REVIEW

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Intrahepatic biliary proliferations: histopathology and potential immunohistochemical markers

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HIGLIGHTS

- Intrahepatic biliary proliferations represent a spectrum varying from reactive to malignant entities.
- Clinical and imaging patterns may be similar, requiring histopathological and immunohistochemistry for precise diagnosis.

ABSTRACT – Intrahepatic biliary proliferations represent a spectrum from reactive (ductular reaction, some with atypical architecture), hamartomatous (von Meyenburg complex), benign (bile duct adenoma) and precursor/borderline entities (biliary intraepithelial neoplasia, intraductal papillary neoplasm of the bile duct) to fully malignant (cholangiocarcinoma) neoplasms. Clinical pictures and even imaging patterns may be similar, requiring refined studies aiming at histopathological and immunohistochemistry for more precise diagnosis, essential for correct patient management. This article discusses updated concepts and definitions of most relevant entities aiming more specifically at the differential diagnosis in practice, focusing on morphology and immunohistochemistry, with a discussion of potential markers to help distinguishing between benign and malignant lesions.

Keywords – Neoplasm; bile ducts; cholangiocarcinoma.

INTRODUCTION

Intrahepatic biliary proliferations are defying entities in routine practice, representing a spectrum varying from reactive lesions (ductular reaction, some with atypical architecture), hamartomatous von Meyenburg complex (VMC) benign bile duct adenoma (BDA), precursor/borderline entities (biliary intraepithelial neoplasia [BilIN], intraductal papillary neoplasm of the bile duct [IPNB], rare cases of malignant transformation in BDA) and fully malignant counterpart (cholangiocarcinoma [CCA], mainly the small duct subtype and its subtypes cholangiolocarcinoma and ductal plate malformation–like). Since clinical and radiological pictures may be very similar, even in present days the need for further histopathological criteria and the development and criterious selection of antibodies for immunohistochemical differential diagnosis is essential.

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Several patterns of biliary epithelial proliferation are now recognized, some of which present histological aspects reminiscent of their original epithelial counterparts. However, even after the publication of 5th Edition of WHO (2019) concerning questions remain regarding nomenclature, histological criteria and the potential for malignant transformation of each of these entities[1].

Advances in the study of intrahepatic biliary proliferations have become more relevant, with emerging studies focusing on the causes, potential of malignant transformation and heterogeneous clinical evolution. Since Chung and Park performed a recent and comprehensive review of intrahepatic cholangiocarcinoma[2], in the present study, the main focus of the present review will be on the remaining biliary proliferations.

Von Meyenburg complex

Rather common lesions[3] with diverse names in the literature, VMC is considered a form of ductal plate malformation[4-6], sometimes related to congenital cystic liver disease[7] and to recurrent cholangitis[8]. The patients are usually asymptomatic, presenting as solitary[9] or multiple nodules[1,4], or even as a multicystic lesion[10]. Recently, an atypical presentation of rapidly enlarging VMC was described[11].

Macroscopically, the lesions are typically subcapsular and well defined nodules[4], usually less than 0.5 cm[5], rarely forming a cyst[2]. The VMC is composed of branching irregular bile ducts with uniform cytological appearance[5], embedded in a fibrous and hyalinized stroma[1,4,6], with variable amounts of lymphocytes[4] (FIGURE 1). The lumina contain amorphous material or inspissated bile[5,12,13]. While this lesion is clearly nonneoplastic, a few reports describe cases showing concomitant CCA[13-20] or even hepatocellular carcinoma (HCC)[21] arising in VMC, but no clear cut mechanism for neoplastic transformation could be demonstrated.

A major point of concern is the similarity of some of these lesions with metastatic carcinoma, especially from the pancreatic duct, a major challenge for surgical pathologists in intraoperative consultations[22-26].

Bile duct adenoma

The origin of this lesion is, as yet, a matter of discussion: Ferrell et al.[5] ascribed its origin to a proliferative response to a localized injury rather than a true neoplastic process - this pattern of reaction to a focal injury might be related to its typical location (as subcapsular lesions, as sequelae of trauma or ischemia) and, accordingly, may appear in livers already presenting late-stage cirrhosis. The recent World Health Organization (WHO) classification, while accepting the hypothesis of postinflammatory/traumatic origin[1,27], also considers the alternative theory ascribing this lesion to a peribiliary gland hamartoma[1,28] or even to a neoplastic lesion driven by BRAF mutation[1,29,30].

BDA usually presents as a single lesion, more frequently subcapsular, flattened, well circumscribed and not encapsulated, ranging from 1 to 20 mm[4].

BDA is composed of uniformly shaped and spaced tubules, branching and densely packed[4]; without evident atypia or mitosis[1,5], and their nuclei are lighter than in normal bile duct[4]. These tubules may contain mucin and may be found in preexisting portal tracts[1]. The interface with the adjacent parenchyma is smooth at low magnification but contains

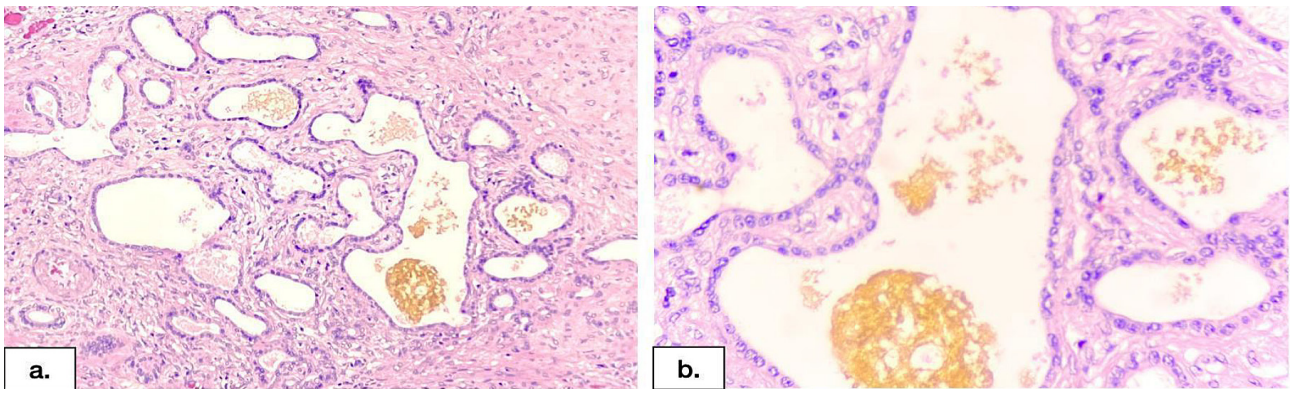

FIGURE 1. Von Meyenburg complex. At low power (a., HE stain, 100x), the ductules are irregularly shaped, branching, with inspissated bile in the lumina; the epithelial cells are low cuboidal, with uniform cytological appearance (b., HE stain, 400x).

interdigitations at higher magnification, appearing jagged[1] (FIGURE 2). Variable amounts of stroma, with sparse lymphocytic cells, is more pronounced at the margins[27]. Microcalcification[31] and hyalinization[4] are occasionally found.

The most recent WHO classification states that the potential for malignant transformation of BDA is questionable, especially since it has not been reported in classic BDA[1]. On the other hand, Zimmerman described this possibility in BDA presenting atypical ductular profiles, with cells showing hyperchromatic large nuclei[4]. Wang et al. published a series of four patients whose lesions had an evident transition between an adenoma and a cholangiocarcinoma, presenting different morphological and immunohistochemical patterns, two of whom presented the BRAF-V600E mutation[32]. Angkathunyakul et al. studied a series of biliary lesions in five patients with alpha-1-antitrypsin deficiency and described these lesions as similar to BDA but with a hepatic progenitor cell phenotype, which may constitute a separate entity on its own[33]. Further longitudinal studies on multicentric series of cases with clinical, morphological and molecular evidence are required.

Biliary adenofibroma

Described by Tsui et al. in 1993 as a 7 cm tubulocystic mass in a 74-year-old woman, biliary adenofibroma (BAF) is now defined as a solid-microcystic epithelial neoplasm lined by non mucin-secreting biliary epithelium supported by fibroblastic stromal scaffolding[1,34] (FIGURE 3).

Later reported cases showed an indolent behavior of BAF[35]. However, several studies have presented further evidence of malignant transformation: Nguyen et al. reported a high-grade dysplasia in BAF[36]. Tsutsui et al. described microcystic changes in the periphery and solid changes in the center as evidence of malignant transformation[37]. Jacobs et al. reported a case with focal low-grade dysplasia[38]. In

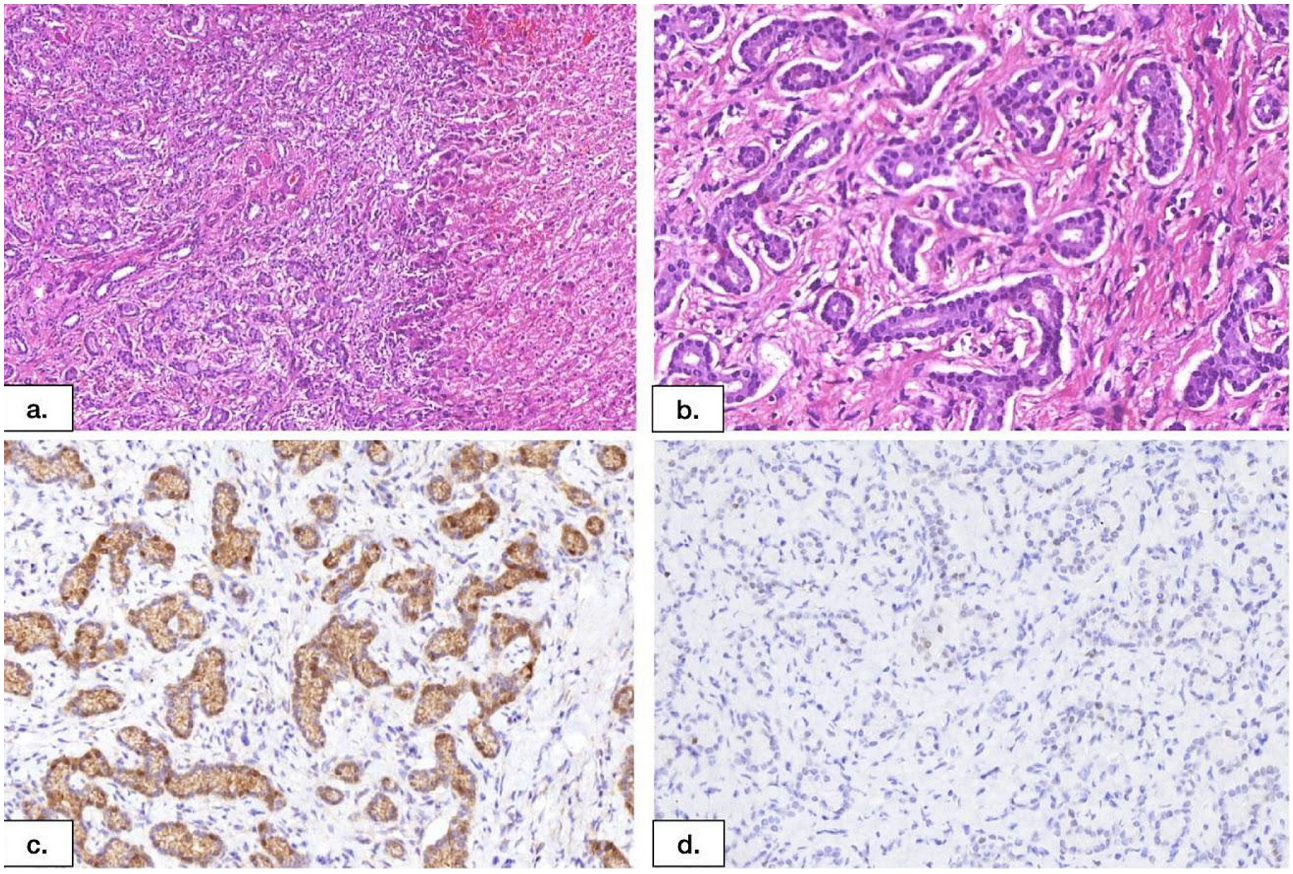

FIGURE 2. Bile duct adenoma. The lesion has a smooth interface with the adjacent parenchyma (a., HE stain, 40x) and the ducts are uniformly shaped, densely packed, with minor cytological atypia (b., HE stain, 100x). The immunohistochemistry is positive to p16 (c., p16 immunostain, 100x) and negative to p53 (d., p53 immunostain, 100x).

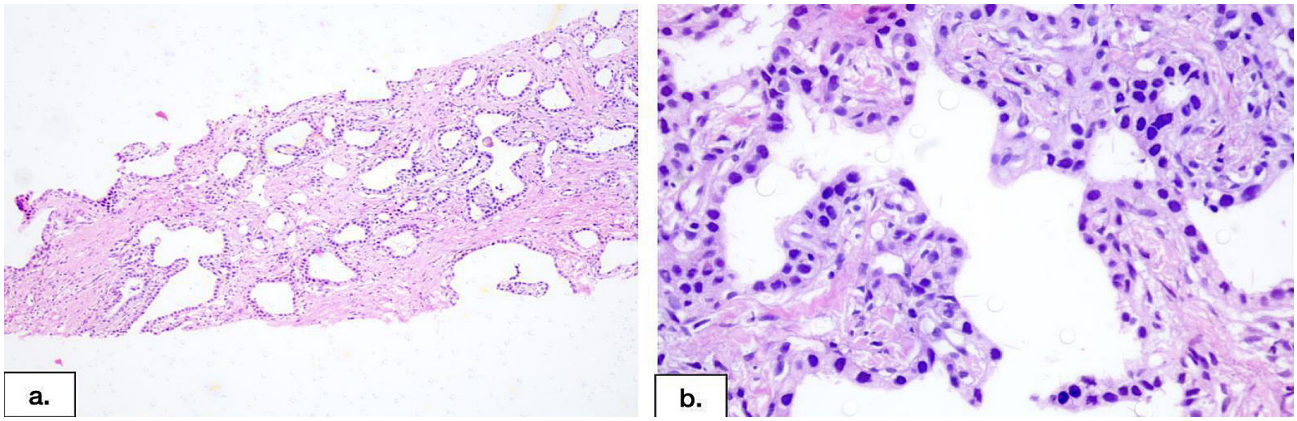

FIGURE 3. Biliary adenofibroma. The lesion has a prominent mesenchymal component, with fibroblastic stromal scaffolding, and the epithelium presentes a microcystic pattern (a., HE stain, 40x). The cells have minimal cytologic atypia (b., HE stain, 400x).

the case studied by Sturm et al., part of the tumor had a distinct pattern, with pseudopapillary projections and cribriform-like growth[39]. Godambe et al. described a case of full transformation of BAF to invasive cholangiocarcinoma[40], and Thompson et al. reported two cases of malignant transformation in male patients[41]. Furthermore, Tsui and Nakanuma estimated that premalignant changes leading to invasive carcinoma are noted in half of the reported cases, presenting both architectural and cytological changes of dysplasia[1]. Taken together, these histological findings provide evidence for a possible malignant transformation in biliary adenofibroma.

Biliary intraepithelial neoplasia

Zen et al.[42] proposed the name BilIN as an attempt to standardize the nomenclature of biliary dysplasia and this concept was further validated in a multicentric study in 2007[43]. It is a micropapillary or flat lesion in the biliary tract that can appear endoscopically as a subtle granularity or thickened mucosa[44], but usually without gross correspondence[42,43,45]. Since it is not detected by current imaging methods and is likely to be found incidentally, the real incidence cannot be precisely determined[46].

BilIN is classified based on the grade of dysplasia: BilIN-1 (mild cellular/nuclear atypia with minimal disturbance of cellular polarity); BilIN-2 (evident atypia with a focal disturbance of cellular polarity); and BilIN-3 (diffuse disturbance of cellular polarity with distinct cellular atypia)[42,47]. The most recent WHO classification presents a two-tiered grading: low grade BilIN (BilIN-1/2) versus high grade BilIN (BilIN-3)[1].

The prevalence is higher in alcoholic or hepatitis C virus (HCV) cirrhosis. It is difficult to affirm that low grades of BilIN are truly neoplastic or reactive processes, as these alterations are found in noncirrhotic explants (although these patients had been exposed to drugs, toxins and viruses). The finding of BilIN in alcoholic and HCV-infected livers has been considered a morphologic support to the rising incidence of cholangiocarcinoma[48].

In the series published by Zen et al.[47], the gastric phenotype (MUC5AC expression) was frequent in BilIN, mainly because of foveolar metaplasia; goblet cell (intestinal) metaplasia and MUC2 expression (intestinal phenotype) were not frequent.

KRAS mutations occur in the early stages of BilIN-1, while p53 mutations are a late-stage event[49]. The progression of BilIN to carcinoma is associated with the absence of MUC2 and increased expression of MUC1/EMA[47]. Positivity for P-cadherin, p53 and CD24 can help distinguishing dysplastic tubules from reactive tubules[50]. During the invasion process, there is a significant reduction in the expression of e-cadherin and beta catenin and an increase in MMP-7 and MT1-MMP, which might represent evidence of epithelial-mesenchymal transition[51].

Intraductal papillary neoplasm of the bile duct (IPNB)

IPNB is characterized by its prominent papillary growth with fibrovascular cores and atypical biliary epithelium leading to a dilatation of large bile ducts identified by imaging methods[52,53]. The presence of intestinal phenotype, with goblet cells and positivity

for MUC2 in IPNB differs from BilIN, although many cases of both; patterns of intraepithelial biliary proliferation may present a gastric phenotype[54].

Adenoma-adenocarcinoma sequence has been fully demonstrated in IPNB, and an invasive component is found in 40–80%[55], associated with increased MUC1/EMA, whereas colloid adenocarcinomas are usually MUC1/EMA-negative[51]. The progression from intraepithelial lesion to invasive adenocarcinomas has been associated with early KRAS mutations, whereas TP53 and SMAD4 mutations have been described as late events[55].

In a series published by Jarnagin et al., the survival of patients resected for invasive hilar cholangiocarcinoma with a component of IPNB was found to be longer than those resected for conventional invasive cholangiocarcinomas[54].

Ductular reaction

Hans Popper et al. (1957) defined ductular reaction as a nonspecific reaction to acute and chronic liver disease with ductular phenotype and found at portal-parenchymal interface[56]. In 2011, Valeer Desmet classified DR as types 1 (fast multiplication of preexisting cholangiocytes as a rescue mechanism against cholestatic parenchymal damage), 2 (activation of hepatic progenitor cells) or 3 (progenitor cell based parenchymal regeneration after liver injury)[57].

More recently, Nejak-Bowen reviewed evidences in the sense that, after liver injury, the hepatocytes are reprogrammed and transdifferentiate in cholangiocytes — an attempt to repair and compensate for the biliary damage[58]. Nakanuma and Ohta characterized the immunohistochemical profile of the typical and atypical ductules, with a stronger expression of keratins and epithelial membrane antigen in the typical ductules[59]. In an attempt to standardize the names and concepts, Roskams et al. suggested the use of the term "ductular reaction" to any reaction with ductular phenotype arising in acute and/or chronic liver disease, not necessarily of ductular origin, discouraging the use of "ductular proliferation" or "oval-like cells"[60]. However, despite coauthoring that standardized nomenclature, Gouw et al. subsequently acknowledged the need to highlight that ductular reactions are diverse processes and have different phenotypes, depending on the causes of the liver disease[61].

Usually, ductular reactions adjacent to hepatocellular carcinomas are not common, and this finding has been used as a criterion for the diagnosis of stromal invasion in early HCC[62]. It is also important to acknowledge that locoregional neoadjuvant therapy may induce exaggerated ductular reactions around tumors with necrosis and stromal fibrosis and even with cytologic atypia, not infrequently mimicking a neoplastic process[63].

Intrahepatic cholangiocarcinoma

Different from the morphological pattern of extrahepatic CCA more frequently found as "large ducts", dilated, presenting mucin secretion at the lumina of the tubules or papillae, 36 to 87% of intrahepatic CCA are classified as small duct CCA[2], presenting a tubular pattern, with low columnar to cuboidal cells in a desmoplastic stroma; small-sized and early lesions may even contain portal tracts and partially preserve the architecture[1], very similar to benign lesions, such as bile duct adenoma or ductular reaction. It can have a ductular component (ductular/cord-like pattern), presenting as innocent-looking tubular formations and thus resembling a ductular reaction[64]. When more than 80% of the lesion is composed of a neoplastic ductular component, the neoplasm is subclassified as a cholangiolocarcinoma (FIGURE 4), while the ductal plate malformation subtype is composed of dilated neoplastic glands with benign looking biliary epithelium in a fibrotic stroma[3].

Immunohistochemical markers in the differential diagnosis of intrahepatic biliary proliferations

Since all the lesions described herein are composed of biliary cells, they are all positive for keratins 7, 8, 18 and 19[1,4]. However, some markers have been described as helpful in discriminating these lesions (TABLE 1): STIP1, SerpinH1, 14-3-3Sigma are faint in the ductular reaction, in contrast to the strong staining seen in cholangiocarcinoma and BDA[65]. HSP27, HSP70, p53, EZH2 and IMP3 are negative (or faintly positive) in BDA and ductular reaction, contrasting with their strong expression in CCA[66-70]. Also, the proliferative index is significantly higher in cholangiocarcinomas when compared to BDA, VMC and ductular reaction[68,71,72].

On the other hand, p16 and BCL2 are negative in

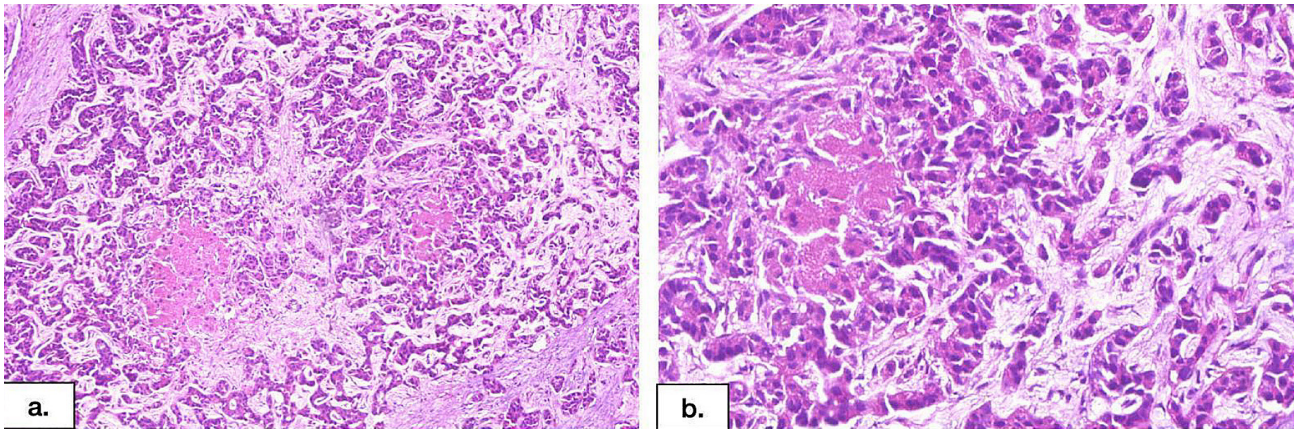

FIGURE 4. Intrahepatic small duct cholangiocarcinoma, cholangiolocarcinoma subtype. The neoplasia is entirely composed of a ductular component, with an infiltrative and anastomosing pattern of growth in an fibrotic stroma (a., HE stain, 40x). The cells display mild pleomorphism and atypia (b., HE stain, 100x).

TABLE 1. Potential markers to distinguish between ductular reaction, bile duct adenoma and cholangiocarcinoma.

	Ductular reaction	Bile duct adenoma	Intrahepatic cholangiocarcinoma, small-duct subtype
p16[66]	+	+	-
CD56/N-CAM[67]	+	+	+
EZH2[66]	-	-	+
p53[50,63]	-	-	+
BCL2[65]	+	+	- (or faintly positive)
HSP27[65]	-	+ (weak)	+ (strong)
HSP70[65]	-	+ (weak)	+ (strong)
STIP1[65]	-	+	+
SerpinH1[65]	-	-	+
14-3-3Sigma[65]	-	+	+
Ki67 [1,69,72]	low	low	high (usually higher than 10%)

CCA, but positive in BDA and ductular reaction[65,66] (FIGURE 5). The expression of CD24 and loss of BAP-1 are common in cholangiocarcinoma[51,73], but more studies comparing with other lesions are required.

Chung et al. showed a higher expression of C reactive protein (CRP) and FGB (fibrinopeptide B) in small duct CCA than in large duct CCA (28% vs 8%, with at least one marker). The proliferation indexes are lower in tumors with a cholangiolocellular component[74].

In an important attempt to identify the site of origin of cholangiocarcinomas, Lok et al. compared the immunoprofile of intrahepatic cholangiocarcinoma to pancreatic ductal carcinoma. In their series, intrahepatic cholangiocarcinomas were more frequently positive for pVHL (71% vs 5%) and negative for S100p (27% vs 95%), maspin (73% vs 100%), MUC5AC (12% vs 67%) and K17 (12% vs 60%), whereas both groups had similar rates of IMP3[75] expression (90%)[76].

Based on the common origin and similarities between pancreatic ductal carcinoma and large duct cholangiocarcinoma[77,78], these markers might also be useful to distinguish between small and large duct tumors (TABLE 2; FIGURE 6 and FIGURE 7).

In the differential diagnosis with extra hepatic lesions, the positivity for albumin in situ hybridization can be useful[75]. However, these data have not been further subject to more comprehensive studies, so this is an area still open for further research.

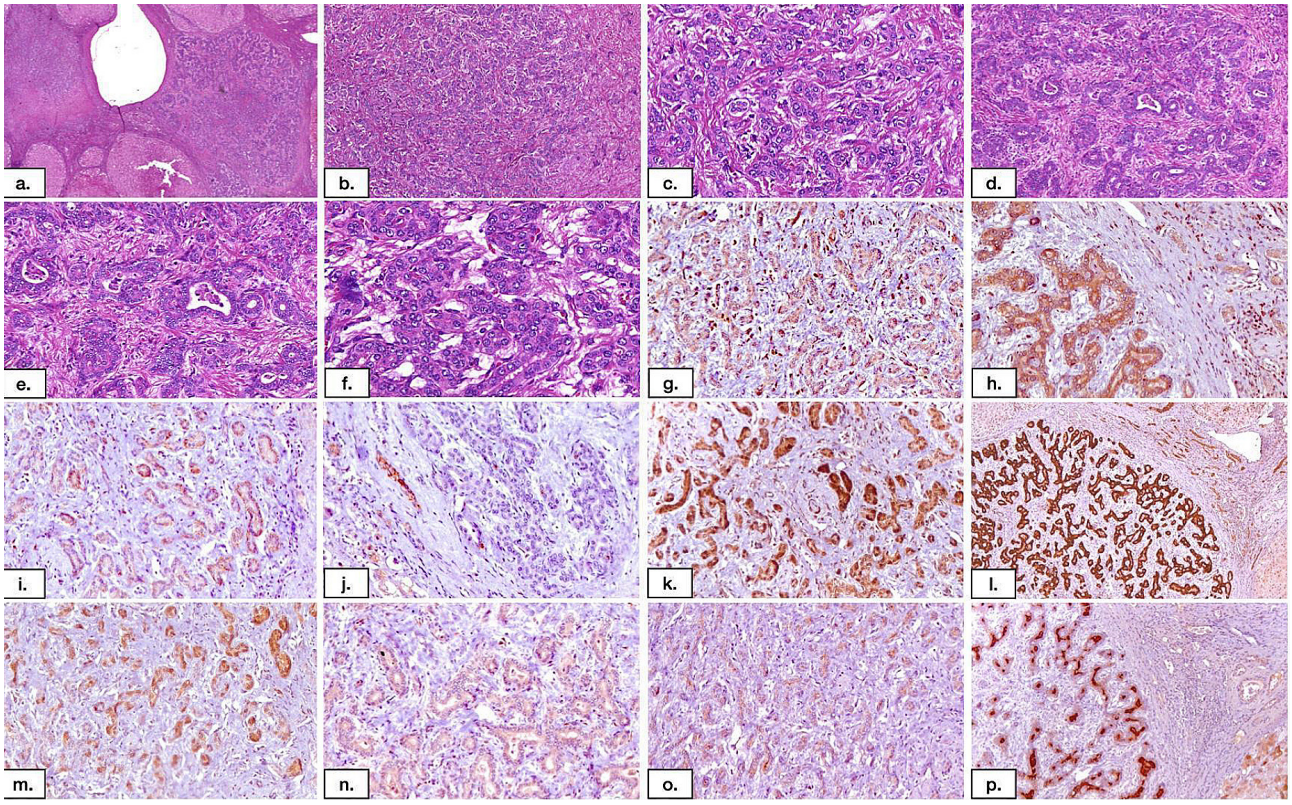

FIGURE 5. Intrahepatic small duct CCA adjacent to a BDA. The lesion is composed of two distinct components: a BDA to the left, and a small duct CCA to the right (a., HE stain, 10x). The BDA has densely packed tubular structures with mild cytological atypia (b., HE stain, 40x; and c., HE stain, 100x), while the CCA has a tubular-cribriform arrangement, with complex architecture, moderate atypia and infiltrative growth (d., HE stain, 40x; and e. and f., HE stain, 100x). Both of them are positive for BCL-2, but the BDA (g., BCL-2 immunostain, 40x) has a weaker staining when compared to CCA (h., BCL-2 immunostain, 100x). CD56 is positive in the BDA (i., CD56 immunostain, 40x), and is faintly positive in the CCA (j., CD56 immunostain, 100x). HSP70 is positive in the BDA (k., HSP70 immunostain, 100x), but the intensity is stronger in the CCA (l., HSP70 immunostain, 40x). p16 is positive in BDA (m., p16 immunostain, 40x) and faintly positive in CCA (n., p16 immunostain, 40x). CRP is weakly positive in BDA (o., CRP immunostain, 40x) and strongly positive in the CCA (p., CRP immunostain, 40x).

TABLE 2. Potential markers to distinguish between small duct and large duct cholangiocarcinoma.

	Small duct cholangiocarcinoma	Large duct cholangiocarcinoma
C reative protein[2,75]	+ (especially if a cholangiolocellular component is present)	-
Fibrinopeptide B[75]	+ (especially if a cholangiolocellular component is present)	-
pVHL[76]	+++	-
S100p[76]	+/-	+++
Maspin[76]	+/-	+++
MUC5AC[76]	+/-	+++
K17[76]	+/-	+++
CD56/N-CAM[2]	+	-

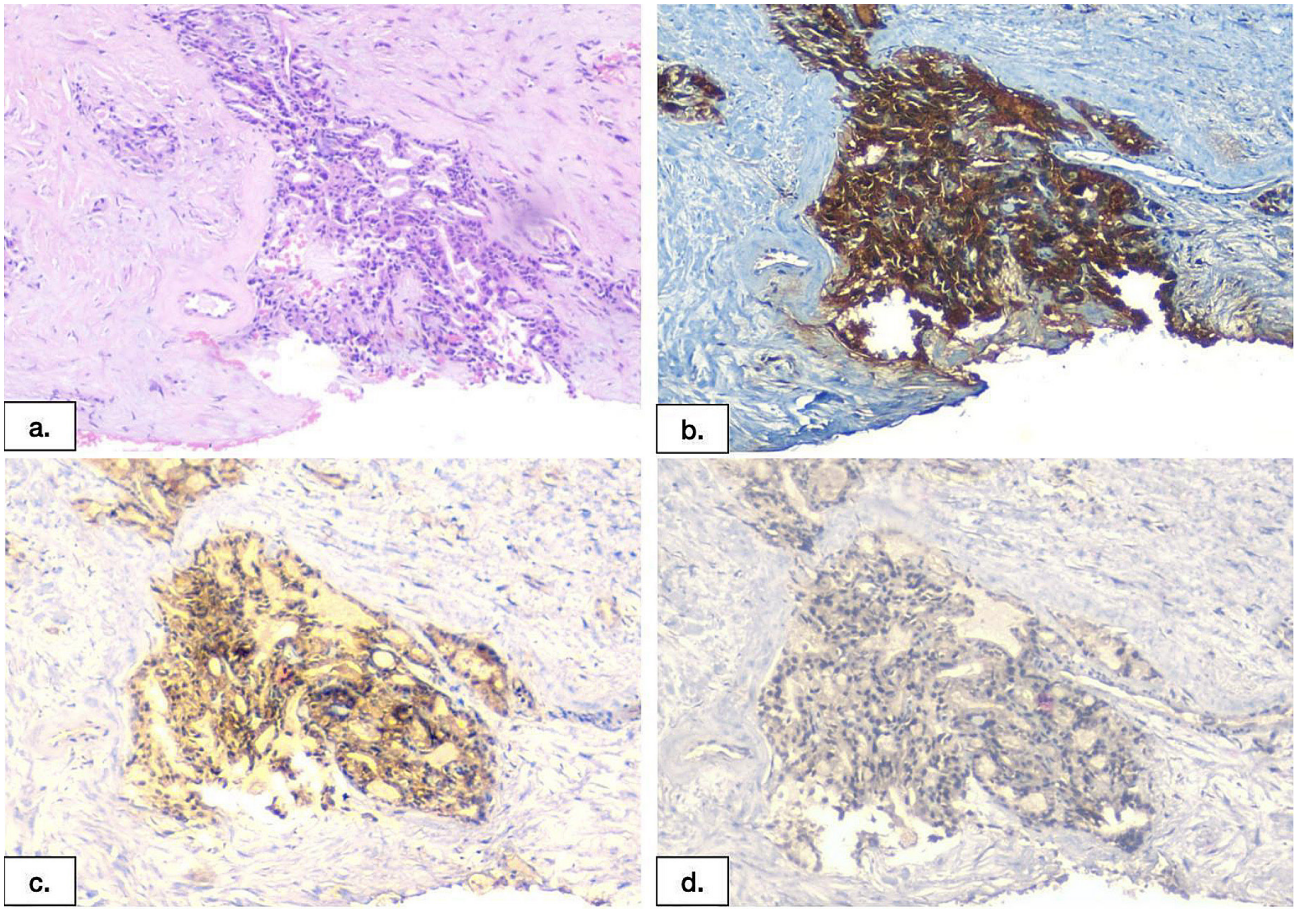

FIGURE 6. Intrahepatic small duct cholangiocarcinoma, NOS. The lesion has a complex tubular-cribriform architecture, displaying moderate atypia with infiltrative pattern (a., HE stain, 100x). On immunohistochemistry, it is positive for CRP (b., CRP immunostain, 100x) and pVHL (c., pVHL immunostain, 100x), and negative por S100p (d., S100p immunostain, 100x).

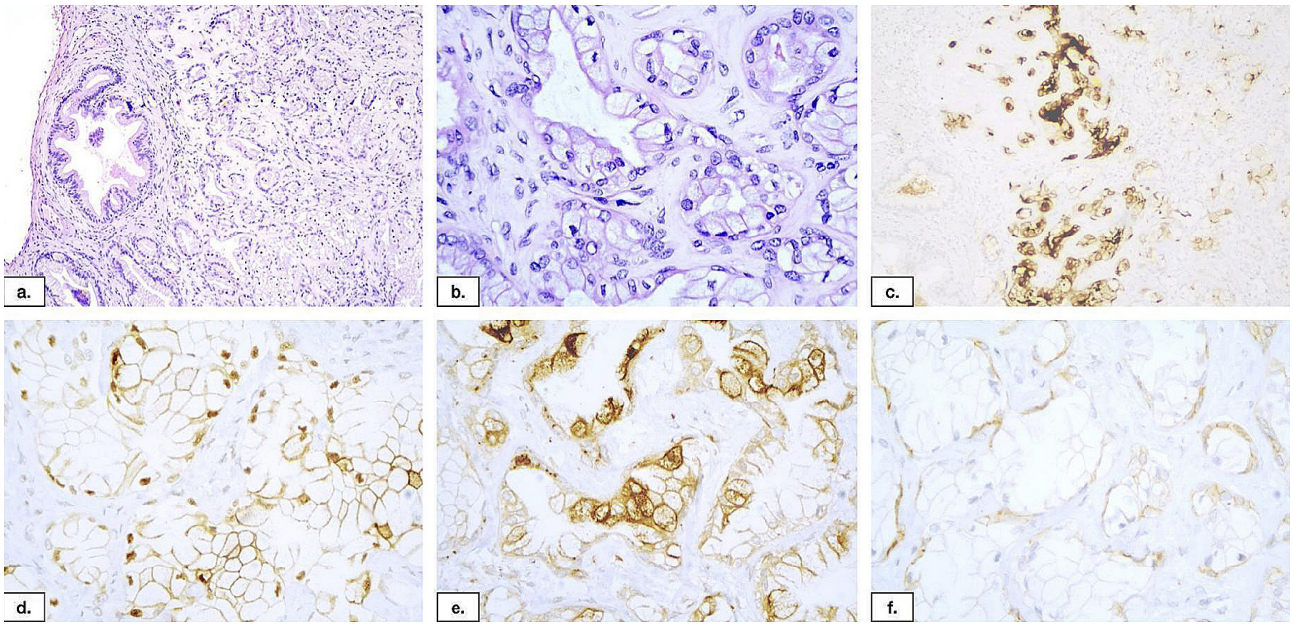

FIGURE 7. Intrahepatic large duct cholangiocarcinoma. The lesion has a complex tubular architecture, with atypical mucinous glandules (a., HE stain, 100x; b. HE stain, 400x). On immunohistochemistry, it is positive for monoclonal CEA (c., monoclonal CEA immunostain, 100x), maspin (d., maspin immunostain, 100x) and MUC5AC (e., MUC5AC immunostain, 100x) and negative for pVHL (f., pVHL immunostain, 100x).

The similarities between histological patterns of intrahepatic biliary proliferations, either benign, premalignant or fully malignant, make them worrisome and challenging even for expert liver pathologists, emphasizing the need for the adoption of detailed histological features. Moreover, the several immunohistochemical/molecular markers recently discovered, once proved useful in further large series of cases submitted to longer follow-up, are expected to aid in the correct diagnosis in routine practice, as seen in a recent review made by Zhang and Wang[79].

The increasing incidence of intrahepatic cholangiocarcinoma[80] and the possibility of new therapies make the correct description of borderline, precursor lesions and subtypes of intrahepatic cholangiocarcinomas even more important.

CONCLUSION

This scenario urges surgical pathologists worldwide to standardize the nomenclature and the criteria to correctly describe all the biliary proliferations seen in surgical practice, and this area is currently a field for multicentric multidisciplinary studies aiming at improving not only the early diagnosis but also searching for a more comprehensive understanding of the carcinogenic transformation of each subtype of cholangiocarcinoma.

Authors' contribution

Hirayama AB: conceptualization, investigation, writing. Mello ES: writing, review. Alves VAF: conceptualization, investigation, writing, review, supervision.

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RESUMO – As proliferações biliares intra-hepáticas representam um espectro que abrange desde entidades reativas (reação ductular, algumas com arquitetura atípica), hamartomatosas (complexo de von Meyenburg), benignas (adenoma de ductos biliares) e precursoras/limítrofes (neoplasia intraepitelial biliar, neoplasia papilar intraductal de ducto biliar) até neoplasias totalmente malignas (colangiocarcinoma). Os quadros clínicos e até mesmo os padrões de imagem podem ser semelhantes, exigindo estudos refinados visando critério histológicos e imuno-histoquímicos para diagnósticos mais precisos, essenciais para o correto manejo do paciente. Este artigo discute conceitos atualizados e definições das entidades mais relevantes visando mais especificamente ao diagnóstico diferencial na prática, com foco na morfologia e imuno-histoquímica, com discussão de potenciais marcadores para ajudar na distinção entre lesões benignas e malignas.

Palavras-chave – Neoplasia; vias biliares; colangiocarcinoma.

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