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Review

Benign biliary neoplasms and biliary tumor precursors

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Summary

Benign biliary tumor are common lesions that are often an incidental finding in subjects who undergo medical imaging tests for other conditions. Most are true neoplasms while few result from reactive or malformative proliferation. Benign tumors have no clinical consequences, although the premalignant nature or potential for malignant transformation is of concern in some cases. The main practical problem for pathologists is the need to differentiate them from malignant biliary tumours, which is not always straightforward.

Premalignant lesions of the bile duct have been described, although their incidence has been poorly characterized. These lesions include biliary mucinous cystic neoplasms, intraductal papillary neoplasms of the bile duct, and biliary intraepithelial neoplasia. In this article, histopathology of benign biliary tumors and biliary tumor precursors is discussed, with a focus on the main diagnostic criteria.

Key words: biliary, bile ducts, benign, neoplasms, precursors

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Introduction

Bile duct neoplasms are a heterogeneous group of benign and malignant tumors, which may arise at any point of the biliary tree. They derive from cholangiocytes and peribiliary glands.

The anatomy of the biliary tree is divided into intrahepatic and extrahepatic portions. Intrahepatic biliary tree begins with the canals of Hering, which connect bile canaliculi to bile ductules, and progressively merges into a system of interlobular, septal, and major ducts, which then coalesce to form the extrahepatic bile ducts. Interlobular bile ducts drain into septal ducts (measuring more than 100 μm in diameter), which in turn drain into segmental ducts (400 to 800 μm in diameter). Segmental ducts continue into the right and left hepatic ducts towards the hepatic hilum where they join, giving rise to the common hepatic duct which finally becomes the common bile duct (7 to 11 cm long and 5 to 10 mm in diameter) after giving off the cystic duct to the gallbladder. The common hepatic duct, the common bile duct (i.e. choledochus), the gallbladder, and the cystic duct are considered as extrahepatic biliary tree [1]. Peribiliary glands are minute structures that are distributed along the intrahepatic large bile ducts and the extrahepatic bile ducts [2]. Intra- and extrahepatic bile ducts are embryologically different: the former originate

Table I. Classification of benign biliary tumors and precursors, according to the 5th edition of the WHO Classification of digestive system tumours [6].

Benign biliary tumors
Bile duct adenoma
Biliary adenofibroma
Mucinous cystic neoplasm of the liver and biliary system
Biliary tumors precursors
Biliary intraepithelial neoplasia
Intraductal papillary neoplasm of the bile ducts

from the remodeling of the ductal plate, while large ducts derive from the elongation of hepatic ducts at hepatic hilum [1].

Small and large biliary ducts also differ from a histological point of view. In fact, small intrahepatic ducts are lined by small cuboidal cholangiocytes, while large ducts are lined by tall cylindrical cholangiocytes and mucin-producing cells. All these differences reflect the different pathological features and biological behavior of biliary neoplasms.

Herein we provide a general overview of benign biliary neoplasms and biliary tumour precursors, focusing on the most important clinical-pathological features, useful in the diagnostic routine. The classification scheme reported in the 5th edition of the WHO Classification of digestive system tumors is provided in Table I. The nomenclature used in this paper is consistent with this classification. Cholangiocarcinoma is the topic of another paper in this special issue of *Pathologica* [3].

Benign biliary neoplasms

Bile duct microhamartoma (von Meyenburg complex)

Bile duct microhamartoma, also called the von Meyenburg complex (VMC), is a ductular liver lesion that is thought to be part of the ductal plate malformation spectrum. It shows a strong association with hepatic fibropolycystic disease, even if most cases occur sporadically [4,5]. VMC is a common lesion, found in about 5% of the general population in a large autoptic series[6]. Although its malformative rather than neoplastic nature, it is reported in this section as it can be a mimicker of a biliary malignant tumor.

VMCs are discrete and well-defined nodules, often multiple and usually less than 0.5 cm in their greatest dimension, and are classically related to portal tracts [4,7].

Microscopically, a VMC is made of rounded or irregularly shaped and dilated bile ducts, containing bile or eosinophilic proteinaceous debris, often with branching and U shapes, embedded in a dense fibrous stroma (Fig. 1). Sometimes, ductal dilation may lead to the development of single or multiple macroscopic cysts. The lining cells are flattened or cuboidal, cytologically uniform, and lack mitoses. They show the typical cholangiocyte immunophenotype, staining positive for cytokeratin (CK) 7, CK8, CK18, and CK19 [4].

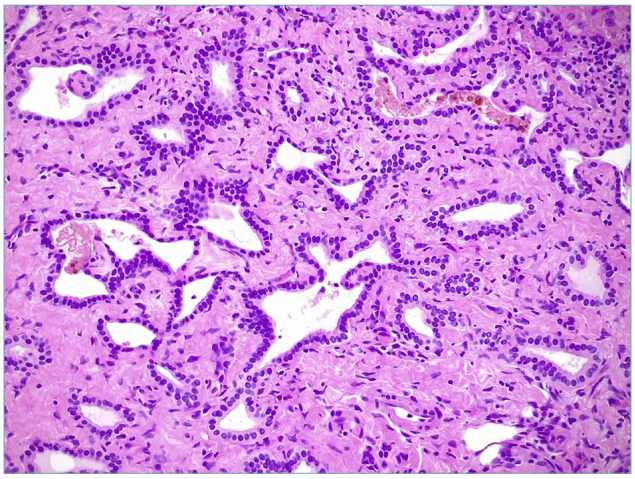

Figure 1. A Von Meyenburg complex, made of irregularly shaped and dilated bile ducts, containing bile and proteinaceous debris, within a dense fibrous stroma (haematoxylin-eosin; original magnification 20x).

VMCs show no significant risk for malignancy, although a few reports suggested the development of cholangiocarcinoma (CCA) in liver with multiple VMCs, particularly in patients with genetic hemochromatosis [8,9].

In the everyday practice of pathologists, it is important to differentiate VMC from CCA or metastatic adenocarcinoma, particularly in frozen sections on incidental findings at surgery. The presence of dilated glands containing bile is the major clue to exclude metastasis, while the complete absence of any cytological atypia is helpful to rule out CCA.

Bile duct adenoma

Bile duct adenoma (BDA) is defined as a benign epithelial lesion made of a proliferation of small, normal-looking bile ducts [7]. Peribiliary gland hamartoma is still an acceptable synonym.

BDA is commonly observed as an incidental finding during abdominal surgery or autopsy, in patients with a wide age range (1.5-99 years, mean 55 years), with no sex predilection [4,10,11].

The biological meaning of BDA is still controversial. It has classically been considered as a reactive process due to inflammation or traumatic injury. However, *BRAF* V600E mutations have been described in high percentage of cases (> 50%), suggesting its neoplastic nature [11-13].

BDAs are usually solitary and subcapsular (nearly 90% of the cases), and more than 90% are less than 10 mm in size. Grossly, BDA appears as a whitish, well-circumscribed, unencapsulated firm nodule [11].

Histological diagnostic criteria include: i) a patternless and disordered proliferation of small, relatively uniformly shaped and spaced tubules and/or ductular structures with an intact basement membrane, in a connective tissue stroma, ii) a single layer of cuboidal to columnar cells with regular nuclei without atypia and mitosis, and iii) absence of infiltrative borders (Fig. 2). Ducts in BDA show no or little lumen, without dilation. The fibrous stroma can be variably dense, collagenized and hyalinized, loose or scant, and may show various degrees of chronic inflammation, including nodular lymphoid aggregates, particularly at the periphery. Normal portal tracts are often enclosed within a BDA, usually near the periphery [4,7]. Clear-cell and oncocytic changes have been described, but their meaning is still unknown [14,15]. BDA epithelial cell phenotype is similar to that of normal bile ducts. They can also express mucin and show a secretory gland cell phenotype, expressing the foregut epithelial antigens D10, 1F6, MUC6, MUC5AC, and TFF2 [4,16,17].

Malignant transformation of a BDA has never been clearly reported. The main BDA clinical issue is the possibility to misdiagnose BDA as a well-differentiated CCA or a pancreatic adenocarcinoma, particularly during intraoperative consultation. In frozen sections, the differential is based on tumor size, cytological and architectural patterns, the presence of pre-existing portal tracts within the lesion, and the presence of invasive features [4,7]. However, it is not always possible to reach a definite diagnosis in frozen section, and even in paraffin-embedded samples diagnosis can be challenging. Thus, immunostainings can be of some help. The keratin profile is not useful, since BDAs and pancreato-biliary cancers share the same immunophenotype, which consists in the expression of CK7, CK8, CK18, and CK19, with a variable expression of CK20. Ki67, EZH2, p53, and p16 may help. In fact, a high proliferation index and a high expression of EZH2 and p53 favor carcinoma. However, it is important to keep in mind that a low proliferation index and a low EZH2 and p53 expression do not exclude malignancy. Interestingly, p16 is constantly express in BDA (Fig. 3), while a subgroup of CCA lacks its expression.

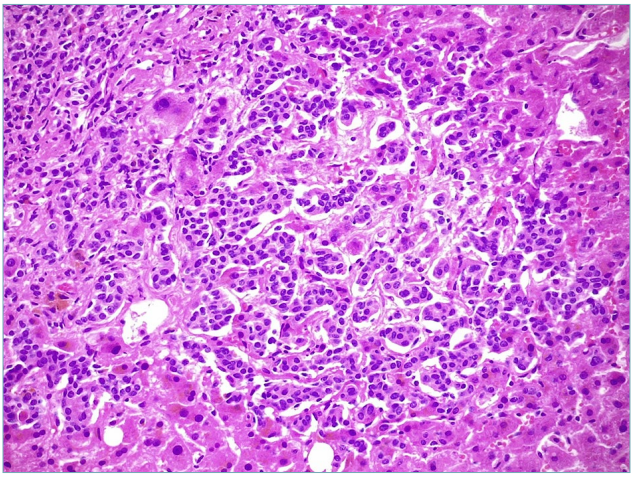

Figure 2. Bile duct adenoma is a disordered proliferation of small and uniform ductular structures within a connective tissue stroma, with no infiltrative borders. Neoplastic cells are cuboidal/columnar and lack atypia and mitosis (hematoxylin-eosin; original magnification 20x).

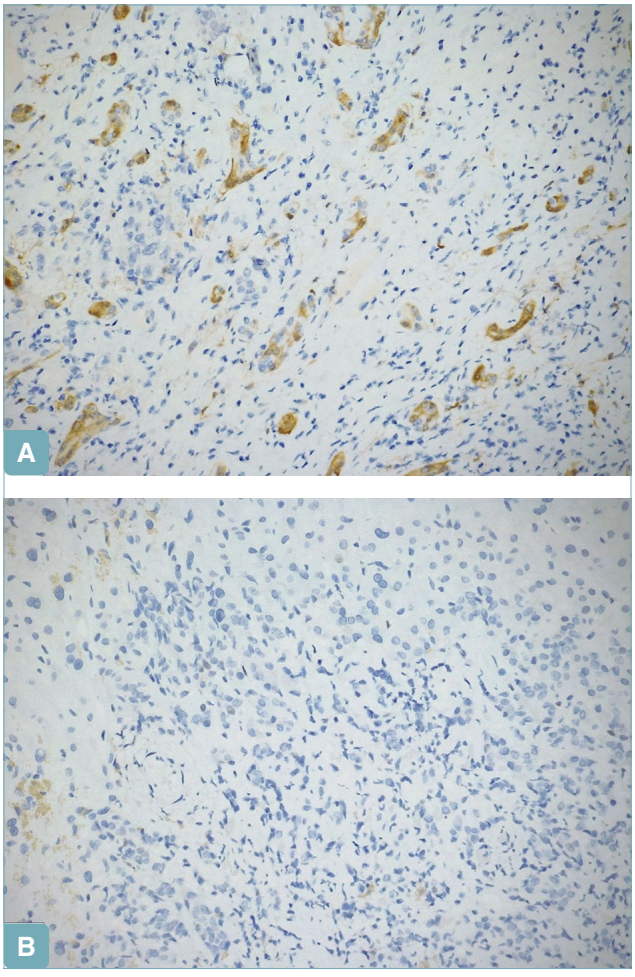

Figure 3. Differently from cholangiocarcinoma, bile duct adenoma shows a diffuse p16 positivity (A), and a wild-type pattern of expression of p53, with only a few positive nuclei (B) (A: p16 immunostain; original magnification 20x; B: p53 immunostain; original magnification 20x).

Therefore, a negative p16 stain supports CCA diagnosis [18,19]. BDA differential diagnoses also include other benign biliary proliferations, such as reactive ductular proliferation (particularly in cirrhosis, when the proliferation may be nodular in shape), VMC, and biliary adenofibroma. BDAs never show cystic changes and never contain bile in gland lumens [7].

Biliary adenofibroma

Biliary adenofibroma (BAF) is a solid epithelial benign liver neoplasia composed of microcystic and tubuloacinar glands lined by non-mucin-secreting biliary epithelial cells, embedded in a fibrous stroma [7]. It is an exceptionally rare benign tumor with a potential risk for malignant transformation and recurrence, if surgical excision is incomplete [20]. In the literature, only 21 cases have been described, so far.

Mean age at presentation is 60 years, with a slight female predominance. Most patients show abdominal pain, while a few lesions are incidental findings [20].

BAF is a primary epithelial tumor with a secondarily induced stroma [21]. Multiple clonal cytogenetic alterations have been described in BAF, supporting its neoplastic nature. Moreover, amplifications of *CCND1* and *ERBB2* and mutations in *CDKN2A* were found in cases showing aggressive behavior and malignant transformation [22].

BAFs are typically solitary and may affect both liver lobes. They are usually large lesions, with a wide diameter range, and are well-defined, round to oval, whitish, and unencapsulated. On cut section, both solid and microcystic areas (with sponge-like appearance) can be recognized, and some lesions may show macrocystic changes [7].

Histologically, BAF is composed of both glandular and stromal components. Glandular structures are typically shaped in acini, tubules, and cysts, and show biliary differentiation, similarly to VMC and BDA. Glands and cysts show variable size and shape, with dilation and branching, sometimes with complex configurations. Epithelial polypoid projections may be present. The glandular component is lined by a cuboidal to low columnar, amphophilic, non-mucin-producing epithelium. Cells show bland round nuclei with inconspicuous nucleoli, and apocrine changes can be seen. Microcysts are typically lined by flattened epithelium, and can be filled with eosinophilic, proteinaceous material and cellular debris, but they do not contain mucin. Only occasional mitotic figures are present, and the proliferation index is low in both epithelial and stromal components. The background stroma is usually abundant, collagenous, and contains bland myofibroblasts. It is frequently present a patchy chronic inflammatory infiltrate. As previously mentioned, epithelial cells have a biliary phenotype, expressing CK7, CK19, epithelial membrane antigen (EMA), and CA19-9 [4,7]. Premalignant changes have been reported in nearly a half of cases, showing epithelial dysplasia and architectural disarray, which consists in complex intracystic papillary proliferation and cribriform pattern. As the behavior of these tumors is poorly understood, patients require close clinical follow-up. Malignant degeneration leads to the development of a conventional adenocarcinoma [20,22-25].

Mucinous cystic neoplasm of the liver and biliary system

Mucinous cystic neoplasm (MCN) of liver and biliary system, formerly known as 'hepatobiliary cystadenoma', is a cyst-forming epithelial neoplasia with no communication with the bile ducts, made of mucin-producing epithelium associated with an underlying ovarian-type stroma. MCN shows either low grade or high grade epithelial dysplasia, and may be associated with an invasive carcinoma [4,7]. Most MCNs are solitary intrahepatic lesions, rarely seen in the extrahepatic bile ducts and in gallbladder [26,27].

MCNs are rare, with an estimated incidence of 1 case/20,000-100,000 per year, consisting in less than 5% of all liver cysts. They occur almost exclusively in women, and the mean age at diagnosis is nearly 50 years, even if MCNs with an associated invasive carcinoma arise in older people[26-29]. Patients typically show symptoms, which include chronic non-specific abdominal pain or discomfort and swelling, a palpable mass, and obstructive jaundice. In old patients, acute symptoms may suggest an invasive component [7,27]. Preoperative diagnosis may be challenging and requires a high degree of suspicion. Serum CA19-9 levels may be increased, particularly in patients with an associated invasive component, while intracystic CA19-9 and carcinoembryonic antigen (CEA) levels may be of help in differential diagnosis with non-neoplastic lesions, since they are higher in MCN [27,30,31]. Abdominal imaging shows large multilocular cystic lesions, with cyst-in-cyst appearance, without any communication with the biliary tree, even though unilocular cystic lesions may be seen in about 10% of patients. Irregular thickness of the cystic wall, enhancing internal septa with mural nodules, and papillary projections suggest an invasive component [27,32]. Etiology of MCN remains unclear, even if its prevalence in middle-aged women point toward a hormonal influence [30].

MCN grossly appears as a multiloculated and well-demarcated cystic lesion, with a fibrous capsule, independent from the biliary tree, even if a polypoid extension into bile duct lumen may be observed [33]. Its inner surface is usually smooth or trabeculated; when an invasive component is present, papillary projections

and white solid areas are seen. Cysts may contain clear serous, mucinous or gelatinous fluid, which can be purulent or hemorrhagic [28,34].

Histological hallmarks are i) mucinous lining epithelium, and ii) specialized ovarian-like stroma. Epithelial cells are usually arranged in a single layer and may be columnar, cuboidal or flattened, with pale eosinophilic to mucinous cytoplasm, bland and basally oriented nuclei, and no mitotic activity. In some cases the epithelium may be non-mucinous, resembling the lining of non-neoplastic bile ducts, and may show intestinal and gastric differentiation as well as squamous metaplasia. Scattered chromogranin- or synaptophysin-positive neuroendocrine cells can be seen. Small polypoid or papillary tufts may be seen along the inner surface. Mucin can be typically demonstrated by the histochemical staining with mucicarmine and Alcian blue. The lining cells have the staining pattern of biliary-type epithelium, with positivity for CK7, CK19, CK8, and CK18. They also express EMA, CEA, and MUC5AC, while CDX2, MUC2 and CK20 are positive in areas with intestinal differentiation. The epithelium may be ulcerated, and extravasation of the cyst fluid into the stroma or wall may occur, with the development of inflammation, xantho-granulomatous reaction, scarring, and calcifications [28,34,35]. Importantly for diagnostic purposes, the entity-defining stroma is present in all MCNs, at least focally. Since it may be focal, an extensive sampling of the lesion is recommended. The stroma is hypercellular, clearly resembling ovarian stroma, which, in turn, is surrounded by more collagenized fibrous tissue (Fig. 4). Stromal cells are characteristically positive for estrogen and progesterone receptors, and α-inhibin, and may be focally luteinized (Fig. 4). Stromal inflammation, hemorrhage, calcification, and necrosis are common [28].

Most MCNs have low/intermediate-grade dysplasia. However, nuclear pleomorphism, loss of polarity, presence of mitotic figures and multilayering of the epithelium define a high-grade dysplasia. The association with invasive adenocarcinoma, which is defined by the infiltration of the underlying stroma by tumor cells, is rare and occurs in nearly 6% of cases; it usually shows tubular or tubulo-papillary patterns and a desmoplastic stromal reaction [28,36]. It is strongly recommended a thorough histological examination of the surgical specimen for grading dysplasia and excluding malignant transformation.

Fine-needle aspiration (FNA) cytology is not helpful in MCN diagnosis, since it can only differentiate benign cyst contents from adenocarcinoma, so the final diagnosis requires histological confirmation and correlation with clinical history and imaging. Moreover, FNA should be avoided if MCN is suspected, since

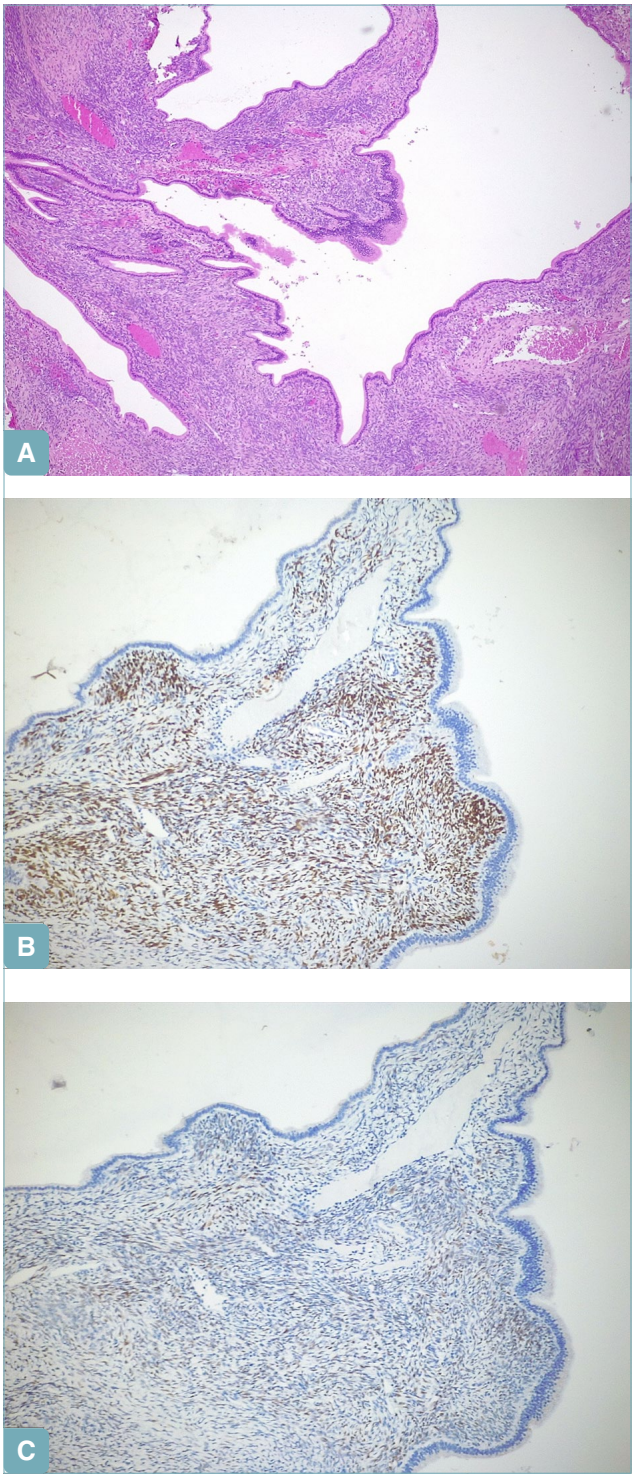

Figure 4. Mucinous cystic neoplasm of liver and biliary system is composed of a mucin-secreting lining epithelium, embedded in a hypercellular specialized ovarian-like stroma (A). Stromal cells stain positive for estrogen (B) and progesterone receptors (C) (A: hematoxylin-eosin; original magnification 5x; B: estrogen receptor immunostain; original magnification 10x; C: progesterone receptor immunostain; original magnification 10x).

intraoperative cyst spillage may lead to peritoneal dissemination. FNA samples usually contain aggregates of bland cuboidal to columnar epithelial cells, with occasional papillary arrangement. Different degrees of nuclear atypia might be observed, if a high-grade dysplastic or carcinomatous component is present [27,37]. The ovarian-type stromal component is typically not seen. The background is usually watery or with abundant thick mucin containing chronic inflammatory cells and histiocytes [27,37]. *KRAS* gene mutations are found in nearly 20% of MCNs, mostly in high-grade dysplastic lesions, being uncommon (5%) in cases with low-grade dysplasia [38].

Many cystic lesions of the liver can mimic MCN, but the finding of ovarian-like stroma confirms MCN diagnosis. The main differential diagnoses include simple bile duct or peribiliary gland cysts, developmental cysts, hydatid cysts, microcystic serous cystadenomas, and cystic liver metastases, which occur mainly from colorectal, ovarian, pancreatic, or lung carcinomas. Endometriosis can simulate MCN, as well. Finally, intraductal papillary neoplasms of the bile ducts can show a cystic appearance and be mimicker of MCN, but the absence of an ovarian-like stroma and the absence of connection with the biliary tree help the pathologist in the diagnosis [4,7,27].

The frequency of MCN malignant transformation has been reported to be as high as 20% to 30%; therefore, surgical resection is indicated. Prognosis is excellent if a complete excision is possible, with an outstanding recurrence-free survival. Marsupialization and fenestration are considered inadequate surgical procedures, with high risk of recurrence. Staging of MCN with an associated adenocarcinoma follows the TNM classification for intrahepatic cholangiocarcinoma or carcinoma of the extrahepatic bile ducts, depending on the neoplastic location. Prognosis of MCN-associated carcinoma seems to be better than conventional intrahepatic cholangiocarcinoma [27,38,39].

Biliary tumor precursors

There are two main types of premalignant lesions of the bile ducts, which are considered as precursors of CCA:

- Biliary intraepithelial neoplasia (BilIN)
- Intraductal papillary neoplasm of the bile ducts (IPNB).

Biliary intraepithelial neoplasia

BilIN is a microscopic, non-invasive, flat or micropapillary lesion confined to the lumen of bile ducts. It is now classified in BilIN with low-grade or high-grade dysplasia[7].

The prevalence of BilIN of the bile ducts outside the setting of invasive carcinoma is not known and difficult to assess. Indeed, BilIN is usually seen as an incidental finding in specimens resected for other reasons, and it is not detectable by imaging [7,40]. BilIN development has been associated with different risk factors, such as lithiasis, familial adenomatous polyposis, primary sclerosing cholangitis, choledocal cysts, and anomalous confluence of pancreato-biliary ducts. BilIN is often found in biliary epithelium adjacent to invasive adenocarcinoma, as well as in cirrhotic livers with non-biliary diseases, such as alcoholic liver disease or non-alcoholic steatohepatitis [41,42]. Persistence of chronic inflammation seems to lead the onset and progression of neoplastic changes in biliary epithelium, with involvement of different molecular pathways. In fact, it is known that *KRAS* mutations are an early event in biliary carcinogenesis, being present in almost 40% of BilINs, while *TP53* mutations occur as a late molecular event [43,44].

Macroscopically, BilIN are usually not evident, and only subtle mucosal changes can be observed, such as mucosal thickening, granularity, and change in color [7,40].

Histologically, BilIN is classified according to the highest degree of nuclear and architectural atypia in low-grade or high-grade. This classification recently replaced the previous three-tiered classification, which included BilIN-1, BilIN-2 (now low-grade BilIN), and BilIN-3 (now high-grade BilIN). In low-grade BilIN, biliary epithelium shows mild cytoarchitectural atypia, with hyperchromatic nuclei with prominent nucleoli, a mildly increased nucleus/cytoplasm ratio with minor nuclear pseudostratification and preserved nuclear polarity (Fig. 5). Peribiliary glands are rarely involved [29,40,45-47].

Low-grade BilIN should not be confused with mucosal reactive changes, which are much more common [40,43]. Hyperplasia or regenerative changes are usually flat, although low-papillary or micropapillary architecture can be observed in association with hepatolithiasis or choledochal cyst. Compared with normal epithelium, the cellularity is only slightly increased. Two very useful clues supporting the reactive nature are: i) the appearance of nuclear membrane, which remains smooth, and ii) the presence of intraepithelial infiltration of neutrophils [48]. Immunoistochemical stain may be of help, since p53 overexpression supports the diagnosis of a dysplastic lesion, even if its absence does not exclude it.

High-grade BilIN more frequently shows micropapillary growth pattern, with taller papillae. Cells display severe nuclear atypia, with irregular, pleomorphic, and bizarre nuclei, and a complete loss of nuclear po-

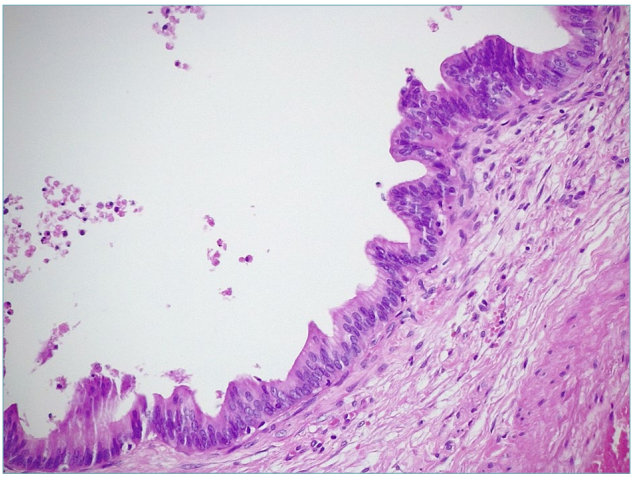

Figure 5. Low-grade biliary intraepithelial neoplasia shows mild cytological atypia, with hyperchromatic nuclei, prominent nucleoli, a mildly increased nucleus/cytoplasm ratio, and minor nuclear pseudostratification, with preserved nuclear polarity (hematoxylin-eosin; original magnification 20x).

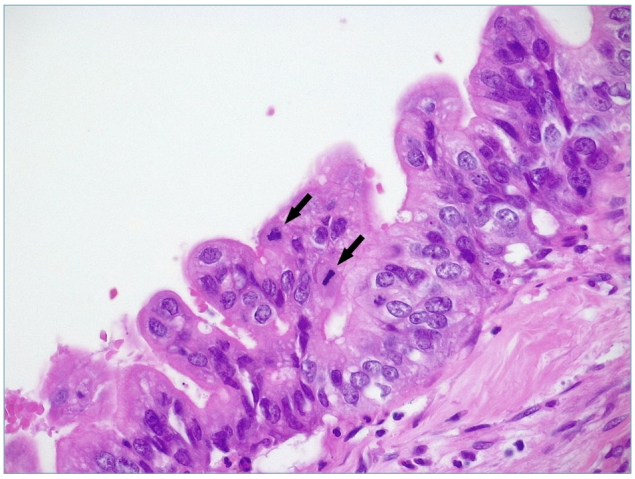

Figure 6. High-grade biliary intraepithelial neoplasia displays severe nuclear atypia, with irregular and pleomorphic nuclei, several mitotic figures (arrows), and a complete loss of nuclear polarization, with complex stratification (hematoxylin-eosin; original magnification 40x).

larity, with complex stratification (Fig. 6). An important feature, which points to the diagnosis of high-grade BilIN, is the evidence of nuclei on the luminal surface (i.e. loss of polarization). Overall, the lesion resembles a malignant one, but with a preserved basement membrane. Mitoses are frequent, with a markedly increased Ki-67 proliferation index, and involvement of the peribiliary glands may occur. Immunohistochemical stains may be of help in distinguishing low-grade from high-grade BilINs (Table II). Epithelial cells may show biliary, intestinal, or gastric phenotype [29,40,45-47].

In FNA specimens, BilIN diagnosis is not considered, and when severely atypical features, similar to those seen in carcinoma, are seen in biliary epithelial cells, it is not possible to differentiate pre-invasive from invasive lesions [49,50].

Intraductal papillary neoplasm of the bile ducts

IPNB is a premalignant lesion with intraductal papillary or villous growth of biliary-type epithelium that may show low- or high-grade dysplasia or have an associated invasive carcinoma [7]. It is considered the biliary counterpart of the intraductal papillary mucinous neoplasm of pancreas, but in liver it is a rarer disease [29,40,47,51,52].

IPNB incidence varies consistently among different geographical regions, representing 7-10% of all bile duct tumors in Europe and North America, and 10-40% in Asian cohorts [51,53]. Patients with IPNB are preferentially men with a median age of 50-70 years, who usually show a recurrent and intermittent pain and cholangitis. IPNB is rarely seen in children, and no tendency for familial aggregation of cases has been described [53-55]. IPNB may be radiologically undetectable, but in most of cases, cholangiography shows filling defects in the biliary tree, due to an intraductal mass, with dilatation of either proximal or distal bile ducts. The prevalent location of IPNB (intrahepatic versus extrahepatic bile ducts) is highly variable among studies [55].

Etiology of IPNB remains unclear in most of the cases, even if known risk factors are primary sclerosing cholangitis, hepatolithiasis, and liver fluke infection (in Asian countries) [51,55,56]. IPNB development and progression from low- to high-grade dysplasia to invasive carcinoma follow a sequential acquisition of molecular

Table II. Immunostains that may be of help in differentiating low-grade from high-grade biliary intraepithelial neoplasia.

Immunostain	Low-grade BilIN	High-grade BilIN
S100	Mildly to moderately increased	Diffusely and strongly positive
p53	Usually wild-type expression	Frequently positive
p16	Relatively preserved	Decreased expression

BilIN: biliary intraepithelial neoplasia.

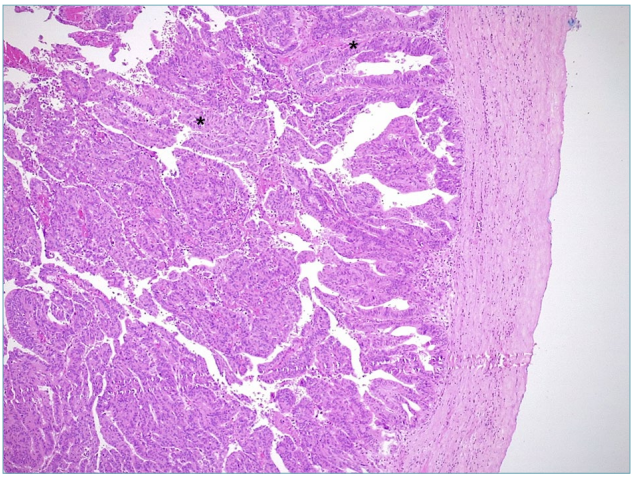

Figure 7. Intraductal papillary neoplasm in a dilated bile duct, with a papillary/villous growth pattern, made of fine fibrovascular cores covered by biliary epithelial cells, with diffuse low-grade and focally high-grade (asterisks) dysplasia (Immunostain-eosin; original magnification 5x).

alterations involving common oncogenic pathways, including *KRAS*, *CDKN2A*, and *TP53* genes [57].

Grossly, IPNB appears as a visible papillary, villous, or polypoid, red-colored, soft mass, predominantly growing in a dilated bile duct lumen, which may be cystic or fusiform in shape. Lesions might be single and isolated or multiple, and mucus hypersecretion can be seen. An invasive component can appear as a mass-forming or nodular lesion [51,55,58].

Histologically, IPNBs are characterized by dilated bile ducts filled with papillary or villous structures with fine fibrovascular cores covered by biliary epithelial cells, with various amount of tubular or glandular components, and lack of an ovarian-type mesenchymal stroma (Fig. 7). Epithelial cells in IPNB are cuboidal or columnar, and may show intestinal, biliary, oncocytic, and gastric-type differentiation, based on morphology and immunophenotype. The biliary subtype is the most common in Western countries, while the oncocytic and gastric types are very rare. Mixtures of different subtypes are observed in about 50% of all IPNBs, so their classification is based on the most prevalent component. The gastric subtype expresses MUC5AC and MUC6, the intestinal one is MUC2-positive, whilst the biliary type frequently expresses EMA (MUC1) [40,51,55,59,60]. Epithelial cells lining IPNB may show variable cytoarchitectural atypia, classified into low-grade and high-grade dysplastic lesions, according to the highest degree. IPNBs with high-grade dysplasia are more frequent in extrahepatic bile ducts. The extension of neoplastic process into peribiliary glands, particularly in the hilar zone, is quite common, and is not considered a feature of invasion. A clear, frank invasion of the stroma is necessary for a definitive diagnosis of an associated invasive adenocarcinoma. About 40-80% of IPNBs show a minimal invasive component at diagnosis, most commonly a tubular adenocarcinoma, sometimes a colloid (mucinous) adenocarcinoma [55,61].

Recently, a group of Japanese and Korean expert pathologists proposed a different classification of IPNB, based on similarities with the pancreatic counterparts. It divides IPNB in two groups. Type 1 IPNB histologically resembles intraductal papillary mucinous neoplasm of the pancreas; it typically develops in the intrahepatic bile ducts, and contains macroscopic mucinous components. Type 2 IPNB has a more complex histological architecture, with irregular papillary branching or foci of solid-tubular components; it involves the extrahepatic bile ducts, and is more frequently associated with invasive cancers [51,62] (Tab. III).

FNA samples from IPNBs are often hypercellular, with sheets of epithelial cells that are commonly arranged in papillary structures with fibrovascular cores. Microglandular structures and mucin may be present, while

Table III. Features of type 1 and type 2 intraductal papillary neoplasm of the bile ducts, according to the Japan-Korea Cooperative Study Group [61].

Feature	Type 1 IPNB	Type 2 IPNB
Preferential location	Intrahepatic bile ducts	Extrahepatic bile ducts
Mucin secretion	Frequent	Rare
Architecture	Regular homogeneous papillae	Irregular complex papillae
Histological subtypes	Gastric, intestinal	Intestinal, pancreatobiliary
Grade	Mostly high grade	Always high grade
Stromal invasion	< 50%, minimal	> 80%, minimal or mild
Similarity to pancreatic IPMN	Similar	Variable
Aggressiveness	Less aggressive	More aggressive
Outcome	More favorable	Worse

IPNB: intraductal papillary neoplasm of the bile ducts; IPMN: intraductal papillary mucinous neoplasm.

cellular crowding is absent. Epithelial cells usually show mild atypia, but not frankly malignant nuclear features. In IPNB with malignant transformation, overt carcinomatous cytological features are seen [49,50].

Differential diagnoses of IPNB include micropapillary biliary intraepithelial neoplasia and intraductal polypoid liver metastasis, predominantly from colorectal cancers [51,63]. The presence of intermixed flat or pseudopapillary intrahepithelial neoplasia are in favor with the diagnosis of biliary intraepithelial neoplasia. Immunohistochemical stains, including CK7, CK19, CK20 and CDX2, are recommended to differentiate IPNB from metastatic colon cancer.

Hepatectomy is the standard therapy of IPNBs confined to the liver. IPNB-associated invasive carcinoma has demonstrated a better prognosis than conventional intrahepatic and extrahepatic cholangiocarcinoma, with an overall survival of nearly 70% at 5 years and a tumor recurrence rate of 47%. The percentage of invasive carcinoma and its depth of invasion correlate with survival. Invasive carcinomas arising from biliary-type IPNB have worse clinic-pathological features and a worse clinical outcome compared to other subtypes. Staging of IPNB-related adenocarcinoma follows the TNM classification for intrahepatic cholangiocarcinoma [39,53-55,64].

A so-called intraductal tubulopapillary neoplasm (ITPN) variant exists, and has a more solid, tubular growth pattern with less papillary frond formation and mucin production. ITPNs are found in different points of the biliary tree, with different frequencies: intrahepatic bile ducts (53%), perihilar bile ducts (30%), and extrahepatic bile ducts (17%). Tumors tend to be larger, with a median size of 5.3 cm. Approximately 40% to 80% of lesions are associated with invasive carcinoma; therefore, ITPNs should undergo a formal oncologic resection, which includes a partial hepatectomy for intrahepatic disease [60,65-67].

References

1 Guido M, Sarcognato S, Sacchi D, et al. The anatomy and histology of the liver and biliary tract. In: D'Antiga L, ed. Pediatric hepatology and liver transplantation. Cham (Switzerland): Springer Nature Switzerland AG 2019, p. 41. https://doi.org/10.1007/978-3-319-96400-3_3

2 Matsubara T, Kozaka K, Matsui O, et al. Peribiliary glands: development, dysfunction, related conditions and imaging findings. Abdom Radiol (NY) 2020;45:416-436. https://doi.org/10.1007/s00261-019-02298-4

3 Sarcognato S, Sacchi D, Fassan M, et al. Cholangiocarcinoma. Pathologica 2021; in press

4 Zen Y, Hübscher SG, Nakanuma Y. Bile duct diseases. In: Burt AD, Ferrell LD, Hübscher SG, eds. MacSween's pathology of the liver. 7th ed. Philadelphia, PA: Elsevier 2018, p. 515.

5 Desmet VJ. Congenital diseases of intrahepatic bile ducts: variations on the theme "ductal plate malformation". Hepatology 1992;16:1069-1083. https://doi.org/10.1002/hep.1840160434

6 Redston MS, Wanless IR. The hepatic von Meyenburg complex: prevalence and association with hepatic and renal cysts among 2843 autopsies [corrected]. Mod Pathol 1996;9:233-237

7 WHO Classification of Tumours Editorial Board. Digestive system tumours. Fifth Edition. Lyon (France): International Agency for Research on Cancer 2019

8 Röcken C, Pross M, Brucks U, et al. Cholangiocarcinoma occurring in a liver with multiple bile duct hamartomas (von Meyenburg complexes). Arch Pathol Lab Med 2000;124:1704-1706. https://doi.org/10.1043/0003-9985(2000)124<1704:COIALW>2.0.CO;2

9 Wisniewski B, Tordjman G, Courillon-Mallet A, et al. Cholangiocarcinoma developing in von Meyenburg complexes in haemochromatosis. Gastroenterol Clin Biol 2002;26:922-924

10 Aishima S, Tanaka Y, Kubo Y, et al. Bile duct adenoma and von Meyenburg complex-like duct arising in hepatitis and cirrhosis: pathogenesis and histological characteristics. Pathol Int 2014;64:551-559. https://doi.org/10.1111/pin.12209

11 Allaire GS, Rabin L, Ishak KG, et al. Bile duct adenoma. A study of 152 cases. Am J Surg Pathol 1988;12:708-715. https://doi.org/10.1097/00000478-198809000-00007

12 Pujals A, Amaddeo G, Castain C, et al. BRAF V600E mutations in bile duct adenomas. Hepatology 2015;61:403-405. https://doi.org/10.1002/hep.27133

13 Pujals A, Bioulac-Sage P, Castain C, et al. BRAF V600E mutational status in bile duct adenomas and hamartomas. Histopathology 2015;67:562-567. https://doi.org/10.1111/his.12674

14 Albores-Saavedra J, Hoang MP, Murakata LA, et al. Atypical bile duct adenoma, clear cell type: a previously undescribed tumor of the liver. Am J Surg Pathol 2001;25:956-960. https://doi.org/10.1097/00000478-200107000-00016

15 Arena V, Arena E, Stigliano E, et al. Bile duct adenoma with oncocytic features. Histopathology 2006;49:318-320. https://doi.org/10.1111/j.1365-2559.2006.02441.x

16 Hughes NR, Goodman ZD, Bhathal PS. An immunohistochemical profile of the so-called bile duct adenoma: clues to pathogenesis. Am J Surg Pathol 2010;34:1312-1318. https://doi.org/10.1097/PAS.0b013e3181ead722

17 Bhathal PS, Hughes NR, Goodman ZD. The so-called bile duct adenoma is a peribiliary gland hamartoma. Am J Surg Pathol 1996;20:858-864. https://doi.org/10.1097/00000478-199607000-00009

18 Tsokos CG, Krings G, Yilmaz F, et al. Proliferative index facilitates distinction between benign biliary lesions and intrahepatic cholangiocarcinoma. Hum Pathol 2016;57:61-67. https://doi.org/10.1016/j.humpath.2016.06.019

19 Sasaki M, Matsubara T, Kakuda Y, et al. Immunostaining for polycomb group protein EZH2 and senescent marker p16INK4a may be useful to differentiate cholangiolocellular carcinoma from ductular reaction and bile duct adenoma. Am J Surg Pathol 2014;38:364-369. https://doi.org/10.1097/PAS.0000000000000125

20 Arnason T, Borger DR, Corless C, et al. Biliary adenofibroma of liver: morphology, tumor genetics, and outcomes in 6 cases. Am J Surg Pathol 2017;41:499-505. https://doi.org/10.1097/PAS.0000000000000773

21 Varnholt H, Vauthey JN, Dal Cin P, et al. Biliary adenofibroma: a rare neoplasm of bile duct origin with an indolent behaviour. Am J Surg Pathol 2003;27:693-698. https://doi.org/10.1097/00000478-200305000-00014

[22] Thompson SM, Zendejas-Mummert B, Hartgers ML, et al. Malignant transformation of biliary adenofibroma: a rare biliary cystic tumor. J Gastrointest Oncol 2016;7:E107-E112. https://doi.org/10.21037/jgo.2016.09.14

[23] Thai E, Dalla Valle R, Evaristi F, et al. A case of biliary adenofibroma with malignant transformation. Pathol Res Pract 2016;212:468-470. https://doi.org/10.1016/j.prp.2015.12.015

[24] Godambe A, Brunt EM, Fulling KH, et al. Biliary adenofibroma with invasive carcinoma: case report and review of the literature. Case Rep Pathol 2016;2016:8068513. https://doi.org/10.1155/2016/8068513

[25] Kaminsky P, Preiss J, Sasatomi E, et al. Biliary adenofibroma: a rare hepatic lesion with malignant features. Hepatology 2017;65:380-383. https://doi.org/10.1002/hep.28818

[26] 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. https://doi.org/10.1038/modpathol.2011.71

[27] Budkule DP, Desai GS, Pande P, et al. Infrequent intrahepatic cystic neoplasm: dilemmas in diagnosis and management. BMJ Case Rep 2019;12:e229058. https://doi.org/10.1136/bcr-2018-229058

[28] Quigley B, Reid MD, Pehlivanoglu B, et al. Hepatobiliary mucinous cystic neoplasms with ovarian type stroma (so-called "hepatobiliary cystadenoma/cystadenocarcinoma"): clinicopathologic analysis of 36 cases illustrates rarity of carcinomatous change. Am J Surg Pathol 2018;42:95-102. https://doi.org/10.1097/PAS.0000000000000963

[29] Matsukuma KE, Yeh MM. Update on the pathology of liver neoplasms. Ann Diagn Pathol 2019;38:126-137. https://doi.org/10.1016/j.anndiagpath.2018.10.005

[30] Lee CW, Tsai HI, Lin YS, et al. Intrahepatic biliary mucinous cystic neoplasms: clinicoradiological characteristics and surgical results. BMC Gastroenterol 2015;15:67. https://doi.org/10.1186/s12876-015-0293-3

[31] Koffron A, Rao S, Ferrario M, et al. Intrahepatic biliary cystadenoma: role of cyst fluid analysis and surgical management in the laparoscopic era. Surgery 2004;136:926-936. https://doi.org/10.1016/j.surg.2004.06.031

[32] Safari MT, Shahrokh S, Miri MB, et al. Biliary mucinous cystic neoplasm: a case report and review of the literature. Gastroenterol Hepatol Bed Bench 2016;9:S88-S92.

[33] Takano Y, Nagahama M, Yamamura E, et al. Prolapse into the bile duct and expansive growth is characteristic behavior of mucinous cystic neoplasm of the liver: report of two cases and review of the literature. Clin J Gastroenterol 2015;8:148-155. https://doi.org/10.1007/s12328-015-0569-8

[34] Albores-Saavedra J, Córdova-Ramón JC, Chablé-Montero F, et al. Cystadenomas of the liver and extrahepatic bile ducts: morphologic and immunohistochemical characterization of the biliary and intestinal variants. Ann Diagn Pathol 2015;19:124-129. https://doi.org/10.1016/j.anndiagpath.2015.03.001

[35] Zhelnin K, Xue Y, Quigley B, et al. Nonmucinous biliary epithelium is a frequent finding and is often the predominant epithelial type in mucinous cystic neoplasms of the pancreas and liver. Am J Surg Pathol 2017;41:116-120. https://doi.org/10.1097/PAS.0000000000000745

[36] Arnaoutakis DJ, Kim Y, Pulitano C, et al. Management of biliary cystic tumors: a multi-institutional analysis of a rare liver tumor. Ann Surg 2015;261:361-367. https://doi.org/10.1097/SLA.0000000000000543

[37] Logroño R, Rampy BA, Adegboyega PA. Fine needle aspiration cytology of hepatobiliary cystadenoma with mesenchymal stroma. Cancer 2002;96:37-42.

[38] Fujikura K, Akita M, Abe-Suzuki S, et al. Mucinous cystic neoplasms of the liver and pancreas: relationship between KRAS driver mutations and disease progression. Histopathology 2017;71:591-600. https://doi.org/10.1111/his.13271

[39] Brierley J, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours. Eighth Edition. Oxford (UK), Hoboken (NJ): John Wiley & Sons Inc. 2017

[40] Geramizadeh B. Precursor lesions of cholangiocarcinoma: a clinicopathologic review. Clin Pathol 2020;13:2632010X20925045. https://doi.org/10.1177/2632010X20925045

[41] Lewis JT, Talwalkar JA, Rosen CB, et al. Precancerous bile duct pathology in end-stage primary sclerosing cholangitis, with and without cholangiocarcinoma. Am J Surg Pathol 2010;34:27-34. https://doi.org/10.1097/PAS.0b013e3181bc96f9

[42] Lee SE, Jang JY, Lee YJ, et al., Korean Pancreas Surgery Club. Choledochal cyst and associated malignant tumors in adults: a multicenter survey in South Korea. Arch Surg 2011;146:1178-1184. https://doi.org/10.1001/archsurg.2011.243

[43] Aishima S, Iguchi T, Fujita N, et al. Histological and immunohistological findings in biliary intraepithelial neoplasia arising from a background of chronic biliary disease compared with liver cirrhosis of non-biliary aetiology. Histopathology 2011;59:867-875. https://doi.org/10.1111/j.1365-2559.2011.04011.x

[44] Hsu M, Sasaki M, Igarashi S, et al. KRAS and GNAS mutations and p53 overexpression in biliary intraepithelial neoplasia and intrahepatic cholangiocarcinomas. Cancer 2013;119:1669-1674. https://doi.org/10.1002/cncr.27955

[45] Basturk O, Hong SM, Wood LD, et al., Baltimore Consensus Meeting. A revised classification system and recommendations from the baltimore consensus meeting for neoplastic precursor lesions in the pancreas. Am J Surg Pathol 2015;39:1730-1741. https://doi.org/10.1097/PAS.0000000000000533

[46] Matthaei H, Lingohr P, Strässer A, et al. Biliary intraepithelial neoplasia (BilIN) is frequently found in surgical margins of biliary tract cancer resection specimens but has no clinical implications. Virchows Arch 2015;466:133-141. https://doi.org/10.1007/s00428-014-1689-0

[47] Zaccari P, Cardinale V, Severi C, et al. Common features between neoplastic and preneoplastic lesions of the biliary tract and the pancreas. World J Gastroenterol 2019;25:4343-4359. https://doi.org/10.3748/wjg.v25.i31.4343

[48] 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. https://doi.org/10.1038/modpathol.3800788

[49] Avadhani V, Hacihasanoglu E, Memis B, et al. Cytologic predictors of malignancy in bile duct brushings: a multi-reviewer analysis of 60 cases. Mod Pathol 2017;30:1273-1286. https://doi.org/10.1038/modpathol.2017.51

[50] Hacihasanoglu E, Memis B, Pehlivanoglu B, et al. Factors impacting the performance characteristics of bile duct brushings: a clinico-cytopathologic analysis of 253 patients. Arch Pathol Lab Med 2018;142:863-870. https://doi.org/10.5858/arpa.2017-0150-OA

[51] Nakanuma Y, Kakuda Y, Uesaka K. Characterization of intraductal papillary neoplasm of the bile duct with respect to the histopathologic similarities to pancreatic intraductal papillary mucinous neoplasm. Gut Liver 2019;13:617-627. https://doi.org/10.5009/gnl18476

[52] Nakanuma Y, Sudo Y. Biliary tumors with pancreatic counterparts. Semin Diagn Pathol 2017;34:167-175. https://doi.org/10.1053/j.semdp.2016.12.013

[53] Luvira V, Somsap K, Pugkhem A, et al. Morphological classification of intraductal papillary neoplasm of the bile duct with survival correlation. Asian Pac J Cancer Prev 2017;18:207-213. https://doi.org/10.22034/APJCP.2017.18.1.207

[54] Luvira V, Pugkhem A, Bhudhisawasdi V, et al. Long-term outcome of surgical resection for intraductal papillary neoplasm of the bile duct. J Gastroenterol Hepatol 2017;32:527-533. https://doi.org/10.1111/jgh.13481

[55] Park HJ, Kim SY, Kim HJ, et al. Intraductal papillary neoplasm of the bile duct: clinical, imaging, and pathologic features. AJR Am J Roentgenol 2018;211:67-75. https://doi.org/10.2214/AJR.17.19261

[56] Hachiya H, Kita J, Shiraki T, et al. Intraductal papillary neoplasm of the bile duct developing in a patient with primary sclerosing cholangitis: a case report. World J Gastroenterol 2014;20:15925-15930. https://doi.org/10.3748/wjg.v20.i42.15925

[57] 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. https://doi.org/10.1038/modpathol.2013.112

[58] Kim H, Lim JH, Jang KT, et al. Morphology of intraductal papillary neoplasm of the bile ducts: radiologic-pathologic correlation. Abdom Imaging 2011;36:438-446. https://doi.org/10.1007/s00261-010-9636-2

[59] 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. https://doi.org/10.1038/ajg.2011.316

[60] Hucl T. Precursors to cholangiocarcinoma. Gastroenterol Res Pract 2019;2019:1389289. https://doi.org/10.1155/2019/1389289

[61] Nakanuma Y, Uesaka K, Miyayama S, et al. Intraductal neoplasms of the bile duct. A new challenge to biliary tract tumor pathology. Histol Histopathol 2017;32:1001-1015. https://doi.org/10.14670/HH-11-892

[62] Nakanuma Y, Jang KT, Fukushima N, et al. A statement by the Japan-Korea expert pathologists for future clinicopathological and molecular analyses toward consensus building of intraductal papillary neoplasm of the bile duct through several opinions at the present stage. J Hepatobiliary Pancreat Sci 2018;25:181-187. https://doi.org/10.1002/jhbp.532

[63] Yamao T, Hayashi H, Higashi T, et al. Colon cancer metastasis mimicking intraductal papillary neoplasm of the extra-hepatic bile duct. Int J Surg Case Rep 2015;10:91-93. https://doi.org/10.1016/j.ijscr.2015.01.053

[64] Kim WJ, Hwang S, Lee YJ, et al. Clinicopathological features and long-term outcomes of intraductal papillary neoplasms of the intrahepatic bile duct. J Gastrointest Surg 2016;20:1368-1375. https://doi.org/10.1007/s11605-016-3103-5

[65] Kanji ZS, Rocha FG. Premalignant lesions of the biliary tract. Surg Clin North Am 2019;99:301-314. https://doi.org/10.1016/j.suc.2018.11.007

[66] Katabi N, Torres J, Klimstra DS. Intraductal tubular neoplasms of the bile ducts. Am J Surg Pathol 2012;36:1647-1655. https://doi.org/10.1097/PAS.0b013e3182684d4f

[67] Schlitter AM, Jang KT, 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. https://doi.org/10.1038/modpathol.2015.61