Received: 17 January 2019 | Revised: 26 February 2019 | Accepted: 27 February 2019

DOI: 10.1111/liv.14093

REVIEW ARTICLE

Liver INTERNATIONAL WILEY

Anatomical, histomorphological and molecular classification of cholangiocarcinoma

Timothy Kendall[1*] | Joanne Verheij[2*] | Eugenio Gaudio[3] | Matthias Evert[4] | Maria Guido[5] | Benjamin Goeppert[6†] | Guido Carpino[7†]

[1]Division of Pathology, University of Edinburgh, Edinburgh, UK

[2]Department of Pathology, Cancer Center Amsterdam, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands

[3]Department of Anatomical, Histological, Forensic Medicine and Orthopedics Sciences, Sapienza University of Rome, Rome, Italy

[4]Institute of Pathology, University of Regensburg, Regensburg, Germany

[5]Department of Medicine – DIMED, University of Padova, Padova, Italy

[6]Department of Pathology, University Hospital Heidelberg and Liver Cancer Center Heidelberg (LCCH), Heidelberg, Germany

[7]Department of Movement, Human and Health Sciences, Division of Health Sciences, University of Rome "Foro Italico", Rome, Italy

Correspondence
Guido Carpino, Department of Movement, Human and Health Sciences, Division of Health Sciences, University of Rome "Foro Italico", Rome, Italy.
Email: guido.carpino@uniroma1.it

Funding information
European Cooperation in Science and Technology, Grant/Award Number: CA18122

Abstract

Cholangiocarcinoma constitutes a heterogeneous group of malignancies that can emerge at any point of the biliary tree. Cholangiocarcinoma is classified into intrahepatic, perihilar and distal based on its anatomical location. Histologically, conventional perihilar/distal cholangiocarcinomas are mucin-producing adenocarcinomas or papillary tumours; intrahepatic cholangiocarcinomas are more heterogeneous and can be sub-classified according to the level or size of the displayed bile duct. Cholangiocarcinoma develops through multistep carcinogenesis and is preceded by dysplastic and in situ lesions. Definition and clinical significance of precursor lesions, including biliary intraepithelial neoplasia, intraductal papillary neoplasms of the bile duct, intraductal tubulopapillary neoplasms and mucinous cystic neoplasm, are discussed in this review. A main challenge in diagnosing cholangiocarcinoma is the fact that tumour tissue for histological examination is difficult to obtain. Thus, a major clinical obstacle is the establishment of the correct diagnosis at a tumour stage that is amenable to surgery which still represents the only curable therapeutic option. Current standards, methodology and criteria for diagnosis are discussed. Cholangiocarcinoma represents a heterogeneous tumour with regard to molecular alterations. In intrahepatic subtype, mainly two distinctive morpho-molecular groups can currently be discriminated. Large-duct type intrahepatic cholangiocarcinoma shows a high mutation frequency of oncogenes and tumour suppressor genes, such as KRAS and TP53 while Isocitrate Dehydrogenase 1/2 mutations and Fibroblast Growth Factor Receptor 2-fusions are typically seen in small-duct type tumours. It is most important to ensure the separation of the given anatomical subtypes and to search for distinct subgroups within the subtypes on a molecular and morphological basis.

Abbreviations: AFP, Alpha-fetoprotein; BilIN, biliary intraepithelial neoplasm; CCA, Cholangiocarcinoma; cHCC-CCA, combined HCC-CCA; CLC, cholangiolocarcinoma; dCCA, distal CCA; ERCP, endoscopic retrograde cholangiopancreatography; FISH, fluorescence in situ hybridization (FISH); HCC, hepatocarcinoma; HepPar-1, hepatocyte in paraffin 1; HISORt, histology, imaging, serology, other organ involvement, response to treatment; iCCA, intrahepatic CCA; IG, intraductal growing; IPNB, intraductal papillary neoplasm of the bile duct; ITPN, intraductal tubulopapillary neoplasms; MCN, mucinous cystic neoplasm; MF, mass-forming; PBG, peribiliary glands; pCCA, perihilar CCA; PI, periductal infiltrating; PSC, Primary Sclerosing Cholangitis; WHO, World Health Organization's.

*These authors share first co-authorship.

†These authors share last co-authorship.

KEYWORDS
cholangiocarcinoma, diagnosis, molecular profile, preneoplastic lesion

1 | ANATOMICAL AND HISTOLOGICAL APPEARANCE OF CHOLANGIOCARCINOMA

Cholangiocarcinoma (CCA) constitutes a heterogeneous group of malignancies that can emerge at any point of the biliary tree.[1] It can arise from epithelial cells in the biliary surface epithelium (ie cholangiocytes) and in peribiliary glands,[2] and possibly also from progenitor cells or even mature hepatocytes.[3]

Key points

- Gross and histological classification of cholangiocarcinoma is based on the anatomy and histology of the biliary tree.
- Specific precursor lesions with variable rates of malignant progression are recognised.
- Adequate diagnosis of CCA may be challenging in daily practice and relevant issues for diagnosis are discussed
- Molecular alterations in cholangiocarcinoma are heterogeneous and correspond to morphological subtypes
- Efforts should be put in developing a classification which combines molecular profile with histo-morphological aspects.

1.1 | Anatomy of the biliary tree

The biliary tree system is highly heterogeneous and varies in size and morphology, ranging from the canals of Hering to the choledochus.[4,5] The biliary tree can be subdivided into intrahepatic and extrahepatic parts (Figure 1A).[4,6] The intrahepatic biliary tree starts at the level of canals of Hering, which connect bile canaliculi between adjacent hepatocytes to bile ductules and interlobular bile ducts.[7] Interlobular bile ducts continue into septal, area and segmental bile ducts. Based on their size, interlobular and septal bile ducts are considered as small intrahepatic bile ducts (<300 μm in diameter); whereas area and segmental are considered as large intrahepatic bile ducts (>300 μm in diameter).[6,8] Small and large intrahepatic ducts further differ in terms of histological and embryological features.[6,8] Small intrahepatic ducts are lined with small and cuboidal-shaped cholangiocytes while the surface epithelium of large ducts is composed of tall and cylindric cholangiocytes and variably contains mucin-producing cells.[6,8] A unique feature of large intrahepatic bile ducts is the presence of glands within the duct wall, known as glands of the biliary tree or peribiliary glands (PBGs).[9,10] Embryologically, small intrahepatic bile ducts originate from the remodelling of the ductal plate, while large ducts derive from the elongation of hepatic ducts at liver hilum.[5] The extrahepatic biliary tree comprises the right and left hepatic ducts, the common hepatic duct, the bile duct (ie choledochus), the cystic duct and the gallbladder.[6] The proximal portion of the extrahepatic bile duct is collectively called the 'perihilar bile ducts'.[4,6,11,12] Peribiliary glands are physiologically distributed along extrahepatic bile ducts and are more numerous in perihilar ducts compared to the choledochus.[13]

1.2 | Anatomical classification of CCA, gross morphology and growth patterns

The classification of CCAs is based on the gross anatomy and histology of the biliary tree.[1] On the basis of anatomical location (Figure 1B), CCA is classified into intrahepatic CCA (iCCA), perihilar CCA (pCCA) and distal CCA (dCCA).[11,14] Anatomically, iCCA is defined as a malignancy located in the periphery of the second-order bile ducts; thus it can arise from segmental bile ducts to smaller branches of the intrahepatic biliary tree.[11] pCCA arises in the right and/or left hepatic duct and/or at their junction[15]; it is clinically also known as Klatskin tumour,[16] although this term should be discouraged. dCCA involves the common bile duct.[1,17]

Intrahepatic CCA can present three main patterns of growth based on its gross appearance (Figure 1C): mass-forming (MF), periductal infiltrating (PI) and intraductal growing (IG).[1,15] MF-type is the most common growth pattern, accounting for about 65% of all iCCA[18]; it presents as a mass lesion in the hepatic parenchyma; MF-type iCCA is generally thought to arise in small intrahepatic bile ducts, and is commonly characterized by central necrosis or scarring.[1,2,15] PI-type iCCA (6% of iCCA) grows longitudinally along the wall of large bile ducts (ie segmental and area) and spreads along the portal tracts[1,2,15]; this growth pattern is associated with progressive wall thickening and development of strictures in affected ducts.[1,2,15] The IG-type (4% of iCCA) presents as a polypoid or papillary tumour growing towards duct lumina. Variable infiltration of liver parenchyma could be present, thus adopting combined features of periductal infiltrating and mass-forming types (PI + MF), representing around 25% of iCCA.[1,2,15]

Macroscopically, pCCA and dCCA have similar aspects; they present as flat or poorly defined nodular sclerosing tumours often with diffuse infiltration into adjacent structures (≈80%) and, less frequently, as intraductal papillary tumours.[2] The latter corresponds to the IG-type and the former to the PI-type of iCCA.[2] PI-type of iCCA and flat/nodular sclerosing type of p/dCCA are often preceded by preinvasive lesions classified as biliary intraepithelial neoplasm (BilIN)[1,2]; similarly, papillary p/dCCA and IG-type iCCA represent the malignant progression of intraductal papillary neoplasm of the bile duct (IPNB).[1,2] No preinvasive lesions of the MF-type iCCA are known.[1,2] Precursor lesions of CCA are detailed in a separate section of this review.

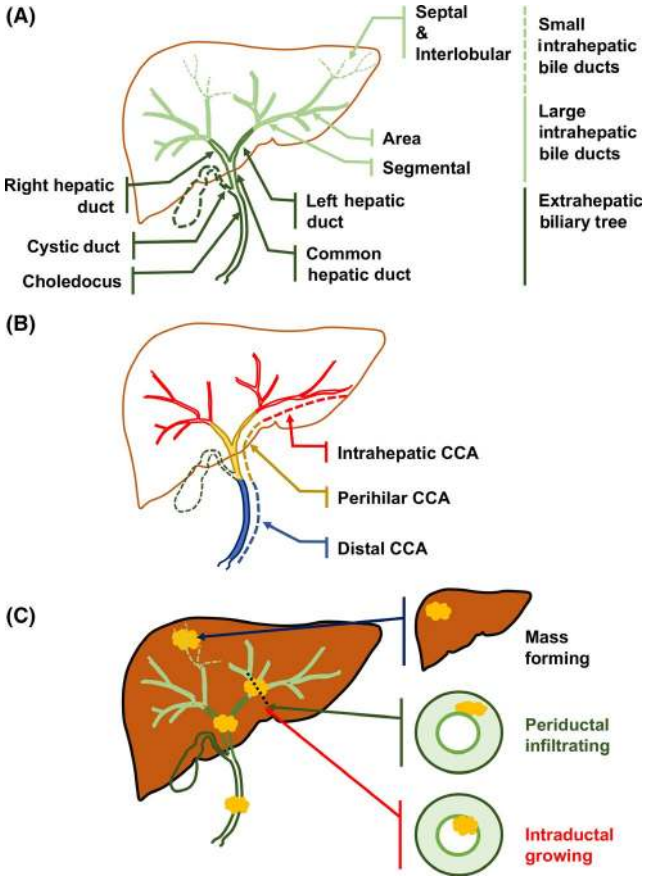

FIGURE 1 (A) The biliary tree is subdivided into the intrahepatic (light green) and extrahepatic parts (dark green). Based on their size, interlobular and septal bile ducts are considered as small intrahepatic bile ducts while segmental and area are considered as large intrahepatic bile ducts. The extrahepatic biliary tree comprises the right and left hepatic ducts, the common hepatic duct, the bile duct (ie choledochus), the gallbladder, and the cystic duct. (B) Based on its location, cholangiocarcinoma (CCA) is classified into intrahepatic CCA, perihilar CCA, and distal CCA. Intrahepatic CCA is a malignancy located proximal to the second-order bile ducts. Perihilar CCA arises in the right and left hepatic duct or at their junction. Distal CCA involves the common bile duct. (C) Intrahepatic cholangiocarcinoma (CCA) can present three main patterns of growth based on its gross appearance: mass-forming (MF), periductal infiltrating (PI), and intraductal growing (IG). MF-type presents as a mass lesion in the hepatic parenchyma. PI-type grows longitudinally along the wall of large bile ducts. The IG-type presents as a polypoid or papillary tumour growing towards duct lumina. Perihilar and distal can present only PI- and IG-growth patterns.

1.3 | Histological aspect and classification of CCA

Histologically, the vast majority of pCCA and dCCA are mucin-producing adenocarcinomas (conventional type) or papillary tumours[2,11]; in contrast, iCCAs are more heterogeneous. Conventional iCCA is an adenocarcinoma with variable morphological aspects of tubular structures, acini formation and micropapillary architecture.[2,11] In general, these tumours are well to moderately differentiated adenocarcinomas formed by columnar to cuboidal epithelial cells, which resemble biliary epithelial cells.[2,11] Desmoplastic stroma and inflammatory reactions frequently occur..[2,11] Mucin production is variably present in the lumen of tubular structures, in the apical side of tumour cells, and in the cell cytoplasm.[2,11] Tumours may show a compressive growth against liver parenchyma or display invasion of hepatocyte plate and sinusoids.[2,11]

As proposed by Nakanuma et al, conventional iCCA can be classified into two main histological subtypes according to the level or size of the displayed bile duct (Figure 2A).[19-23] *Small bile duct type iCCA* may derive from small intrahepatic bile ducts, progenitor cells and mature hepatocytes and presents as small-sized tubular or acinar adenocarcinoma with nodular growth and invading liver parenchyma; these tumours show no or minimal mucin production.[3,19-23] In general, small bile duct type iCCA has a MF growth pattern and is peripherally located.[2] *Large bile duct type iCCA* arises from large intrahepatic (ie segmental and area) bile ducts[20] or from associated peribiliary glands[24,25]; it is constituted by mucin-producing columnar tumour cells arranged in a large-duct or papillary architecture.[19,20] Tumour elements spread along the affected duct with aspects of duct wall and liver parenchyma invasion.[19,20] Large bile duct type iCCA has usually a PI or, less frequently, IG growth patterns and shows a more central location.[19,20] Several investigators introduced different nomenclatures and proposed distinct criteria for distinguishing the two above-mentioned histological subtypes.[19,20] Remarkably, the distinction between small and large bile duct types does not only have histopathological implications but individuates iCCA subtypes with different clinico-pathological and molecular features[19,20]; these aspects are discussed later in this review.

The gross and histological features of large bile duct type iCCA are similar to those of p/dCCA.[2,15,27] Actually, the distinction between iCCA and pCCA at the second-order biliary branches is somehow artificial and seems to take into account surgical implications more than anatomical, embryological and molecular aspects.[14,23] Moreover, the precise anatomical origin of some large tumours can be challenging, as there is no clear evidence whether they are by definition intrahepatic or perihilar on gross examination.[2,23] These aspects corroborate the necessity to develop a combined histomorphological or morpho-molecular classification of CCA.[3] Undoubtedly, the standardization of nomenclature and diagnostic criteria is strongly encouraged and will require international consensus among pathologists, surgeons and clinicians.

Beside conventional and rare variants, cholangiolocarcinoma (CLC) is a further histological variant of iCCA and consists of malignant ductular-like structures in an anastomosing ('antler-like') pattern, embedded in a dense, hyalinized stroma (Figure 2B).[14,20,28] Actually, areas of CLC could be found in both large bile duct type and small bile duct type conventional iCCA[19,20]; in the former case, tubular adenocarcinoma resembling reactive ductules are focally present at the tumour-liver interface.[19,20] Remarkably, the World Health Organization's (WHO) Classification included CLC in the group of so-called *mixed tumours*, liver cancers showing a

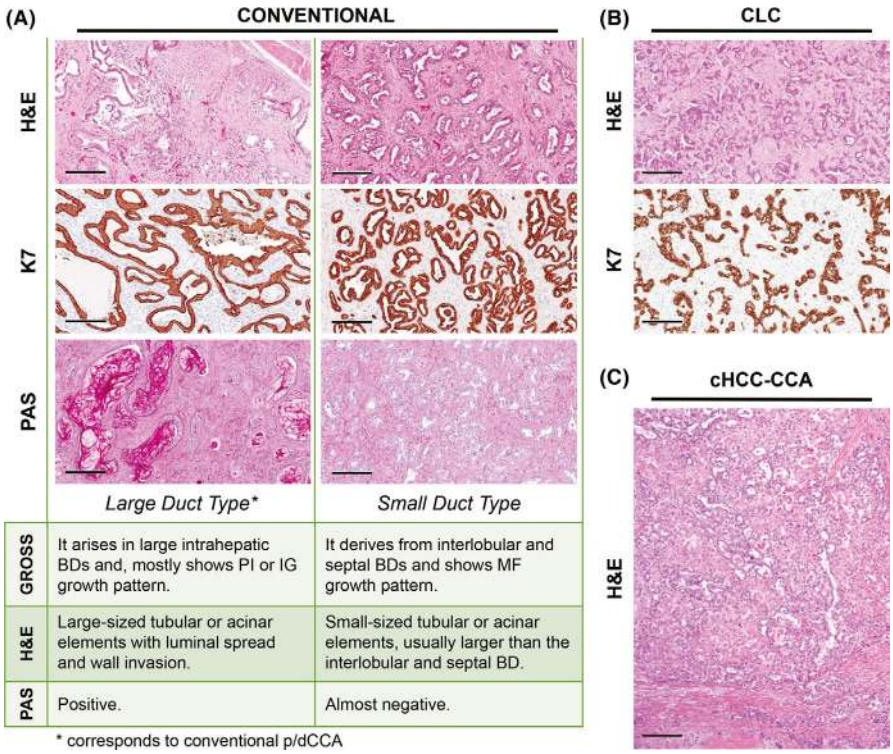

FIGURE 2 (A) Conventional intrahepatic (i) CCA is an adenocarcinoma with variable morphological aspects of tubular structures, acini formation, and micropapillary architecture. Conventional iCCA can be classified into two main histological subtypes: *small bile duct type iCCA* presents as small-sized tubular or acinar adenocarcinoma with no or minimal mucin production. *Large bile duct type iCCA* is constituted by mucin-producing columnar tumour cells arranged in a large-duct or papillary architecture; large bile duct type iCCA latter corresponds histologically to conventional perihilar (p) and distal (d) CCA. (B) Beside conventional, *cholangiolo-carcinoma* (CLC) is a further histological variant of iCCA and consists of malignant ductular-like structures in an anastomosing pattern embedded in a dense, hyalinized stroma. (C) Combined (c) HCC-CCA is composed of typical HCC and typical iCCA, which may be intermixed or separated. H&E: Hematoxylin & Eosin; K7: cytokeratin 7; PAS: Periodic-Acid od Schiff; BD: bile ducts; PI: periductal infiltrating; IG: intraductal growing; MF: mass forming. Scale bars = 200 μm

mixed phenotype with varying degrees of both CCA and hepatocarcinoma (HCC) features.[29] Mixed tumours, representing about 2% of all primary liver cancers, are termed combined HCC-CCA (cHCC-CCA) and, by definition, are composed of typical HCC and typical iCCA, which may be separate or intermixed (Figure 2C).[30] A recent international consensus recommended that the diagnosis of cHCC-CCA be based on routine histopathology; immunostains may support the diagnosis, but are not essential.[30] Molecular characterization of cHCC-CCA is still premature. A subgroup of cHCC-CCA has been demonstrated to show stem/progenitor features, down-regulation of the hepatocyte differentiation programme and a commitment to the biliary lineage.[31] CLC may be a component of cHCC-CCA or a 'pure' tumour when more than 80% of the neoplasm shows the CLC features. CLC is still a poorly understood type of liver cancer, showing clinico-pathological, radiological and molecular differences from both CCA and HCC.[30] Recent molecular data support defining CLC as a distinct biliary-derived molecular entity with no HCC traits.[32] A further subtype of liver cancer with features intermediate between hepatocytes and cholangiocytes has been described; the suggested terminology for this tumour is *intermediate cell carcinoma*. Immunostains support features of both hepatocytic and cholangiocytic lineage within individual cells.[33]

Among rare variants, lymphoepithelioma-like CCA is worthy to be mentioned because it could represent a distinct model of interaction between the immune system and neoplastic cells.[34] This subtype is defined as a tumour composed of undifferentiated epithelial cells with a prominent lymphoid infiltrate and is characterized by lower rates of recurrence after surgery and better overall survival. Data on this tumour subtype remain limited[35]; however, comprehensive studies could offer precious insights for immunotherapeutic strategies.

2 | PRECURSOR LESIONS OF CCA

With the exception of MF-iCCA, where the existence of precursor lesions is unknown,[1,2] CCA develops through multistep carcinogenesis and is preceded by dysplastic and in situ lesions (Figure 3). Although precursor lesions specific to the site can be identified alongside invasive malignancy in many cases, in situ dysplastic lesions cannot always be found. Biliary epithelial neoplasia (BilIN) and intraductal papillary neoplasms of the bile duct (IPNB) are well-defined precursor lesions, and intraductal tubulopapillary neoplasms (ITPN) and mucinous cystic neoplasm (MCN) have been more recently accepted as premalignant.

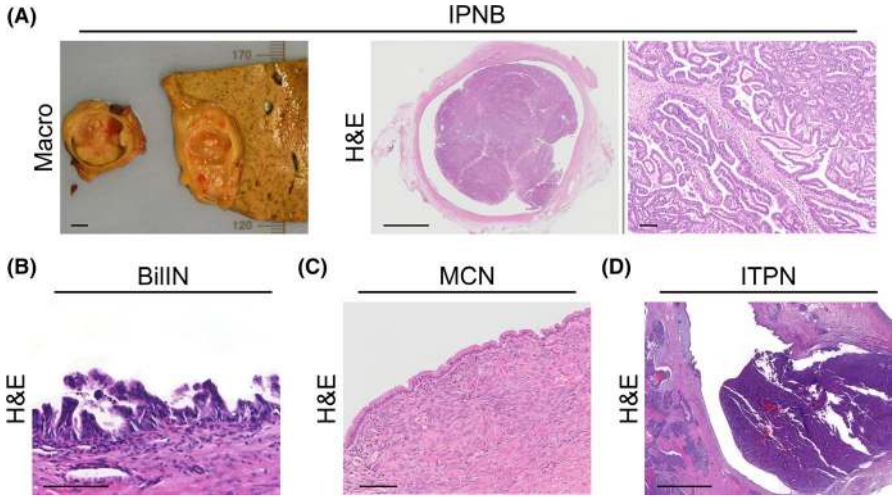

FIGURE 3 Precursor lesions. (A) Intraductal papillary neoplasm of the bile duct (IPNB) is an intraductal lesion with dysplastic epithelium, analogous to pancreatic intraductal papillary mucinous neoplasms (IPMN). Scale Bars = left and centre panel 5 mm, right panel 100 μm. (B) Biliary epithelial neoplasia (BilIN) is flat or micropapillary dysplasia within the biliary tree, whose nomenclature is analogous to that described at other sites. Scale Bar = 100 μm. (C) Mucinous cystic neoplasms (MCN) of the bile duct are similar to their pancreatic and ovarian counterparts, usually simple biliary type epithelium without significant atypia overlying characteristic ovarian-type stroma. Scale Bar = 100 μm. (D) Intraductal tubulopapillary neoplasms (ITPN) show the same solid or nodular intraductal growth, tubular pattern and focal necrosis as equivalent pancreatic lesions. Scale bar = 1 mm

Rare cases of von Meyenburg complexes associated with CCA are reported in the literature. [36,37] However, given the prevalence of von Meyenburg complexes in adults is 5.6%[38] and the number of reported cases is so low, their occasional co-existence with CCA is likely a reflection of their prevalence rather than representing evidence for a premalignant role.

2.1 | Biliary epithelial neoplasia

Biliary epithelial neoplasia (BilIN) is flat or micropapillary dysplasia within the biliary tree, whose nomenclature is analogous to that described at other sites.[39] In common with flat intraepithelial neoplasia at other sites, BilIN can be classified based on the degree and intraepithelial extent of cellular and nuclear atypia into three grades, BilIN-1-3.[40,41] BilIN-3 is considered to be carcinoma in situ.

In brief, BilIN-1 shows mild cellular and nuclear atypia or enlargement, and little loss of cellular polarity. BilIN-2 shows more obvious changes in addition to the loss of polarity, but not amounting to carcinoma in situ. BilIN-3 shows widespread loss of polarity, often with cellular and nuclear atypia. Inter-observer agreement using this system of classification is moderate.[40] This classification has been validated as independent of underlying chronic biliary injury.

The diagnostic challenges posed by flat dysplasia in the biliary tree are common to those faced at other sites. Distinguishing BilIN-1 from reactive atypia can occasionally be difficult, and the presence of intraepithelial neutrophils may favour a reactive diagnosis. The use of 'indefinite for dysplasia' has been proposed in cases where sufficient doubt precludes definitive classification.

Although classification of BilIN is based upon cellular and architectural morphology, immunohistochemical features, reflecting underlying mutational status, have also been suggested as an adjunct to aid diagnosis. For example, expression of mucins MUC1, MUC2 and MUC5AC, and expression of cell cycle-related proteins such as cyclin D1, p21 and p53 increases with progression towards invasive malignany.[42,43] CD15 expression has recently been described as a potential aid to distinguish dysplastic from non-dysplastic biliary lesions.[44]

Intra- and extra-hepatic CCAs arise more frequently in patients with a chronic biliary injury. This may be secondary to liver fluke infection (*Clonorchis sinensis* and *Opisthorchis viverrini)*, in patients with Primary Sclerosing Cholangitis (PSC), or a consequence of chronic biliary stone disease or cysts. The development of dysplasia also shows similar relationships with the underlying cause.

Dysplasia was identified in 37% of explants from patients with PSC who received liver transplantation for medical reasons. This was typically manifest as flat dysplasia although additional micropapillary or papillary dysplasia was frequently found, and a variety of metaplastic changes were also present. Dysplasia in these cases was usually limited to 1-3 ducts but was more widespread in some cases.[45]

In patients with PSC-associated CCA, BilIN was more frequently identified than in PSC explants without CCA; 83% of explants contained BilIN-2 or -3, and greater numbers of ducts with dysplasia were also identified in the setting of co-existent CCA.[45]

BilIN has been documented in patients with non-biliary chronic liver disease, particularly those with alcohol or chronic HCV infection.[46,47] Although less formally described, the development of CCA in patients with liver fluke infection is also likely to develop through a sequence of biliary epithelial hyperplasia and dysplasia.[48] Biliary dysplasia has also been documented in association with sporadic cases of CCA.[49]

2.2 | Intraductal papillary neoplasms of the bile duct

Intraductal papillary neoplasms of the bile duct (IPNBs) can be considered the biliary manifestation of the classical adenoma-carcinoma sequence of the intestine. This category comprises a range of previous terminologies and entities. They may arise within intra- or extrahepatic ducts and may be multiple. They can present clinically as a consequence of large duct obstruction, with cholestatic derangement of liver function tests and jaundice.

IPNBs are yellow, friable papillary lesions found within the duct system. They can be single or multiple, and they typically have fine fibrovascular stalks. The cytological features of the neoplastic epithelium allow classification of IPNBs into pancreaticobiliary, intestinal, gastric and oncocytic types in the manner used for the equivalent pancreatic lesions, intraductal papillary mucinous neoplasms (IPMN).

Pancreaticobiliary and intestinal subtypes are most common, although their frequency varies with geography.[50,51] The intestinal subtype resembles a classical colonic neoplasm. Dysplasia is most often high-grade, however it can be of any grade in the neoplastic papillary epithelium, and epithelium of the duct in which the IPNB arises often shows flat dysplasia.[52,53]

An associated invasive CCA can be identified in approximately half of the cases of IPNB.[50] Invasive CCA can be found in association with any subtype although is more often associated with the pancreaticobiliary subtype. The morphology of the associated malignancy varies depending on the subtype of IPNB with which it is associated. Invasive carcinomas associated with the pancreaticobiliary, gastric and oncocytic subtype are typically tubular adenocarcinoma, and intestinal subtype associated invasive carcinomas are often mucinous adenocarcinomas. The pattern of mucins and immunohistochemical phenotype also differ between IPNB subtypes.[52]

IPNBs are reportedly more frequent in the Far East, without a significant inheritable risk or gender predilection.[55] Their geographic distribution reflects their frequent development in patients with hepatolithiasis and liver fluke infection, both much more common in those areas.[51,56] In the West, they are usually sporadic. The most common clinical presentations are right upper quadrant abdominal pain, acute cholangitis and obstructive jaundice. Acute cholangitis is observed in 5%-59% of patients with IPNB, in contrast to CCA.

Where IPNBs exhibit dysplasia of any grade but without structural atypia or areas suspicious for stromal invasion into papillary cores, the prognosis after resection is excellent. Lesions with structural atypia or those suspicious for papillary stromal invasion have a better outcome than those with concurrent invasive carcinoma.[54]

2.3 | Mucinous cystic neoplasm

Mucinous cystic neoplasms (MCN) of the bile duct (MCN-B) represent <5% of cystic lesions within the liver[57] and are similar to their pancreatic and ovarian counterparts. They are associated with the development of invasive carcinoma, as in the pancreas.

MCN-Bs are usually multilocular cysts with septation, or show a cyst-in-cyst appearance on pre-operative imaging.[57] The epithelium is usually simple biliary type epithelium without significant atypia, overlying characteristic ovarian-type stroma. Focal flat or micropapillary dysplasia can be observed in a minority of cases, usually incidentally identified by microscopic examination.

The rate of invasive malignancy is low. Where invasion is present it is usually confined to the cystic neoplasm rather than extending into the hepatic parenchyma. Cases are identified by greater epithelial atypia and stromal invasion.

MCNs show a strong female predominance and are found in a generally younger age range than IPNBs (21-69 years).[57] They present with non-specific features associated with all liver cysts, for example, right upper quadrant or epigastric pain and abdominal fullness, although incidental identification is common.

Cases of non-invasive MCN have an excellent prognosis when completely resected. Where invasion is present, the prognosis remains good if restricted to within the lesion,[58] although good quality follow-up data is lacking.

CCA has been documented in patients with polycystic liver disease,[59,60] and it has been suggested that the pathogenesis in these cases may be dysplasia within existing benign cysts.[62] However, the literature does not indicate an increased rate of CCA in these patients, nor that cysts should be should be formally considered to be premalignant lesions per se.

2.4 | Intraductal tubulopapillary neoplasms

Intraductal tubulopapillary neoplasms (ITPN) have recently been described in the pancreas as a distinct intraductal neoplasm. Their bile duct counterparts have subsequently been identified.[63,64]

In the pancreas, these are solid and nodular lesions that dilate pancreatic ducts. They demonstrate necrosis and high-grade dysplasia, and little mucin production.[65] Invasion was identified in three of the 10 cases originally described. MUC5AC expression is absent in these neoplasms.

Fewer biliary ITPNs have been described. They occur in a population with a mean age of 60, without gender differences. Biliary ITPNs show the same intraductal growth, tubular pattern and focal necrosis as in the pancreas, and have been documented to grow up to 15 cm.

One of the biliary cases first described was associated with lung metastases, indicating the malignant potential of these lesions,[66] and subsequent case series have confirmed a high risk of malignancy, demonstrating invasive carcinoma, typically tubular carcinoma, in 70%-80% of cases.[53,67] Despite this high rate of associated malignancy, overall ITPNs have a prognosis that is favourable when compared with IPNBs. This may reflect earlier diagnosis effected by the large in situ intraductal component, or inherent differences in the molecular background.[53]

3 | CURRENT PROBLEMS IN DIAGNOSTIC PATHOLOGY OF CCA

It is challenging for clinicians, radiologists and pathologists to establish the diagnosis of CCA. The difficult diagnosis potentially results in delayed surgery, which has a negative impact on the chance of curative treatment. Moreover, a pathologically confirmed diagnosis is often required before starting chemotherapy in patients for whom surgery is not an option.

One of the main issues in the diagnosis of CCA is the fact that tumour tissue for histological examination is difficult to obtain, particularly in pCCA and dCCA.[68] Bile duct brush cytology during endoscopic retrograde cholangiopancreatography (ERCP) is the reference standard used in daily practice, but yields a low sensitivity (range of 20%-55%).[68,69] This is due to the fact that the bile duct may be difficult to access. Moreover, a periductal growth pattern of CCA and pronounced stromal reaction may compromise the technical procedure and harvesting of lesional cells and/or tissue. A particular challenging issue is distinguishing malignant strictures from benign strictures in patients with PSC, which is one of the most important risk factors for CCA. In PSC, strictures develop in the context of inflammation and fibrosis. In benign strictures, inflammation causes reactive cytological changes that may mimic neoplasia. Specificity for cytology in patients without PSC varies between 82% and 100%.[69,70] Additional molecular techniques, including fluorescence in situ hybridization (FISH) for detection of polysomy with fluorescently labeled DNA probes to the pericentromeric regions of chromosomes 3, 7 and 17, and/or to chromosomal band 9p21 deletion may be helpful in increasing sensitivity (up to 50%-70%) while maintaining specificity, particularly in non PSC-patients and if combined with conventional brush cytology.[69,70]

Cell blocks may be helpful to improve cytological evaluation. Additional molecular techniques, such as FISH or DNA flow cytometry data, may indicate dysplasia rather than invasive cancer in PSC patients.[69,72] Results should always be interpreted in the clinical context and the presence of additional risk factors, such as a dominant stricture on imaging and/or elevation of CA19.9 in serum, increases the probability of CCA in PSC patients.[71]

Other inflammatory diseases, such as IgG4-associated cholangitis, are also mimickers of CCA, resulting in a substantial number of unnecessary liver resections. IgG4 cholangitis is treated with steroids and typically shows a striking/prompt response. Treatment response helps in establishing the diagnosis.[73] A recent study showed that inflammation alone was found in 15% of resections for presumed pCCA, of which 42% could be diagnosed as IgG4-related cholangitis.[74] However, numbers of IgG4 positive plasma cells may be increased in the presence of pancreatobiliary malignancies.[75] Additional histological criteria, described in the context of IgG4-related disease, such as storiform fibrosis, obliterative phlebitis and dense lymphoplasmacytic infiltrates may improve correct interpretation of an increase in numbers of IgG4 positive plasma cells.[76] Again, histological findings must be interpreted in the clinical setting. Composite scores, such as HISORt criteria (histology, imaging, serology, other organ involvement, response to treatment) are used for diagnosis.[77] Blood-based diagnostics may provide a window of opportunity in differentiating between malignant and benign strictures. New diagnostic tests, such as IgG/IgG4 RNA ratio, may improve pre-operative diagnosis and avoid unnecessary surgery. IgG/IgG4 RNA ratio was shown to have a sensitivity of 94% and specificity of 99% for the diagnosis of IgG4-related disease, when compared to ratios in patients with PSC or pancreaticobiliary malignancies.[78]

Only a minority of CCAs occur in the liver (<10%, iCCA). Histological mimickers of iCCA include intrahepatic metastases from other primary tumours, such as gastric or pancreatic carcinomas. Immunohistochemical staining for markers such as CRP, possibly in combination with N-cadherin, may be of help in correctly identifying the tumour of origin if the clinical work-up is unclear. CRP was shown to be more frequently positive in iCCA than in liver metastasis of other primary tumours.[79] Furthermore, the CLC subtype of iCCA, which morphologically resembles reactive ductular proliferation, frequently expresses NCAM (CD56) and vimentin with increased expression of p53 and ki-67. However, the distinction between CLC and reactive ductular reaction may remain challenging, since NCAM expression is seen in ductular reaction as well.[28,80]

Finally, the distinction between a solid iCCA and HCC may be difficult solely on the basis of morphology. Immunohistochemical markers that are indicative of hepatocellular differentiation include HepPar-1 (hepatocyte in paraffin 1), Arginase-1, Alpha-fetoprotein (AFP), pCEA (canalicular) and CD10 (canalicular). Markers indicative of biliary differentiation include CK7, CK19 and epithelial membrane antigen (EMA).[29,30] Serum biomarkers, including CA 19-9, alpha-fetoprotein and glypican-3 are of limited value in distinguishing both tumours.[81] HCC and iCCA have, at least partially, common risk factors and overlapping oncogenic pathways. Correct diagnosis is important because of different treatment strategies. In 2%-5% of primary liver tumours, mixed features of both CCA and HCC are seen (cHCC-CCA). Thus, extensive sampling is necessary to decrease the potential contribution of tumour heterogeneity, the latter being a particular issue in pre-operative biopsies. Agreement on diagnosis, including morphological characteristics in relation to the use of additional immunohistochemical stains for the identification of hepatocellular and/or biliary differentiation, and agreement on nomenclature and reporting is very important for a deeper understanding of cHCC-CCA, for future research strategies and multicenter collaboration.[3,29,30,82]

After surgical excision, correct pathology reporting of CCA-specimens is of utmost importance. For CCA in general, there is room for improvement in terms of prognostic value of clinicopathological features.[83,84] The definition used for R-status (0 or <1 mm) and the different margins evaluated are not always clearly described in studies. In resection specimens of pCCA, it has been shown that important prognostic histological features are missing in a substantial number of pathology reports, confirming the complexity of adequate reporting. Chatelain et al reviewed 263 reports from 22 hepatopancreaticobilairy centers. Tumour differentiation was

missing in 27% of cases, vascular invasion in 45% and infiltration of the bile duct surgical margins in 4% of the reports. Moreover, distances between the tumour and the vessel margin, liver margin and the periductal soft tissue circumferential margin were not specified in 87%, 79% and 89% of cases respectively.[85] The inadequacy of reporting may be key to explaining the finding that even after R0 resection (local) recurrence rates have been reported to be more than 50%.[86] The prognostic value of the new, 8th edition of the American Joint Committee on Cancer staging system also deserves further study, since a recent study showed that overall prognostic performance of the 8th edition was not markedly improved over the 7th edition.[87]

The inclusion of all potentially relevant parameters, such as resection and dissection planes in the pathology report is essential for adequate staging, for the correct interpretation of residual disease status, and last but not least for scientific research. In this modern area of molecular research and novel (molecular) treatments, it is of great importance that the basis pathology data are translated and reported in an accurate way. Consensus among all clinicians involved in diagnosis and treatment of CCA, and a coordinated approach both in the clinical and research setting, is warranted to improve diagnosis, prognosis and treatment options in CCA.

3.1 | Molecular pathology of CCA

After a long period of standstill, numerous new molecular alterations in CCA have been discovered within the last few years. Recent advances are largely due to the employment of technical innovations in high-throughput molecular analyses and the more or less strict separation of CCA subtypes. Evolving molecular data and the understanding of underlying mechanisms are not only helpful for improving the characterization of CCA and its subtypes but might also pave the way for personalized medicine for these rare cancer types in most countries. For future studies it is most important to ensure the separation of the given anatomical subtypes and to search for distinct subgroups within the subtypes on a molecular and morphological basis. This goal of a new morpho-molecular classification of CCA can only be reached if clinicopathologically well-characterized cohorts are used.

Current knowledge of genomic and epigenomic alterations characterizes CCA as a highly heterogeneous tumour; however, available molecular data are partly conflicting. Several reasons might account for this: geographical, ethnic and etiological differences of study populations, usage of different detection methods and misclassifications (eg pCCA and iCCA or dCCA and pancreatic adenocarcinoma). Therefore, the status quo of molecular alterations in CCA is imprecise, and further work is required to accomplish more accurate data by employing better clinicopathologically characterized and more homogeneous (eg monoetiological) study populations.

3.2 | Molecular alterations of intrahepatic CCA

Partly due to the reasons mentioned, there is a high variation of mutation frequencies in iCCA. Nonetheless, the anatomical differences described above (large vs small-duct type iCCA) are reflected in the molecular picture we have seen so far, ie mainly two distinctive morpho-molecular groups of iCCA can currently be discriminated. Large-duct type iCCAs show a high mutation frequency of oncogenes and tumour suppressor genes, such as KRAS (15%-30%) and TP53 (10%-40%).[22,88,89] Large-duct type iCCAs typically lack IDH1/2 mutations and FGFR2-fusions, features typically seen in small-duct iCCA. Apart from these high-frequency mutations, other genes, such as BRAF, BAP1, PIK3CA, GNAS, ARID1A, SMAD4, PTEN, MDM2, EGFR, ERBB2/HER2 and many more, are mutated; however usually in a much lower frequency in most cohorts. Some of them, although usually low in frequency, are easy to test and might serve as a putative therapeutical target with available drugs that have proven efficacy in other tumour types (eg for ERBB2 and BRAF mutations).[92,93] Microsatellite instability (MSI) is another putative predictive and therapeutically relevant marker, since it has been shown that DNA-mismatch repair-deficient tumours are significantly more responsive to immune checkpoint blockade. MSI was detected in up to 30% of CCA, particularly in liver-fluke associated tumours,[95] whereas, in non-liver-fluke associated CCA MSI seems to be a rare event.[96]

In contrast, small-duct type iCCAs are typically of mass-forming type and show IDH1/2 mutations (10%-30%) and FGFR2-fusions (10%-25%).[88-91] From a diagnostic point of view, these molecular alterations fairly restricted to iCCA can be used to improve the classification of anatomical 'borderline cases', for example, to discriminate perihilar from intrahepatic CCA. Additionally, BAP1 mutations were found to be restricted to iCCA in some studies and BAP1 and IDH mutations were also found in HCC, suggesting an overlap of iCCA, cHCC-CCAs and HCC respectively a common cell-of-origin for at least some of these mass-forming, small-duct type iCCAs.[97,100,101] As cHCC-CCAs represent a separated tumour type, according to the current WHO classification, and mostly vary in their molecular profile significantly to iCCA, this will not be further discussed in this review.[30] Furthermore, TP53 mutations may be used as a surrogate marker for malignancy (eg by immunohistochemistry to discriminate untypical bile duct adenomas (BDA) from iCCA). It is noteworthy that BDA often (~50%) exhibit BRAF mutations, and this feature should not be misinterpreted as a surrogate marker for malignancy.

Recent studies have tried to address the etiological distinctiveness of CCA and have found significant genomic and epigenomic landscape changes between liver fluke and non-liver fluke associated CCA.[104,105] Further studies focusing on molecular cholangiocarcinogenesis of well-characterized CCA cohorts with distinct etiology (eg PSC-associated CCA) are needed, and are likely to highlight differences between monoetiological and conventional/idiopathic CCA cohorts.

3.3 | Molecular alterations of extrahepatic CCA

Extrahepatic CCA largely shares the heterogenic molecular pattern with large-duct type iCCA, though frequencies of some gene

mutations vary significantly (eg KRAS mutation frequency seems to be higher in pCCA and dCCA compared to iCCA).[22,89-91] In particular, IDH1/2 and BAP1 mutations and FGFR2-fusions are typically absent in extrahepatic CCA.[88-91]

3.4 | Molecular alterations of precursor lesions

Little is known on molecular alterations of precursor lesions. Clearly-defined biliary precursor lesions, such as intraductal papillary neoplasias of the bile duct (IPNB), intraductal tubulopapillary neoplasias of the bile duct (ITPN), and biliary intraepithelial neoplasias (BilIN), have been the focus of studies searching for early molecular alterations in cholangiocarcinogenesis. However, most studies were conducted as a single gene approach, and systematic genome-wide screening approaches are lacking, to date. For IPNB, involvement of common molecular pathways have been described, for instance, KRAS mutations were described in up to 46%.[50,107] Another study found recurrent mutations in the Wnt/β-catenin signaling pathway in IPNB.[108] However, the few studies available are already conflicting, as GNAS mutations were found in 50% of IPNB in one study, whereas another study showed a low GNAS mutation level of <5%.[107,109] Due to difficulties in microdissecting BilIN lesions, there is no meaningful study on molecular alterations in BilIN available to date. For ITPN, similar alterations could be detected in a study of 20 cases; however CDKN2A/p16 mutations were found in a high proportion (44%) in this cohort.[53]

3.5 | Epigenetic alterations in cholangiocarcinogenesis

CCA is a highly epigenetic regulated tumour type. Epigenetic mechanisms involved in gene regulation typically include histone modification, DNA methylation and noncoding RNAs. However, not all of these mechanisms have been studied sufficiently in human CCA cohorts. Systematic studies focusing on DNA methylation changes are available and genome-wide methylation patterns in CCA were first described in 2014.[104,110,111]

For a more detailed description of epigenetic and genetic alterations in cholangiocarcinogenesis, see the other reviews in this issue.

4 | CONCLUDING REMARKS

Morphologically, CCA is a heterogeneous group of malignancies; this histo-morphological heterogeneity is strictly associated with cell of origin, pathogenesis, underlying liver disease and molecular alterations. These aspects corroborate the necessity to develop a combined morpho-molecular classification of CCA. Up to date, no international consensus on histological classification is present and the standardization of nomenclature and diagnostic criteria is strongly required. The creation of networks such as the European Network for the Study of Cholangiocarcinoma (ENS-CCA) could represent positive experiences to set up collaborative efforts, registry creation and consensus in the management and treatment of this tumour. Furthermore, experimental models and clinical studies do not take in full consideration differences in CCA with distinct morphological features. Finally, the application of novel tools on histological images (eg deep convolutional neural network[112]) could help in tumour classification and be relevant in stratify patients' prognosis and predict mutation status.

ACKNOWLEDGEMENTS

Authors of this review are members of the European Network for the Study of Cholangiocarcinoma (ENS-CCA) and participate to the initiative COST Action EURO-CHOLANGIO-NET granted by the COST Association (CA18122).

CONFLICT OF INTEREST

None to declare.

ORCID

Guido Carpino https://orcid.org/0000-0001-8570-2519

REFERENCES

1. Banales JM, Cardinale V, Carpino G, et al. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol*. 2016;13:261-280.
2. Nakanuma Y, Kakuda Y. Pathologic classification of cholangiocarcinoma: New concepts. *Best Pract Res Clin Gastroenterol*. 2015;29:277-293.
3. Sia D, Villanueva A, Friedman SL, Llovet JM. Liver cancer cell of origin, molecular class, and effects on patient prognosis. *Gastroenterology*. 2017;152:745-761.
4. Roskams TA, Theise ND, Balabaud C, et al. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology*. 2004;39:1739-1745.
5. Roskams T, Desmet V. Embryology of extra- and intrahepatic bile ducts, the ductal plate. *Anat Rec*. 2008;291:628-635.
6. Nakanuma Y, Hoso M, Sanzen T, Sasaki M. Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. *Microsc Res Tech*. 1997;38:552-570.
7. Carpino G, Renzi A, Franchitto A, et al. Stem/progenitor cell niches involved in hepatic and biliary regeneration. *Stem Cells International*. 2016;2016.
8. Carpino G, Cardinale V, Renzi A, et al. Activation of biliary tree stem cells within peribiliary glands in primary sclerosing cholangitis. *J Hepatol*. 2015;63:1220-1228.
9. Carpino G, Cardinale V, Onori P, et al. Biliary tree stem/progenitor cells in glands of extrahepatic and intraheptic bile ducts: An anatomical in situ study yielding evidence of maturational lineages. *J Anat*. 2012;220:186-199.
10. Cardinale V, Wang Y, Carpino G, et al. The biliary tree-a reservoir of multipotent stem cells. *Nature Reviews Gastroenterology and Hepatology*. 2012;9:231-240.
11. Krasinskas AM. Cholangiocarcinoma. *Surg Pathol Clin*. 2018;11:403-429.

12. Blechacz B, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology*. 2008;48:308-321.
13. Cardinale V, Wang Y, Carpino G, et al. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology*. 2011;54:2159-2172.
14. Blechacz B, Komuta M, Roskams T, Gores GJ. Clinical diagnosis and staging of cholangiocarcinoma. *Nat Rev Gastroenterol Hepatol*. 2011;8:512-522.
15. Nakanuma Y, Sato Y, Harada K, Sasaki M, Xu J, Ikeda H. Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. *World J Hepatol*. 2011;2:419-427.
16. Oliveira IS, Kilcoyne A, Everett JM, Mino-Kenudson M, Harisinghani MG, Ganesan K. Cholangiocarcinoma: classification, diagnosis, staging, imaging features, and management. *Abdominal Radiology*. 2017;42:1637-1649.
17. DeOliveira ML, Schulick RD, Nimura Y, et al. New staging system and a registry for perihilar cholangiocarcinoma. *Hepatology*. 2011;53:1363-1371.
18. Vijgen S, Terris B, Rubbia-Brandt L. Pathology of intrahepatic cholangiocarcinoma. *HepatoBiliary Surg Nutr*. 2017;6:22-34.
19. Akita M, Fujikura K, Ajiki T, et al. Dichotomy in intrahepatic cholangiocarcinomas based on histologic similarities to hilar cholangiocarcinomas. *Mod Pathol*. 2017;30:986-997.
20. Komuta M, Govaere O, Vandecaveye V, et al. Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. *Hepatology*. 2012;55:1876-1888.
21. Aishima S, Kuroda Y, Nishihara Y, et al. Proposal of progression model for intrahepatic cholangiocarcinoma: Clinicopathologic differences between hilar type and peripheral type. *Am J Surg Pathol*. 2007;31:1059-1067.
22. Liau JY, Tsai JH, Yuan RH, Chang CN, Lee HJ, Jeng YM. Morphological subclassification of intrahepatic cholangiocarcinoma: etiological, clinicopathological, and molecular features. *Mod Pathol*. 2014;27:1163-1173.
23. Hayashi A, Misumi K, Shibahara J, et al. Distinct clinicopathologic and genetic features of 2 histologic subtypes of intrahepatic cholangiocarcinoma. *Am J Surg Pathol*. 2016;40:1021-1030.
24. Cardinale V, Wang Y, Carpino G, Reid LM, Gaudio E, Alvaro D. Mucin-producing cholangiocarcinoma might derive from biliary tree stem/progenitor cells located in peribiliary glands. *Hepatology*. 2012;55:2041-2042.
25. Igarashi S, Sato Y, Ren XS, Harada K, Sasaki M, Nakanuma Y. Participation of peribiliary glands in biliary tract pathophysiologies. *World J Hepatol*. 2013;5:425-432.
26. Carpino G, Cardinale V, Folseraas T, et al. Neoplastic transformation of peribiliary stem cell niche in cholangiocarcinoma arisen in primary sclerosing cholangitis. *Hepatology*. 2018. https://doi.org/10.1002/hep.30210.
27. Nakanuma Y, Harada K, Sasaki M, Sato Y. Proposal of a new disease concept "biliary diseases with pancreatic counterparts". Anatomical and pathological bases. *Histol Histopathol*. 2014;29:1-10.
28. Komuta M, Spee B, Borght SV, et al. Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. *Hepatology*. 2008;47:1544-1556.
29. Bosman FT. WHO classification of tumours of the digestive system - NLM Catalog - NCBI. In *WHO Classification of Tumours of the Digestive System*. Editors: Bosman FT, Carneiro F, Hruban RH, Theise ND. Lyon, France: International Agency for Research on Cancer; 2010:225-227.
30. Brunt E, Aishima S, Clavien P-A, et al. cHCC-CCA: consensus terminology for primary liver carcinomas with both hepatocytic and cholangiocytic differentation. *Hepatology*. 2018;68:113-126.
31. Coulouarn C, Cavard C, Rubbia-Brandt L, et al. Combined hepatocellular-cholangiocarcinomas exhibit progenitor features and activation of Wnt and TGFβ signaling pathways. *Carcinogenesis*. 2012;33:1791-1796.
32. Moeini A, Sia D, Zhang Z, et al. Mixed hepatocellular cholangiocarcinoma tumors: Cholangiolocellular carcinoma is a distinct molecular entity. *J Hepatol*. 2017;66:952-961.
33. Brunt EM, Paradis V, Sempoux C, Theise ND. Biphenotypic (hepatobiliary) primary liver carcinomas: the work in progress. *Hepatic Oncol*. 2015;2:255-273.
34. Solinas A, Calvisi DF. Lessons from rare tumors: hepatic lymphoepithelioma-like carcinomas. *World J Gastroenterol*. 2015;21:3472-3479.
35. Labgaa I, Stueck A, Ward SC. Lymphoepithelioma-like carcinoma in liver. *Am J Pathol*. 2017;187:1438-1444.
36. Röcken C, Pross M, Brucks U, Ridwelski K, Roessner A. Cholangiocarcinoma occurring in a liver with multiple bile duct hamartomas (von meyenburg complexes). *Arch Pathol Lab Med*. 2000;124:1704-1706.
37. Jain D, Sarode VR, Abdul-Karim FW, Homer R, Robert ME. Evidence for the neoplastic transformation of Von-Meyenburg complexes. *Am J Surg Pathol*. 2000;24:1131-1139.
38. Redston MS, Wanless IR. The hepatic von Meyenburg complex: prevalence and association with hepatic and renal cysts among 2843 autopsies [corrected]. *Mod Pathol An Off J United States Can Acad Pathol Inc*. 1996;9:233-237.
39. Sato Y, Sasaki M, Harada K, et al. Pathological diagnosis of flat epithelial lesions of the biliary tract with emphasis on biliary intraepithelial neoplasia. *J Gastroenterol*. 2014;49:64-72.
40. Zen Y, Adsay NV, Bardadin K, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. *Mod Pathol*. 2007;20:701-709.
41. Zen Y, Aishima S, Ajioka Y, et al. Proposal of histological criteria for intraepithelial atypical/ proliferative biliary epithelial lesions of the bile duct in hepatolithiasis with respect to cholangiocarcinoma: Preliminary report based on interobserver agreement. *Pathol Int*. 2005;55:180-188.
42. Sato Y, Harada K, Sasaki M, Nakanuma Y. Histological characterization of biliary intraepithelial neoplasia with respect to pancreatic intraepithelial neoplasia. *Int J Hepatol*. 2014;2014:1-7.
43. Moschovis D, Bamias G, Delladetsima I. Mucins in neoplasms of pancreas, ampulla of Vater and biliary system. *World J Gastrointest Oncol*. 2016;8:725.
44. Walter D, Herrmann E, Winkelmann R, et al. Role of CD15 expression in dysplastic and neoplastic tissue of the bile duct – a potential novel tool for differential diagnosis of indeterminate biliary stricture. *Histopathology*. 2016;69:962-970.
45. Lewis JT, Talwalkar JA, Rosen CB, Smyrk TC, Abraham SC. Precancerous bile duct pathology in end-stage primary sclerosing cholangitis, with and without cholangiocarcinoma. *Am J Surg Pathol*. 2010;34:27-34.
46. Wu TT, Levy M, Correa AM, Rosen CB, Abraham SC. Biliary intraepithelial neoplasia in patients without chronic biliary disease: Analysis of liver explants with alcoholic cirrhosis, hepatitis C infection, and noncirrhotic liver diseases. *Cancer*. 2009;115:4564-4575.
47. Rougemont L, Genevay M, McKee TA, Gremaud M, Mentha G, Rubbia-Brandt L. Extensive biliary intraepithelial neoplasia (BilIN) and multifocal early intrahepatic cholangiocarcinoma in non-biliary cirrhosis. *Virchows Arch*. 2010;456:711-717.
48. Choi BI, Han JK, Hong ST, Lee KH. Clonorchiasis and cholangiocarcinoma: etiologic relationship and imaging diagnosis. *Clin Microbiol Rev*. 2004;17:540-552.
49. Hughes NR, Pairojkul C, Royce SG, Clouston A, Bhathal PS. Liver fluke-associated and sporadic cholangiocarcinoma: an immunohistochemical study of bile duct, peribiliary gland and tumour cell phenotypes. *J Clin Pathol*. 2006;59:1073-1078.

50. Schlitter AM, Born D, Bettstetter M, et al. Intraductal papillary neoplasms of the bile duct: stepwise progression to carcinoma involves common molecular pathways. *Mod Pathol*. 2014;27:73-86.
51. Kim KM, Lee JK, Shin JU, et al. Clinicopathologic features of intraductal papillary neoplasm of the bile duct according to histologic subtype. *Am J Gastroenterol*. 2012;107:118-125.
52. Zen Y, Fujii T, Itatsu K, et al. Biliary papillary tumors share pathological features with intraductal papillary mucinous neoplasm of the pancreas. *Hepatology*. 2006;44:1333-1343.
53. Schlitter AM, Jang K-T, Klöppel G, et al. Intraductal tubulopapillary neoplasms of the bile ducts: clinicopathologic, immunohistochemical, and molecular analysis of 20 cases. *Mod Pathol*. 2015;28:1249-1264.
54. Jung G, Park KM, Lee SS, Yu E, Hong SM, Kim J. Long-term clinical outcome of the surgically resected intraductal papillary neoplasm of the bile duct. *J Hepatol*. 2012;57:787-793.
55. Fukumura NY, Cong H, Hara K, Kakuda Y, Nakanuma Y. Intraductal papillary neoplasm of the bile duct. *Pathol Bile Duct*. 2017;19:163-175.
56. Rocha FG, Lee H, Katabi N, et al. Intraductal papillary neoplasm of the bile duct: a biliary equivalent to intraductal papillary mucinous neoplasm of the pancreas? *Hepatology*. 2012;56:1352-1360.
57. Zen Y, Pedica F, Patcha VR, et al. Mucinous cystic neoplasms of the liver: a clinicopathological study and comparison with intraductal papillary neoplasms of the bile duct. *Mod Pathol*. 2011;24:1079-1089.
58. Nakajima T, Sugano I, Matsuzaki O, et al. Biliary cystadenocarcinoma of the liver. A clinicopathologic and histochemical evaluation of nine cases. *Cancer*. 1992;69:2426-2432.
59. Ikeda N, Naito T, Isonuma H, Dambara T, Hayashida Y. Polycystic kidney complicated by cholangiocellular carcinoma presenting as fever of unknown origin. *Gen Med*. 2005;6:23-27.
60. Delis SG, Bakoyiannis A, Triantopoulou C, Paraskeva K, Athanassiou K, Dervenis C. Obstructive jaundice in polycystic liver disease related to coexisting cholangiocarcinoma. *Case Rep Gastroenterol*. 2008;2:162-169.
61. Sasaki M, Katayanagi K, Watanabe K, Takasawa K, Nakanuma Y. Intrahepatic cholangiocarcinoma arising in autosomal dominant polycystic kidney disease. *Virchows Arch*. 2002;441:98-100.
62. Chebib FT, Hogan MC. Extrarenal manifestations of autosomal dominant polycystic kidney disease: polycystic liver disease. In Cowley BD, J, Bissler JJ, eds. *Polycystic Kidney Disease: Translating Mechanisms into Therapy*. , New York, NY: Springer; 2018:171-195.
63. Zen Y, Amarapurkar AD, Portmann BC. Intraductal tubulopapillary neoplasm of the bile duct: Potential origin from peribiliary cysts. *Hum Pathol*. 2012;43:440-445.
64. Nakagawa T, Arisaka Y, Ajiki T, et al. Intraductal tubulopapillary neoplasm of the bile duct: a case report and review of the published work. *Hepatol Res*. 2016;46:713-718.
65. Yamaguchi H, Shimizu M, Ban S, et al. Intraductal tubulopapillary neoplasms of the pancreas distinct from pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am J Surg Pathol*. 2009;33:1164-1172.
66. Park HJ, Jang KT, Heo JS, La CY, Han J, Kim SH. A potential case of intraductal tubulopapillary neoplasms of the bile duct. *Pathol Int*. 2010;60:630-635.
67. Katabi N, Torres J, Klimstra DS. Intraductal tubular neoplasms of the bile ducts. *Am J Surg Pathol*. 2012;36:1647-1655.
68. Walter D, Peveling-Oberhag J, Schulze F, et al. Intraductal biopsies in indeterminate biliary stricture: Evaluation of histopathological criteria in fluoroscopy- vs. cholangioscopy guided technique. *Dig Liver Dis*. 2016;48:765-770.
69. Brooks C, Gausman V, Kokoy-Mondragon C, et al. Role of fluorescent in situ hybridization, cholangioscopic biopsies, and EUS-FNA in the evaluation of biliary strictures. *Dig Dis Sci*. 2018;63:636-644.
70. Liew ZH, Loh TJ, Lim T, et al. Role of fluorescence in situ hybridization in diagnosing cholangiocarcinoma in indeterminate biliary strictures. *J Gastroenterol Hepatol*. 2018;33:315-319.
71. Rizvi S, Eaton J, Yang JD, Chandrasekhara V, Gores GJ. Emerging technologies for the diagnosis of perihilar cholangiocarcinoma. *Semin Liver Dis*. 2018;38:160-169.
72. Vannas MJ, Boyd S, Färkkilä MA, Arola J, Isoniemi H. Value of brush cytology for optimal timing of liver transplantation in primary sclerosing cholangitis. *Liver Int*. 2017;37:735-742.
73. Hubers LM, Beuers U. IgG4-related disease of the biliary tract and pancreas: clinical and experimental advances. *Curr Opin Gastroenterol*. 2017;33:310-314.
74. Roos E, Hubers LM, Coelen R, et al. IgG4-associated cholangitis in patients resected for presumed perihilar cholangiocarcinoma: a 30-year tertiary care experience. *Am J Gastroenterol*. 2018;113:765-772.
75. Harada K, Shimoda S, Kimura Y, et al. Significance of immunoglobulin G4 (IgG4)-positive cells in extrahepatic cholangiocarcinoma: molecular mechanism of IgG4 reaction in cancer tissue. *Hepatology*. 2012;56:157-164.
76. Deshpande V, Zen Y, Chan J, et al. Consensus statement on the pathology of IgG4-related disease. *Mod Pathol*. 2012;25:1181-1192.
77. Ghazale A, Chari ST, Zhang L, et al. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. *Gastroenterology*. 2008;134:706-715.
78. Doorenspleet ME, Hubers LM, Culver EL, et al. Immunoglobulin G4+B-cell receptor clones distinguish immunoglobulin G 4-related disease from primary sclerosing cholangitis and biliary/pancreatic malignancies. *Hepatology*. 2016;64:501-507.
79. Yeh Y-C, Lei H-J, Chen M-H, et al. C-Reactive Protein (CRP) is a promising diagnostic immunohistochemical marker for intrahepatic cholangiocarcinoma and is associated with better prognosis. *Am J Surg Pathol*. 2017;41:1630-1641.
80. Kozaka K, Sasaki M, Fujii T, et al. A subgroup of intrahepatic cholangiocarcinoma with an infiltrating replacement growth pattern and a resemblance to reactive proliferating bile ductules: "Bile ductular carcinoma". *Histopathology*. 2007;51:390-400.
81. Macias R, Banales JM, Sangro B, et al. The search for novel diagnostic and prognostic biomarkers in cholangiocarcinoma. *Biochim Biophys Acta - Mol Basis Dis*. 2018;1864:1468-1477.
82. Yeh MM. Pathology of combined hepatocellular-cholangiocarcinoma. *J Gastroenterol Hepatol*. 2010;25:1485-1492.
83. Burt AD, Alves V, Bedossa P, et al. Data set for the reporting of intrahepatic cholangiocarcinoma, perihilar cholangiocarcinoma and hepatocellular carcinoma: recommendations from the International Collaboration on Cancer Reporting (ICCR). *Histopathology*. 2018;73:369-385.
84. Bagante F, Merath K, Squires MH, et al. The limitations of standard clinicopathologic features to accurately risk-stratify prognosis after resection of intrahepatic cholangiocarcinoma. *J Gastrointest Surg*. 2018;22:1-9.
85. Chatelain D, Farges O, Fuks D, Trouillet N, Pruvot FR, Regimbeau JM. Assessment of pathology reports on hilar cholangiocarcinoma: the results of a nationwide, multicenter survey performed by the AFC-HC-2009 study group. *J Hepatol*. 2012;56:1121-1128.
86. Komaya K, Ebata T, Yokoyama Y, et al. Recurrence after curative-intent resection of perihilar cholangiocarcinoma: analysis of a large cohort with a close postoperative follow-up approach. *Surg (United States)*. 2018;163:732-738.
87. Kang S-H, Hwang S, Lee Y-J, et al. Prognostic comparison of the 7th and 8th editions of the American Joint Committee on Cancer staging system for intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Sci*. 2018;25:240-248.

88. Nakamura H, Arai Y, Totoki Y, et al. Genomic spectra of biliary tract cancer. *Nat Genet*. 2015;47:1003-1010.
89. Borger DR, Tanabe KK, Fan KC, et al. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. *Oncologist*. 2012;17:72-79.
90. Wang P, Dong Q, Zhang C, et al. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. *Oncogene*. 2013;32:3091-3100.
91. Kipp BR, Voss JS, Kerr SE, et al. Isocitrate dehydrogenase 1 and 2 mutations in cholangiocarcinoma. *Hum Pathol*. 2012;43:1552-1558.
92. Goeppert B, Frauenschuh L, Renner M, et al. BRAF V600E-specific immunohistochemistry reveals low mutation rates in biliary tract cancer and restriction to intrahepatic cholangiocarcinoma. *Mod Pathol*. 2014;27:1028-1034.
93. Andersen JB, Spee B, Blechacz BR, et al. Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. *Gastroenterology*. 2012;142(1021–1031):e15.
94. Shafizadeh N, Grenert JP, Sahai V, Kakar S. Epidermal growth factor receptor and HER-2/neu status by immunohistochemistry and fluorescence in situ hybridization in adenocarcinomas of the biliary tree and gallbladder. *Hum Pathol*. 2010;41:485-492.
95. Limpaiboon T, Krissadarak K, Sripa B, et al. Microsatellite alterations in liver fluke related cholangiocarcinoma are associated with poor prognosis. *Cancer Lett*. 2002;181:215-222.
96. Goeppert B, Roessler S, Renner M, et al. Mismatch repair deficiency is a rare but putative therapeutically relevant finding in non-liver fluke associated cholangiocarcinoma. *Br J Cancer*. 2018. https://doi.org/10.1038/s41416-018-0199-2
97. Lowery MA, Ptashkin R, Jordan E, et al. Comprehensive molecular profiling of intrahepatic and extrahepatic cholangiocarcinomas: potential targets for intervention. *Clin Cancer Res*. 2018;24:4154-4161.
98. Graham RP, Barr Fritcher EG, Pestova E, et al. Fibroblast growth factor receptor 2 translocations in intrahepatic cholangiocarcinoma. *Hum Pathol*. 2014;45:1630-1638.
99. Arai Y, Totoki Y, Hosoda F, et al. Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. *Hepatology*. 2014;59:1427-1434.
100. Andrici J, Goeppert B, Sioson L, et al. Loss of BAP1 expression occurs frequently in intrahepatic cholangiocarcinoma. *Med (United States)*. 2016;95:e2491.
101. Mosbeh A, Halfawy K, Abdel-Mageed WS, Sweed D, Rahman M. Nuclear BAP1 loss is common in intrahepatic cholangiocarcinoma and a subtype of hepatocellular carcinoma but rare in pancreatic ductal adenocarcinoma. *Cancer Genet*. 2018;224–225:21-28.
102. Lee JH, Shin DH, Park WY, et al. IDH1 R132C mutation is detected in clear cell hepatocellular carcinoma by pyrosequencing. *World J Surg Oncol*. 2017;15:82.
103. Saha SK, Parachoniak CA, Ghanta KS, et al. Mutant IDH inhibits HNF-4α to block hepatocyte differentiation and promote biliary cancer. *Nature*. 2014;513:110-152.
104. Jusakul A, Cutcutache I, Yong CH, et al. Whole-genome and epigenomic landscapes of etiologically distinct subtypes of cholangiocarcinoma. *Cancer Discov*. 2017;7:1116-1135.
105. Chaisaingmongkol J, Budhu A, Dang H, et al. Common molecular subtypes among Asian hepatocellular carcinoma and cholangiocarcinoma. *Cancer Cell*. 2017;32(57–70):e3.
106. Sturm P, Baas IO, Clement MJ, et al. Alterations of the p53 tumor-suppressor gene and K-ras oncogene in perihilar cholangiocarcinomas from a high-incidence area. *Int J Cancer*. 1998;78:695-698.
107. Sasaki M, Matsubara T, Nitta T, Sato Y, Nakanuma Y. GNAS and KRAS mutations are common in intraductal papillary neoplasms of the bile duct. *PLoS One*. 2013;8:e81706.
108. Fujikura K, Akita M, Ajiki T, Fukumoto T, Itoh T, Zen Y. Recurrent mutations in APC and CTNNB1 and activated Wnt/β-catenin signaling in intraductal papillary neoplasms of the bile duct. *Am J Surg Pathol*. 2018;42:1674-1685.
109. Matthaei H, Wu J, Dal Molin M, et al. GNAS codon 201 mutations are uncommon in intraductal papillary neoplasms of the bile duct. *HPB*. 2012;14:677-682.
110. Farshidfar F, Zheng S, Gingras M-C, et al. Integrative genomic analysis of cholangiocarcinoma identifies distinct IDH-mutant molecular profiles. *Cell Rep*. 2017;18:2780-2794.
111. Goeppert B, Konermann C, Schmidt CR, et al. Global alterations of DNA methylation in cholangiocarcinoma target the Wnt signaling pathway. *Hepatology*. 2014;59:544-554.
112. Coudray N, Ocampo PS, Sakellaropoulos T, et al. Classification and mutation prediction from non–small cell lung cancer histopathology images using deep learning. *Nat Med*. 2018;24:1559-1567.

How to cite this article: Kendall T, Verheij J, Gaudio E, et al. Anatomical, histomorphological and molecular classification of cholangiocarcinoma. *Liver Int*. 2019;39(Suppl. 1):7–18. https://doi.org/10.1111/liv.14093