCCA, pCCA, and dCCA is based on the 8th edition of AJCC staging system [44] (Tabs. IV-VI).

Pathological report of resected cholangiocarcinoma

A standardized approach to cancer reporting is highly recommended in resected CCAs, as in any tumor setting. A comprehensive and accurate pathology report is a prerequisite to adequate cancer staging and outcome prediction. Nowadays, cancer reports must include many elements necessary for clinical management, and with the advent of targeted therapies and personalized medicine, its complexity is even significantly increasing [55]. It has been demonstrated that the adoption of histopathological reporting models lead to improvements in the reporting of key prognostic factors by pathologists [56,57].

The International Collaboration on Cancer Reporting (ICCR) is an alliance formed by the Royal College of Pathologists of Australasia, the Royal College of Pathologists of the United Kingdom, the College of American Pathologists, the Canadian Partnership Against Cancer, the European Society of Pathology, and the American Society of Clinical Pathology, with the aim to develop an evidence-based reporting data set for each cancer site (http://www.iccr-cancer.org/). Lately, the ICCR data set for reporting liver tumors, including iCCA and pCCA, has been updated and it is now freely available for worldwide use at the ICCR website (http://www.iccr-cancer.org/datasets/published-datasets/digestive-tract/liver). This dataset includes items agreed to be essential to the pathological reporting, but additional data may be included according to local needs and to guarantee clarification.

Table III. A recently proposed molecular classification of cholangiocarcinoma [53]. Specific genetic alterations are related to anatomical and histomorphological classifications of cholangiocarcinoma.

SBD iCCA-specific	iCCA-specific	Shared by LBD iCCA and eCCA[#]	eCCA-specific[#]
IDH1/2	*EPHA2*	*KRAS*	*ARID1B*
FGFR2	*BAP1*	*TP53*	*PRKACA*
		SMAD4	*BRAF*
		GNAS	*MDM2*
		NRAS/MRAS	

[#]: eCCA includes perihilar and distal cholangiocarcinoma.

SBD: small bile duct; iCCA: intrahepatic cholangiocarcinoma; LBD: large bile duct; eCCA: extrahepatic cholangiocarcinoma.

Table IV. TNM staging classification of intrahepatic cholangiocarcinoma[44].

T - Primary tumor	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ (intraductal tumor)
T1a	Solitary tumor ≤ 5 cm in greatest dimension WITHOUT VI
T1b	Solitary tumor > 5 cm in greatest dimension WITHOUT VI
T2	Solitary tumor WITH intrahepatic VI *OR* multiple tumors with or without VI
T3	Tumor perforating the visceral peritoneum
T4	Tumor involving local extrahepatic structures by direct hepatic invasion
N - Regional lymph nodes*	
Nx	Regional lymph nodes cannot be assessed
N0	NO regional lymph nodes metastasis
N1	Regional lymph nodes metastasis
M - Distant metastasis	
M0	NO distant metastasis
M1	Distant metastasis

*: in a regional lymphadenectomy specimen, ≥6 lymph nodes should be histologically evaluated.
VI: vascular invasion.

Table V. TNM staging classification of perihilar cholangiocarcinoma[44].

T - Primary tumor	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ (intraductal tumor)
T1	Tumor confined to the bile duct, extension up to the muscle layer or fibrous tissue
T2a	Tumor invades beyond the wall of the bile duct to surrounding adipose tissue
T2b	Tumor invades adjacent hepatic parenchyma
T3	Tumor invades unilateral branches of the portal vein or hepatic artery
T4	Tumor invades the main portal vein or its branches bilaterally *OR* the common hepatic artery *OR* unilateral second-order biliary radicals with contralateral portal vein or hepatic artery involvement
N - Regional lymph nodes*	
Nx	Regional lymph nodes cannot be assessed
N0	NO regional lymph nodes metastasis
N1	Metastases to 1-3 regional lymph nodes
N2	Metastases to ≥4 regional lymph nodes
M - Distant metastasis	
M0	NO distant metastasis
M1	Distant metastasis

*: in a regional lymphadenectomy specimen, ≥15 lymph nodes should be histologically evaluated.

We strongly support the use of this dataset in the everyday routine management of CCA, as a guide to ensure that any important data could not be missed in the final histological report.

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Table VI. TNM staging classification of distal cholangiocarcinoma [44].

T - Primary tumor	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ (intraductal tumor)
T1	Tumor invades bile duct wall to a depth <5 mm
T2	Tumor invades bile duct wall to a depth of 5 mm up to 12 mm
T3	Tumor invades bile duct wall to a depth >12 mm
T4	Tumor involves the coeliac axis, the superior mesenteric artery and/or the common hepatic artery
N - Regional lymph nodes*	
Nx	Regional lymph nodes cannot be assessed
N0	NO regional lymph nodes metastasis
N1	Metastases to 1-3 regional lymph nodes
N2	Metastases to ≥4 regional lymph nodes
M - Distant metastasis	
M0	NO distant metastasis
M1	Distant metastasis

*: in a regional lymphadenectomy specimen, ≥12 lymph nodes should be histologically evaluated.

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