Annals of Diagnostic Pathology 38 (2019) 126–137

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Review Article

Update on the pathology of liver neoplasms

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A B S T R A C T

Many advances have developed in the pathology of liver tumors in the recent decade. Examples of these advances include the use of glutamine synthetase in the diagnosis of focal nodular hyperplasia, subtyping of hepatocellular adenomas using molecular and immunohistochemical methods, the unraveling of the fusion transcript between the *DNAJB1* gene and the *PRKACA* gene in fibrolamellar carcinoma, and the more unified classification and terminology in intrahepatic bile duct tumors and their precursor lesions. Nevertheless, challenges still remain, e.g., the differential diagnosis between well-differentiated hepatocellular carcinoma and hepatocellular adenoma; distinction among poorly differentiated hepatocellular carcinoma, cholangiocarcinoma and metastatic neoplasm; terminology of the combined hepatocellular carcinoma-cholangiocarcinoma, etc.

This review aims to address updates in the pathologic diagnosis and clinical relevance of tumors of the liver and intrahepatic bile ducts in adults and their differential diagnosis and diagnostic pitfalls.

1. Introduction

In the last few years several significant imaging, molecular, and therapeutic advances have occurred in the field of primary liver neoplasms (e.g., hepatocellular carcinoma, cholangiocarcinoma). These discoveries have changed not only the nature of the specimens received for tissue diagnosis but also the types of information needed by the physicians for clinical management, particularly identification of morphologic subtypes of these common tumors. Specific events leading to this re-engineered role for surgical pathologists include: (1) implementation of the Liver Imaging and Data Reporting System (LI-RADS) by the field of radiology, (2) whole exome sequencing of hepatocellular carcinoma and cholangiocarcinoma, (3) a proliferation of promising clinical trials showing efficacy of broad spectrum tyrosine kinase and other small molecule inhibitors against primary liver carcinomas, (4) clarification of the clinical and molecular features of the various hepatocellular adenomas and their risk of malignant progression, and (5) the molecular definition of fibrolamellar carcinoma [1-4].

The aim of this review is to synthesize well-established pathomorphologic and clinical features with the aforementioned new discoveries, emphasizing the clinical value of the additional complexity inherent in such progress. On a more practical level, we present our approach to pathologic diagnosis of the primary liver neoplasms and highlight potential diagnostic pitfalls. We also address general histopathologic features unique to current TNM staging of this category of tumors. Finally, the theme of understanding the clinical backdrop of the lesion is emphasized - as in our experience this information is far more important than whether a panel of immunostains slightly favors one neoplasm over another.

2. Hepatocellular neoplasms

Hepatocellular neoplasms can be broadly divided from a diagnostic point of view into 3 main challenges: (1) distinguishing well-differentiated hepatocellular carcinoma (HCC) from hepatocellular adenoma (HCA) and non-neoplastic liver, (2) identifying rare but clinically important subtypes of HCC (e.g., scirrhous, fibrolamellar, cirrhotomimetic), and (3) distinguishing poorly differentiated HCC from metastasis and poorly differentiated cholangiocarcinoma (CCA). We discuss our diagnostic approach to these specific issues, with emphasis on the particular challenges of needle biopsy.

2.1. The mass-directed liver biopsy

Biopsy material is inherently challenging to interpret due to limited sampling. Additionally, because radiologists now have specific criteria upon which to diagnose HCC in high-risk populations when the imaging features are characteristic (e.g., LI-RADS system [5]), lesions that now undergo biopsy are by definition more atypical or difficult to classify. Regardless of the imaging findings, our approach is to first rely on

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https://doi.org/10.1016/j.anndiagpath.2018.10.005
Received 6 October 2018; Accepted 9 October 2018

Table 1
Helpful features to distinguish well-differentiated hepatocellular neoplasms from non-neoplastic liver.

Non-lesional	Lesional/neoplastic
Alternating portal tracts and central venules	No obvious portal tracts
Generally homogeneous appearance	Unpaired or "naked" arteries
Prominent inflammation or necrosis at one edge (may represent edge of lesion)	2 cytologically distinct areas on a biopsy
	Abnormal thickening of hepatic plates
	Hepatic rosettes
	"Incomplete" portal tracts
	Fatty nodule in a non-fatty liver
	"Stromal invasion" of hepatocytes into portal tract
	Prominent, hyperchromatic sinusoidal lining cells

patient-specific factors to establish a clinical differential diagnosis. Is the patient male or female? How old is the patient? Does the patient have a history of liver disease or cirrhosis? Is there a single mass or is the process multifocal? The clinical differential diagnosis thus derived informs the approach to histologic evaluation.

2.2. Classifying well-differentiated hepatocellular lesions

If the tissue sampled is clearly hepatocellular, the 3 key questions are: (1) Is the tissue lesional? (2) If lesional, is the tissue neoplastic? (3) If neoplastic, is it malignant? Features used to distinguish lesional from non-lesional tissue on routine H&E are listed in Table 1. If the tissue is lesional, the differential diagnosis includes primarily focal nodular hyperplasia, dysplastic nodule, HCA, and HCC. In addition to these, it is worth noting that low grade angiosarcoma growing along sinusoids can be a subtle histologic mimic of a well-differentiated hepatocellular lesion. As such, a quick assessment of the character of the sinusoidal lining is useful. Since each of the above possibilities has a characteristic clinical setting, the patient's medical history is important for prioritizing the differential diagnosis. For example, if the patient has no evidence of cirrhosis, HCC may not be the top choice, (although not infrequent, such as in hepatitis B patients). If the patient is a female of child bearing age, HCA is more likely. If the patient has cirrhosis, HCC and dysplastic nodule are more likely. A prioritized differential diagnosis is necessary for selection of ancillary stains and establishing criteria for interpretation of those stains. Frequently useful stains in the differential diagnosis of well-differentiated hepatocellular neoplasms are: reticulin, CD34, and Ki67.

Reticulin is the single most helpful tool in the differential diagnosis of a well-differentiated hepatocellular lesion in that if clearly abnormal it strongly points to HCC (Fig. 1). That being said, if one is not familiar with the spectrum of normal/non-neoplastic reticulin staining in liver, its interpretation can be challenging. If normal or non-lesional liver is present within the biopsy, this is helpful as it serves as an internal control. Normal/non-lesional liver demonstrates linear arrays of hepatic plates of 1–2 cells in thickness, bounded by reticulin on the sinusoidal surfaces. In contrast, HCC demonstrates consistent widening of the hepatic plates, typically 3 or more cells in thickness, and has a disorganized appearance. In place of linear hepatic plates, "nests" of hepatocytes bounded by reticulin may be seen. Of note, generally speaking, hepatic plate widening must be relatively diffuse within an abnormal area to be diagnostically useful. If only one or two hepatic plates are focally expanded, this could represent a regenerative change or sampling issue. Additionally, areas with steatotic hepatocytes can also demonstrate reticulin loss, which is not indicative of neoplasia.

CD34 can also be a helpful stain, although several caveats apply. In the normal liver, sinusoids are not lined by true endothelium, and thus are not highlighted by CD34. In HCC "capillarization" of sinusoids may be seen; that is, endothelial cells may grow along the sinusoids of the lesion. When diffuse sinusoidal staining by CD34 correlates with morphologically abnormal areas on H&E or reticulin stain, this enhances visualization of the lesional area and helps to support HCC. Hepatocellular adenomas may have sinusoidal CD34 staining as well, but it is typically more patchy in distribution. Occasionally, however, HCAs can show diffuse CD34 sinusoidal staining, and alternatively not all HCCs demonstrate diffusely CD34-positive sinusoids. CD34 is also frequently seen in along the sinusoids in zone 1 in normal liver, on the surface of cirrhotic nodules, and in a patchy distribution (described as "inflow pattern") [6] in focal nodular hyperplasia. Thus, awareness of these diagnostic pitfalls is necessary when assessing the pattern of CD34 expression. If diagnostic ambiguity between HCA and HCC remains, Ki67 stain can be informative. HCAs do not show increased proliferation (< 2%) whereas HCCs show increased Ki67 [2], often > 10%.

Although glypican-3 is documented to be relatively sensitive for HCC in distinction from non-neoplastic lesions, it is less useful for needle biopsies of well-differentiated hepatocellular lesions, as it has decreased sensitivity for well-differentiated HCCs and can be positive in a subset of high grade dysplastic nodules and regenerative nodules [7]. Additionally, while there is no significant disadvantage to performing this stain if it is readily available, in the absence of supportive morphologic findings, a positive glypican-3 stain is insufficient for the diagnosis of HCC.

If the tissue is abnormal for any of the reasons noted in Table 1, and yet reticulin, CD34, and Ki67 fail to show corresponding abnormalities, focal nodular hyperplasia may be a consideration in a non-cirrhotic patient. In contrast, in a cirrhotic patient, a dysplastic nodule is more likely. Definitive diagnosis of focal nodular hyperplasia on biopsy can be challenging; however, the glutamine synthetase immunostain can be helpful, in that strong, but patchy expression (described as "map-like" when viewed on whole tissue section) supports the diagnosis, whereas in normal liver glutamine synthetase expression is restricted to 1 to 3 cell layers around the central venules. Of note, HCC and HCA can demonstrate prominent glutamine synthetase staining, which can be difficult to distinguish from the "map-like" pattern of focal nodular hyperplasia, particularly on biopsy material. Thus, awareness of the limitations of the glutamine synthetase stain is important for appropriate interpretation.

In contrast to HCAs in which the clinical setting is usually a female of child bearing age without cirrhosis, high grade dysplastic nodules enter into the differential diagnosis of a patient with cirrhosis. This diagnosis is challenging because it is essentially made by exclusion of other possibilities. Although a combination of HSP70, glypican-3, and glutamine synthetase has been proposed to distinguish dysplastic nodules from HCC [8,9], this panel of stains is not particularly successful in distinguishing between high grade dysplastic nodules and (very) well-differentiated HCC, the types of lesions most difficult to distinguish histologically. Because data suggest patients with dysplastic nodules are at significant risk for development of HCC, current clinical guidelines recommend repeat biopsy and/or surveillance to evaluate for interval growth [10]. Thus, the diagnosis of high grade dysplastic nodule is functionally a diagnosis of uncertainty. As such, for liver biopsies, we prefer to describe lesions that fall short of HCC but that have clinical and/or morphologic features concerning for HCC as "atypical" and mention the possibility of a high grade dysplastic nodule and HCC in the comment. This approach is practical and acknowledges the possibility that the biopsy may not be representative of the entire lesion. A variety of terms have been proposed for this category of lesion (e.g., hepatocellular neoplasm of uncertain malignant potential/HUMP, atypical hepatocellular lesion [11]). As long as the terminology followed by a clear comment conveys the appropriate risk of malignancy, any of these terms seems appropriate.

2.2.1. Subclassifying hepatocellular adenomas (HCA)

If the morphologic findings along with the clinical history support a

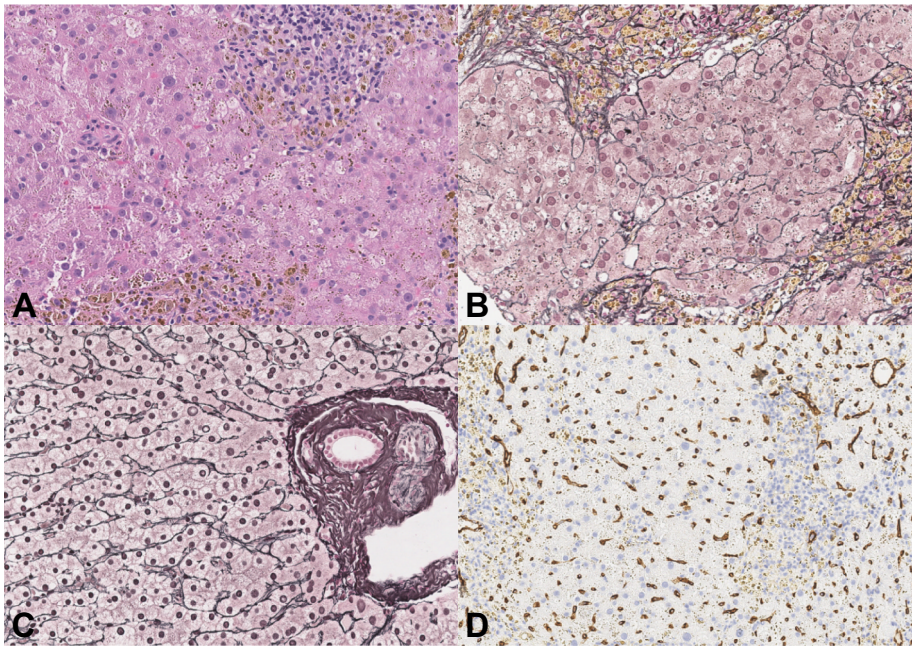

Fig. 1. Well-differentiated hepatocellular carcinoma. (A) The tumor is clearly hepatocytic. However, the normal hepatic plate architecture and portal tracts are lost. (B) Reticulin stain highlights loss of the normal hepatic plate architecture. (C) In this section of normal liver, included for comparison, the reticulin stain outlines the sinusoids and highlights the 1–2 cell thickness of normal hepatic plates. (D) CD34 stain highlights sinusoidal endothelial cells diffusely within the carcinoma.

HCA (e.g., 80–90% of HCAs occur in young to middle aged women), additional prognostic information can be obtained by its subclassification.

Four established categories of HCA currently exist, all of which pertain to estrogen-driven tumors (which comprise the vast majority of HCAs). They are: (1) inflammatory, (2) HNF1a-inactivated, (3) beta-catenin activated, and (4) unclassified (Table 2). The following section highlights the features necessary to categorize HCAs. Of note, subclassification requires access to several immunostains with a narrow range of diagnostic utility, which may not be readily available to all surgical pathologists. An alternative, pragmatic approach is to assess for beta-catenin activation, the most prognostically relevant feature, particularly as current clinical guidelines do not rely on subclassification for therapeutic decision-making [12]. Beta-catenin activation - which can be seen in inflammatory, HNF1a-inactivated (very rarely), and beta-catenin activated subtypes - increases risk of malignant progression. It is best assessed by using immunostains for both beta-catenin and glutamine synthetase, a downstream target of the beta-catenin pathway. Nuclear beta-catenin expression can be focal (even a single immunoreactive cell is considered positive); however, if a tumor is negative for beta-catenin, this does not completely exclude beta-catenin activation. Concomitant use of glutamine synthetase is helpful, as diffuse expression (> 90%) is supportive of clinically significant beta-catenin activation. A key pitfall is that focal nodular hyperplasia can also show prominent (albeit typically more patchy) glutamine synthetase expression, as mentioned above. Thus, exclusion of focal nodular hyperplasia prior to interpretation of the glutamine synthetase stain in this scenario is useful.

2.2.1.1. Inflammatory HCA. Inflammatory type adenomas are characterized by areas of sinusoidal dilatation (formerly described as “telangiectatic”), patchy chronic inflammation, and scattered pseudoportal tracts (i.e., bile ductules and adjacent artery surrounded by a fibrous collar). These adenomas are the most common type of HCA (up to 50%) and arise in the setting of steatosis and steatohepatitis of metabolic or alcoholic origin (Fig. 2A–C). The aforementioned constellation of histologic findings is characteristic and may be sufficient for the diagnosis of inflammatory type HCA. However, if there is diagnostic concern, immunostains for serum amyloid A protein (SAA) and C-reactive protein (CRP) can be performed and at least one should be convincingly positive. Of note, both stains may have a high background, and thus it is best to evaluate the stains in areas with lesional and non-lesional tissue, if available. SAA and CRP can also be positive in HCC, and accordingly should be interpreted in the appropriate morphologic setting. As approximately 10% of inflammatory HCAs are beta-catenin positive, beta-catenin and glutamine synthetase stains are recommended.

2.2.1.2. HNF1a-inactived HCA. HNF1a-inactived HCAs are the second most common HCA (approximately 30–40%). Classically, they demonstrate fatty change, which is present against a non-fatty background, a feature that often facilitates microscopic identification of the tumor (Fig. 2E). However, because foci of fat are also frequently seen in inflammatory type adenomas (as well as HCC), it is necessary to confirm the diagnosis of HNF1a-inactived adenoma by demonstrating loss of liver fatty acid binding protein (LFABP) expression. (LFABP is a downstream target of HNF1A activation and serves as a sensitive biomarker of this pathway.) Evaluating the immunostain in an area with both lesional and non-lesional tissue is optimal for comparison; there should be a sharp contrast in LFABP expression between the adenoma (complete absence of staining) and the background liver (diffuse strong staining) (Fig. 2F). Of note, HCC and fibrolamellar carcinoma can also show loss of LFABP expression, and thus absence of LFABP expression alone is not pathognomonic for HNF1a-inactivated HCA. Although it is worthwhile to evaluate for beta-catenin activation, it is rare in this subtype of HCA, and overall, HNF1a-inactivated HCAs have the least risk of malignant progression.

2.2.1.3. Beta-catenin activated HCA. Beta-catenin activated HCAs account for approximately 10% of HCAs. This category of adenoma may show cytological abnormalities and very focal pseudoacinar or pseudoglandular pattern that falls short of a diagnosis of HCC. However, it is not associated with other specific morphologic features and is instead defined by immunohistochemical evidence of beta-catenin activation, in the absence of markers indicative of inflammatory type or HNF1a-inactivated subtypes. Thus, by definition, beta-catenin activated HCAs must lack expression of SAA, CRP, and show intact LFABP. Beta-catenin (Fig. 2E) and glutamine synthetase stains (as described above) are important for making this diagnosis.

Table 2
Clinicopathologic features of benign hepatocytic lesions in the differential diagnosis of hepatocellular carcinoma.

	FNH	High grade dysplastic nodule	Heptaocellular adenoma			
			Inflammatory	HNF1a-inactivated	Beta-catenin activated	Unclassified
Clinical	Frequently women; due to vascular abnormality	Arises in cirrhotic background	Young, middle aged women; steatosis, steatohepatitis; can be multiple	Young, middle aged women; can be multiple	Young, middle aged women, but can also occur in men	Young, middle aged women
Morphologic	Central scar, ductular reaction, thick-walled blood vessels	Mild cytologic atypia, focal pseudoglandular formation, retained hepatic plate architecture	Pseudoportal tracts, chronic inflammation, sinusoidal congestion	Diffuse steatosis	No specific morphologic features (may show occasional pseudoglands and cytological atypia)	No specific morphologic features
Molecular/IHC	"Map-like" expression of glutamine synthetase	Can have beta-catenin mutation	*IL6ST* mutation	*HNF1A* mutation	*CTNNB1* exon 3 deletion (beta-catenin)	Sonic hedgehog pathway activation; argininosuccinate synthetase-1 expression

FNH = focal nodular hyperplasia; IHC = immunohistochemistry.

2.2.1.4. Unclassified HCA. Although the remaining HCAs (approximately 10%) are currently not further classified, new data suggest that almost half of these show activation of the sonic hedgehog pathway and are associated with increased risk of hemorrhage [13]. Additionally, expression of argininosuccinate synthetase-1, primarily seen in this category of HCAs, has been identified as a marker of increased risk of hemorrhage [14].

2.3. Poorly differentiated HCC versus poorly differentiated intrahepatic CCA

If a solitary or dominant lesion is present in the liver without evidence of primary tumor elsewhere in the body, the differential diagnosis is essentially between HCC (Fig. 3) and intrahepatic CCA (Fig. 4). Epithelioid hemangioendothelioma should also be considered, particularly when the patient is young and no history of liver disease is noted. The distinction between these possibilities is important for both early and advanced lesions - not only for prognosis but also for clinical management. Specifically, hilar lymphadenectomy is performed for early (resectable) CCA, given its propensity to spread to regional lymph nodes, whereas lymphadenectomy is not performed for HCC as it does not typically spread in this fashion. For advanced (unresectable) tumors, the chemotherapeutic regimens also are vastly different: CCA is currently treated with platinum and gemcitabine [15], whereas HCC, which is unresponsive to conventional chemotherapy, is treated with the multikinase inhibitor sorafenib.

In the pathologic assessment of a solitary liver tumor, clinical and histologic features should be considered (Table 3). With regard to immunohistochemical work-up, when the tumor is poorly differentiated, a panel of immunostains that includes hepatocellular markers (e.g., HepPar-1, arginase, and glypican-3) as well as cytokeratins that immunolabel but are not specific for biliary epithelium (e.g., CK7, CK19) is recommended. A mucin stain, such as Alcian blue or mucicarmine, is also useful, although it is important to note that not all cholangiocarcinomas elaborate mucin. One major caveat is that while arginase is more frequently positive in poorly differentiated HCCs than HepPar-1, it is also more frequently expressed in poorly differentiated cholangiocarcinomas [16,17]. In contrast, glypican-3 expression is uncommon in CCA [18,19]. With regard to biliary type keratins, poorly differentiated HCCs typically lack CK7 and CK19 (or if present their expression is often patchy) [20-22], whereas poorly differentiated CCAs tend to retain expression of these cytokeratins [20]. One important pitfall is that scirrhous HCC (a subtype of HCC discussed further below) is frequently negative for HepPar-1 and can be positive for CK7 and CK19. In this situation (and also in the setting of poorly differentiated HCC), arginase and glypican-3 are more sensitive markers of hepatocytic differentiation than HepPar-1 [18]. If epithelioid hemangioendothelioma or epithelioid angiosarcoma (the third most common malignancy in liver) is a concern, CD31 and CD34 are generally sufficient for their evaluation. An additional consideration is combined hepatocellular carcinoma-cholangiocarcinoma (HCC-CCA), which may manifest as a liver tumor with an unexpected or seemingly conflicting immunoprofile [23] (discussed further below).

2.4. Poorly differentiated HCC versus metastatic neoplasm

As mentioned above, clinical and histologic features should be considered when evaluating a poorly differentiated carcinoma of uncertain origin (Table 3). The panel approach is similarly recommended, to include the 3 hepatocellular markers (HepPar-1, arginase, and glypican-3) as well as CK7 and CK20 and judicious use of specific markers of tissue of origin (e.g., TTF-1, CDX2, PAX8, GATA3) based on the clinical setting. One caveat is that HepPar-1, glypican-3 and arginase all can stain a small subset of non-hepatocellular carcinomas and adenocarcinomas [24-26]. As such, caution must be taken when interpreting these stains, and clinical findings should be considered (e.g., single or

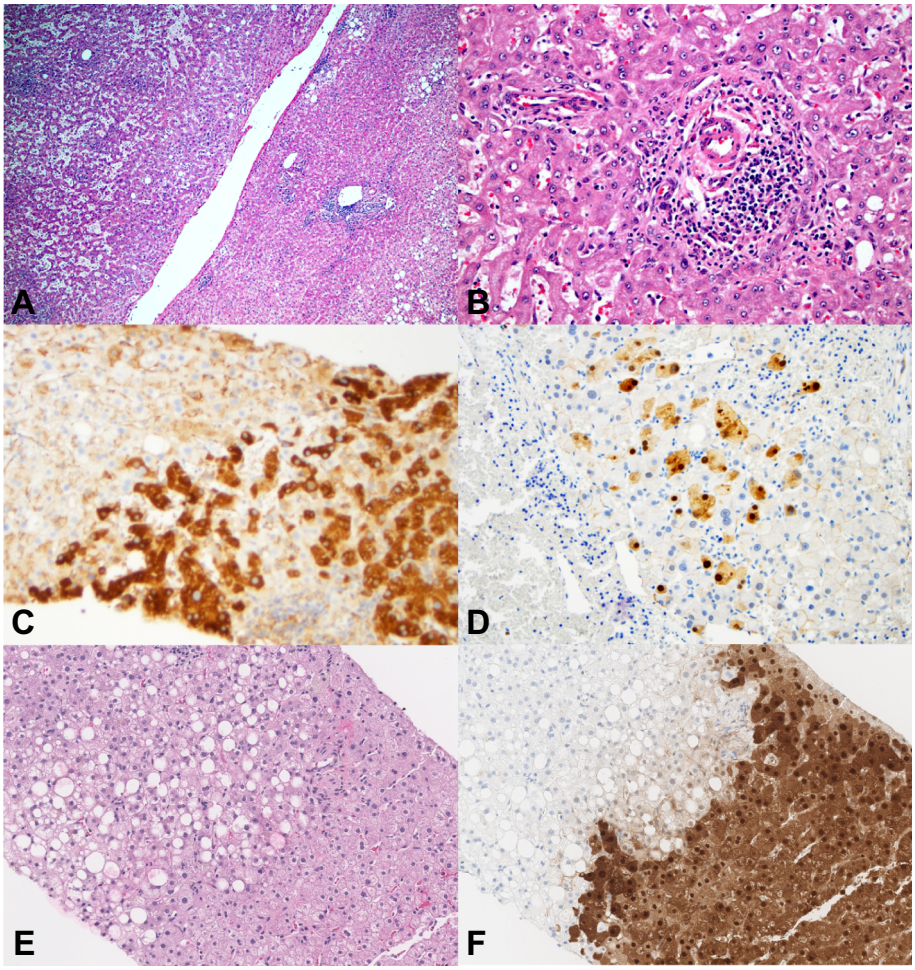

Fig. 2. Hepatocellular adenoma (HCA).
(A) Inflammatory HCA (left) shows telangiectatic change. The background liver (right) commonly shows fatty change due to underlying metabolic syndrome or excessive alcohol use. (B) In inflammatory HCA, pseudoportal tracts consising of bile ductules, unpaired arteries, and a surrounding predominantly mononuclear cell inflammatory infiltrate are present. (C) Expression of C-reactive protein (CRP) is present in inflammatory HCA (right) but not in the background liver (left). Note the pseudoportal tract (bottom right). (D) Positive nuclear and cytoplasmic beta-catenin staining in beta-catenin activated HCA. (E) In HNF1a-inactivated HCA, the tumor is characterized by prominent fatty change (left), which distinguishes it from the background liver (right). (F) Loss of liver fattty acid binding protein (LFABP) expression is characteristic of HNF1a-inactivated HCA.

multiple lesions, imaging suggesting a dominant mass in another organ, background cirrhosis) when arriving at a diagnosis or narrowing the diagnostic possibilities. Also of note, CK19 is not particularly informative in the differential diagnosis of metastatic carcinoma in the liver, as it is positive in a number of common neoplasms (e.g., lung, breast, thyroid, stomach, pancreas) as well as CCA and a subset of HCCs (as mentioned above).

2.5. Rare subtypes of HCC

A large number of HCC subtypes have been characterized, most of which are rare (< 2% of all HCC). These include: steatohepatitic, clear cell, scirrhous, cirrhotomimetic, granulocyte colony-stimulating factor-producing, lymphocyte-rich/lymphoepithelioma-like, sarcomatoid, and fibrolamellar. Additionally, subtypes with more than one line of differentiation include carcinosarcoma, combined HCC-neuroendocrine carcinoma, and combined HCC-CCA. For the most part, the subtypes are readily identifiable as variants of HCC (upon adequate sampling) and currently undergo the same management as typical HCC (with exceptions highlighted below). However, accurate subtyping of HCCs is increasingly important in this age of precision medicine, as the molecular defects underlying the individual tumor subtypes as well as their distinct behaviors are being used to tailor therapy to the specific patient and tumor. On an equally important and more practical level for the pathologist, a familiarity with these subtypes helps to avoid diagnostic pitfalls that have the potential to fundamentally undermine clinical management. Discussed here are those subtypes that pose particular diagnostic challenges for the pathologist.

2.5.1. Fibrolamellar carcinoma

This tumor is characterized by a proliferation of large hepatocytes with abundant granular eosinophilic cytoplasm and round nuclei with prominent, centrally placed nucleoli (Fig. 5A-C). Overall, the cells have a uniform appearance, lacking significant pleomorphism. The neoplastic cells may be arranged in all of the same patterns as that seen in typical HCC (e.g., nests, trabeculae, pseudoglands) and are characteristically interrupted by broad, often linear bands of collagen. In some cases, however, the fibrous bands may not be prominent. The background liver is uniformly non-cirrhotic, a feature that can help to prevent misdiagnosis. Pale bodies (light pink, round intracytoplasmic globules with crisp borders, known to contain fibrinogen) are characteristic but not entirely specific. Fibrolamellar carcinomas typically express HepPar-1 and arginase, but unlike most other HCCs, they show diffuse expression of CK7 and patchy granular expression of CD68. Recent studies have identified activation of protein kinase A as the defining molecular feature of this tumor, the vast majority of which demonstrate fusion of the *DNAJB1* and *PRKACA* genes [27]. A separate genetic defect in the regulatory subunit of the protein kinase A complex, *PRKAR1A*, was also recently identified as the underlying genetic defect in a small subset of cases, some of which are associated with the Carney complex [28]. The main entity in the differential diagnosis of fibrolamellar carcinoma is scirrhous HCC (Fig. 5D), which has a distinct clinical presentation (discussed further below). From a diagnostic perspective, the most important clinical feature of fibrolamellar carcinoma is its age distribution. The majority occurs in patients between the ages of 10 and 35 years, and only rare cases have been documented in patients over 40 years old. Although the prognosis is thought to be similar to that of typical HCC in a non-cirrhotic background [29], fibrolamellar

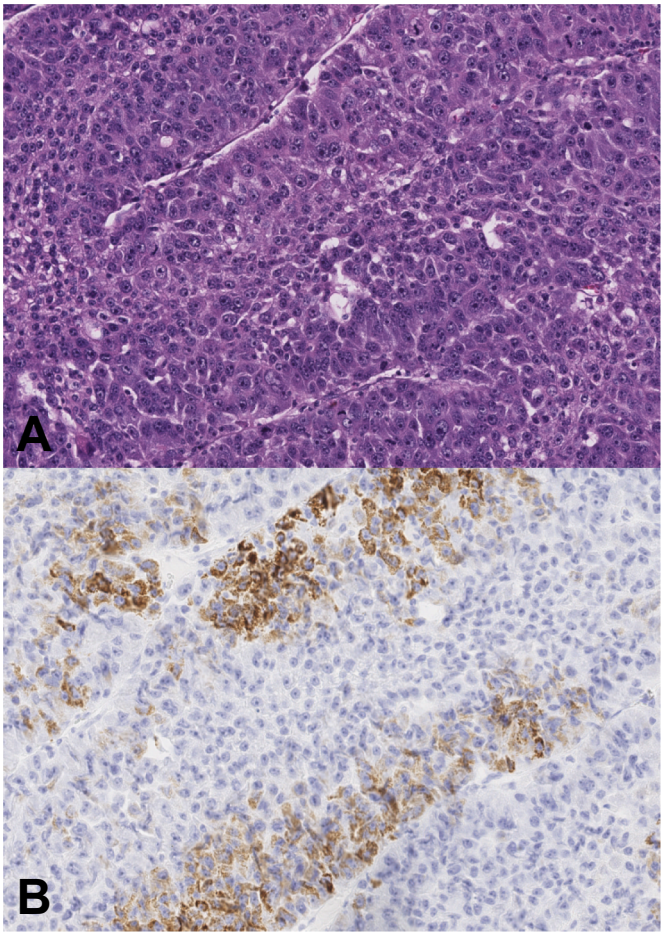

Fig. 3. Poorly differentiated hepatocellular carcinoma (HCC). (A) In contrast to well-differentiated HCC, tumor cells typically have higher nucleus to cytoplasm ratios, greater cytologic atypia, and less granular and less eosinophilic cytoplasm - which make identification of the tumor as hepatocytic more challenging. (B) Poorly differentiated HCC is less often positive for HepPar-1 than well-differentiated HCC, and when positive, HepPar-1 staining can be patchy, as in this example. Concurrent use of arginase and glypican-3 (not shown) are recommended when poorly differentiated HCC is a possibility, as these stains have higher sensitivity for poorly differentiated HCC.

carcinomas have a greater propensity for lymph node metastasis, which surprisingly does not correlate with poor prognosis [30]. Moreover, resection of involved lymph nodes has been associated with long term survival [31]. Thus, for clinical management, it is essential to distinguish these tumors from other forms of HCC. If the histologic features, immunophenotype, or clinical setting are not classic, fluorescence in situ hybridization for the *DNAJB1-PRKACA* gene fusion [32] or sequence-based testing for *PRKACA* or *PRKAR1A* mutations is advised.

2.5.2. Scirrhous HCC

Scirrhous HCC is a hepatocellular neoplasm with prominent intratumoral fibrosis involving at least 25% of the surface area of the tumor [33]. The cells are characterized by moderate to abundant eosinophilic granular cytoplasm and round nuclei with prominent nucleoli, arranged in nests and cords (Fig. 5D). This architectural pattern - set within the background of intratumoral fibrosis - can morphologically simulate cholangiocarcinoma. Furthermore, the neoplastic cells are less frequently positive for HepPar-1 [18,34,35] than typical HCC and can show CK7 and CK19 expression (although often in a patchy distribution). In such cases, arginase and glypican-3 can be helpful for clarification of hepatocellular origin, as 100% of scirrhous HCCs have been shown to be positive for either or both arginase and glypican-3 [18]. Another important entity in the differential diagnosis of scirrhous HCC is fibrolamellar carcinoma. In addition to prominent fibrosis and hepatocytic morphology, scirrhous HCC can demonstrate pale bodies, thus morphologically mimicking fibrolamellar carcinoma. It is important to remember, however, that scirrhous HCC occurs in an older age group, with the average age at diagnosis over 50 years [18,36,37] and that a significant subset occur in the setting of cirrhosis and/or chronic hepatitis. As noted above, molecular testing for protein kinase A gene defects can be useful to distinguish it from fibrolamellar carcinoma.

2.5.3. Cirrhotomimetic HCC

This subtype of HCC (also referred to as diffuse type HCC) is cytologically and architecturally identical to typical HCC, but differs in its distribution pattern in the liver. Rather than a single dominant mass with a few satellite nodules in close proximity, cirrhotomimetic HCC is composed of numerous (often greater than 20) small tumor nodules typically measuring less than 2 cm that are dispersed throughout the liver. Imaging characteristically underestimates the tumor burden due to misinterpretation of tumor foci as background cirrhosis, and grossly the liver may appear simply cirrhotic, without a definitive mass lesion, thus evading radiological detection [38]. Large “dominant” masses may also be present; these represent coalescence of smaller tumor foci. Histologically, the “dominant” lesions appear as multiple adjacent circumscribed nodules of tumor each invested by a fibrous capsule, giving the overall lesion an irregular, knobby contour. Interestingly, the dispersed pattern of tumor growth has been proposed to result from tumor invasion of the main portal vein and subsequent tumor seeding [39]. This subtype of HCC is associated with a poor prognosis [40], a finding compatible with its presumed hematogenous route of spread and generally extensive involvement of the liver. Although most cases arise in the setting of cirrhosis, rarely this pattern may be seen in non-cirrhotic liver.

2.5.4. Combined HCC-neuroendocrine carcinoma

Combined HCC-neuroendocrine carcinoma is characterized by a tumor with clear morphologic and immunophenotypic evidence of hepatocellular differentiation in continuity with morphologic and immunophenotypic evidence of a high grade neuroendocrine carcinoma. The neuroendocrine component may be either small cell type or large cell type. This tumor is very rare; in one series it accounted for 0.5% of all resected hepatic tumors [41], and most published reports are single cases [41-47]. In the majority of the reported cases, the tumors occurred in males between the ages of 50 to 75 years, and most were associated with risk factors for HCC (e.g., Hepatitis B, Hepatitis C, fibrosis, cirrhosis). As with other biphenotypic high grade neuroendocrine malignancies, the prognosis appears to be driven by the high grade neuroendocrine component, and regional lymph node and distant metastasis demonstrating purely neuroendocrine differentiation have been documented in some cases. The main diagnostic pitfall for this entity is related to sampling. On needle biopsy, if only neuroendocrine carcinoma is identified (or recognized), this may lead to a search for an extrahepatic primary. On the other hand, diagnosis of typical HCC would underestimate the biologic potential of the tumor and potentially result in suboptimal local or systemic therapy (e.g., platinum therapy may be used).

2.6. American Joint Committee on Cancer (AJCC) staging of HCC

HCC and intrahepatic cholangiocarcinoma are relatively unique in the spectrum of tumors addressed in the AJCC staging manual in that lymphovascular invasion plays a major role in the T classification of the tumor. This approach is supported by data that show lymphovascular invasion is the most important independent prognostic factor of overall survival following transplantation for HCC [48]. Specifically, the presence of microscopic (microvascular) invasion distinguishes pT1 from

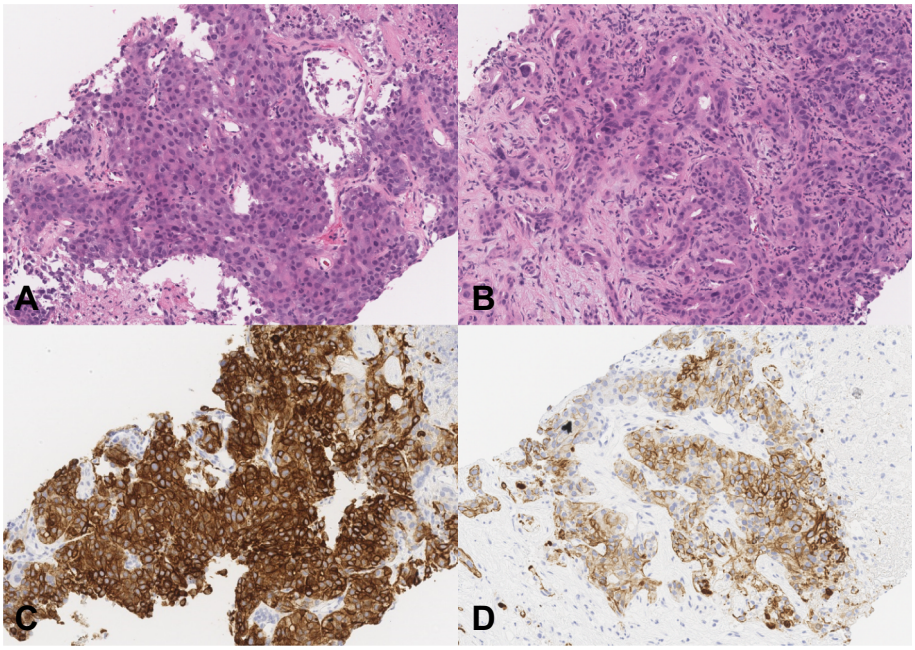

Fig. 4. Poorly differentiated cholangiocarcinoma (CCA). (A) This area of the CCA demonstrates a sheet-like proliferation of tumor cells with a moderate amount of amphophilic cytoplasm and uniform round nuclei, suggesting the possibility of hepatocellular carcinoma. (B) In other areas of the biopsy, however, true gland formation was identified. Note the anastomosing gland pattern. (C) The tumor demonstrates diffuse strong expression of CK7. (D) The tumor also demonstrates diffuse, moderate staining for CK19. HepPar-1 and glypican-3 stains were negative (not shown).

pT2 (and also clinical stage I from clinical stage II). Thus, generous sampling of the tumor-non-tumor junction is recommended, as this area is the most likely to harbor microvascular invasion [33].

Overall, up to 50% of resected HCCs demonstrate microscopic evidence of lymphovascular invasion [48,49]. Tumor size correlates with presence of microvascular invasion, and multiple tumors, which comprise approximately 20% of all resections for HCC, are more likely to manifest lymphovascular invasion (up to 77%, depending on largest tumor size) compared to solitary lesions [49]. Thus, microvascular invasion in HCC is common.

Lastly, it is important to note that major vascular invasion for HCC staging includes only first order branches of the portal vein (i.e., right and left) and hepatic vein (i.e., right, middle, and left) [50,51]. In contrast, for cholangiocarcinoma, first and second order branches of the portal vein and hepatic artery and first order branches of the hepatic vein are designated as major vascular invasion [50].

3. Intrahepatic biliary neoplasms

In the second half of this review, we focus on intrahepatic biliary neoplasms, specifically cholangiocarcinoma (CCA) and its precursors. Key concepts include: (1) the morphologic and molecular classification of the CCA, (2) essential features of pathologic assessment of CCA that impact staging, (3) and the evolving definition of combined hepatocellular-cholangiocarcinoma and other primary liver carcinomas.

3.1. Intrahepatic cholangiocarcinoma (CCA)

From a diagnostic perspective, the majority of CCAs are not difficult to identify, and the most important consideration on biopsy is that of metastatic adenocarcinoma (as discussed previously). Once the diagnosis of CCA has been established, distinction of two main classes is useful: (1) large duct type, and (2) small duct type [52,53]. Although historically emphasis has been placed on the gross features of the tumor for classification (e.g., periductal, mass-forming, peripheral, central), emerging evidence suggests that glandular morphology corresponds with distinct molecular defects - with accompanying therapeutic consequences. Additionally, it is well known that classification of CCA according to gross features is problematic when evaluating large tumors, as they may outgrow their original pattern and destroy adjacent structures. Finally, separation of CCA into morphologically distinct subtypes enhances discovery of the unique molecular pathways of biliary carcinogenesis.

3.1.1. Large duct type

Morphologically, large duct type (also known as “bile duct” type) CCA is characterized by large, variably dilated glands with mucin elaboration (either intracytoplasmic or extracellular) (Fig. 6A). These tend to be more centrally located tumors, and recapitulate the epithelium of larger bile ducts and/or the mucinous epithelium of the peribiliary glands. Stromal fibrosis, when present, appears desmoplastic (i.e., loose spindle cells investing neoplastic glands). This subtype of CCA is associated with chronic biliary disease and precursor biliary lesions such as

Table 3
Clinicopathologic features of poorly differentiated hepatocellular carcinoma, poorly differentiated cholangiocarcinoma, and metastatic carcinoma.

	Hepatocellular carcinoma	Metastatic carcinoma	Cholangiocarcinoma
Background liver	Background cirrhosis	Non-cirrhotic background liver	Non-cirrhotic background liver (more common than cirrhotic)
Tumor microenvironment	Limited stromal reaction within tumor or at interface with non-neoplastic liver	Desmoplasia	Prominent intra-tumoral and peritumoral fibrosis (often)
Morphologic architecture	Nodular/macrotrabecular pattern	Variety of patterns	Anastomosing gland pattern
Mucin	None	Subset	Large duct type (often)
Pattern of metastasis	Lung, bone common	Variable	Lymph node, lung common
Serum markers	Elevated serum AFP (subset)	Variable	Elevated serum CA 19-9

AFP = alpha fetoprotein; CA 19-9 = carbohydrate antigen 19-9.

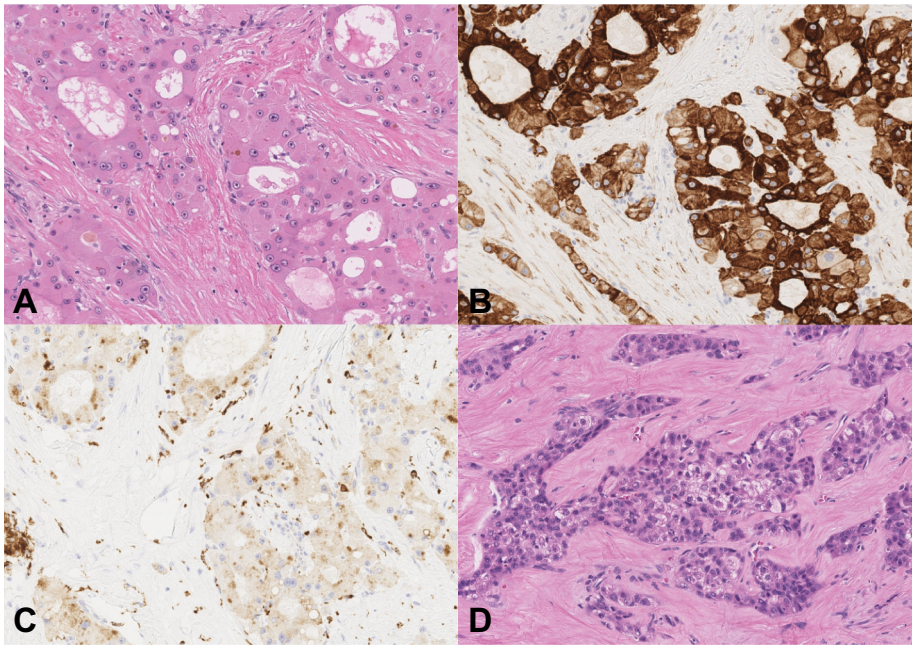

Fig. 5. Fibrolamellar carcinoma and scirrhous hepatocellular carcinoma (HCC). (A) Fibrolamellar carcinoma is characterized by abundant eosinophilic cytoplasm, prominent nucleoli, and dissecting bands of fibrosis. The tumor cells can be arranged in the same architectural patterns as those seen in typical HCC. This particular example has prominent pseudoglandular architecture. (B) Fibrolamellar carcinoma typically demonstrates diffuse strong expression of CK7. (C) It also characteristically demonstrates expression of CD68. (D) Scirrhous HCC has prominent intratumoral fibrosis and can be immunopositive for CK7. As such, cholangiocarcinoma and fibrolamellar carcinoma may enter into the differential diagnosis. The hepatoid cytomorphology, relatively focal expression of CK7, and lack of CD68 positivity are usually sufficient to distinguish it from these other possibilities, however.

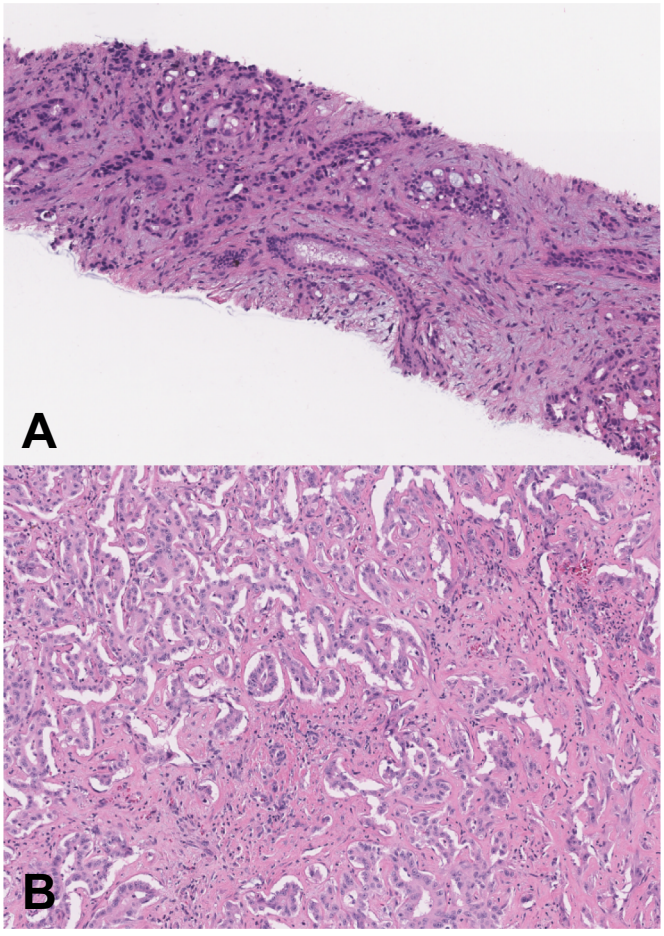

Fig. 6. Subtypes of intrahepatic cholangiocarcinoma (CCA). (A) Large duct type intrahepatic CCA is characterized by mucin-producing glands haphazardly arranged in a loose, desmoplastic stroma. (B) In contrast, small duct type CCA is characterized by small, non-mucinous glands lined by cuboidal epithelium set within a dense, collagenous stroma.

biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of bile duct (IPNB). Large duct type CCAs express S100P (not to be confused with S100B, the melanocytic/neural marker) and trefoil factor 1 (TFF1), an immunoprofile distinct from that of the small duct type. On a molecular level, this subtype has been shown to be associated with *KRAS* mutations [53] and *MDM2* amplification [54], both of which represent targetable gene defects. In particular, selumitinib, an inhibitor of the MAP kinase signaling pathway (downstream of *KRAS*) has shown promise as single agent therapy and in conjunction with conventional chemotherapy for metastatic biliary cancer [55,56]. Additionally, MDM2 inhibitors are in the early phase of clinical validation for use against solid tumors, with several phase 1 clinical trials currently open and recruiting patients (Clinicaltrials.gov identifiers: NCT01877382; NCT03449381; NCT03362723; NCT02935907; NCT02143635).

3.1.2. Small duct type

Small duct type (also referred to in the literature as cholangiolar, cholangiolocellular, and occasionally cholangiocellular) CCA is characterized by small, cuboidal cells with uniform round nuclei, arranged in small tubules and anastomosing (staghorn-like) glands (Fig. 6B). In contrast to the loose spindle cell background stroma of desmoplasia, the neoplastic cells are typically surrounded by a dense collagenous (or sclerotic) stroma. Unlike large duct type, this subtype is associated with chronic *liver* disease, cirrhosis, and viral hepatitis. Small duct type CCAs characteristically express CD56 (also known as neural cell adhesion molecule/NCAM), N-cadherin, and C-reactive protein (CRP). On a molecular level, these tumors are associated with *FGFR* translocations and *IDH1* and *IDH2* mutations [53], all of which are actionable gene defects. In fact, several FGFR inhibitors are currently in clinical trials, with many showing impressive results [57,58] (Clinical trials.gov identifiers: NCT02924376, NCT02265341, NCT03230318). Early clinical trials for IDH1 and IDH2 inhibitors have also shown promising results [59,60], and a subsequent phase 2 trial for solid tumors harboring *IDH2* mutation has just been completed (NCT02273739).

3.2. Rare variants of CCA

In addition to the two broad subtypes above, rare variants of CCA exist that do not fit precisely into either class. Some may represent

examples of the morphologic spectrum of the large or small duct type, whereas others likely are truly distinct and possess unique molecular drivers. Variants include (but are not limited to): lymphoepithelioma-like carcinoma, sarcomatoid, and undifferentiated type.

3.2.1. Lymphoepithelioma-like CCA

This rare variant is characterized by a glandular to syncytial proliferation of monotonous cells with prominent intraepithelial and stromal lymphocytes. Similar to its counterpart in the nasopharynx and stomach, viral integration of Epstein-Barr virus (EBV) is present within the tumor cells. Confirmation of EBV by in situ hybridization or other ancillary test is essential for the diagnosis, particularly as such tumors are thought to have a more favorable prognosis. Distinction of this tumor from lymphoepithelioma-like HCC is important as well and can be accomplished by immunohistochemistry for hepatocellular markers (HepPar-1, arginase, glypican-3).

3.2.2. Sarcomatoid CCA

Sarcomatoid CCA describes a subset of CCAs that demonstrate areas of mesenchymal morphology (e.g., spindle cell, rhabdoid). These areas are typically seen in association with glandular areas, aiding in its identification as CCA. The mesenchymal areas express both cytokeratins and vimentin. Specific types of sarcomatoid CCA include those with predominantly spindle cell morphology [61], those with rhabdoid morphology [62,63], and those that elaborate granulocyte colony-stimulating factor. These tumors are more aggressive than typical CCA, a finding consistent with their poor differentiation/de-differentiated phenotype.

3.2.3. Undifferentiated carcinoma

Undifferentiated carcinoma is characterized by pleomorphic or sarcomatoid cells by definition lacking any evidence of typical CCA. Keratin expression may be only focal. This is a diagnosis of exclusion, and as such, HCC and metastasis must be ruled out. Although it is recommended that it be designated as distinct from CCA [64], the presence of high grade biliary intraepithelial neoplasia (BilIN) in the background liver suggests a biliary origin.

3.3. Precursors of CCA

Three main types of precursor lesions have been established for intrahepatic CCA: (1) biliary intraepithelial neoplasia (BilIN), (2) intraductal papillary neoplasm of bile duct (IPNB), and (3) mucinous cystic neoplasm (MCN). BilIN is a form of flat dysplasia that can arise anywhere in the biliary tract. It is most commonly encountered in the larger intrahepatic bile ducts and the extrahepatic biliary tree and, like its pancreatic counterpart, pancreatic intraepithelial neoplasia, most commonly develops in the setting of chronic inflammation. Three grades of BilIN are recognized, based on the degree of cytologic and architectural atypia [65,66]. BilIN-1 is characterized by a single layer of biliary epithelium with mild nuclear enlargement and cellular crowding (Fig. 7A). Nuclear chromasia is not significantly increased, and mitotic figures are not identified. Intestinal or pyloric gland metaplasia may be present. The atypical epithelium may demonstrate a scalloped appearance (pseudopapillary) due to the cellular crowding and slight disorganization of the epithelial layer. BilIN-2 is characterized by more significant nuclear enlargement, cellular crowding, and increased nuclear chromasia. Mitotic figures are typically rare. Architecturally, the epithelium is more disorganized, with changes including occasional loss of nuclear polarity, mild nuclear stratification, and development of papillary projections. BilIN-3 is characterized by more significant cytologic atypia (e.g., nuclear enlargement, irregular nuclear membranes, diffuse loss of nuclear polarity, epithelial tufting, complex papillary structures, and cribriform gland formation). Mitotic figures may be seen. IPNB (also known as papillary carcinoma in situ and intraductal growth type CCA - when not associated with invasion through the basement membrane of the bile duct) is histologically analogous to intraductal papillary mucinous neoplasm of the pancreas. IPNB is characterized by radiologically identified ductal dilatation resulting from an intraductal epithelial proliferation (Fig. 7B). The spectrum of epithelial changes is essentially identical to that seen in BilINs; however, the cytologic and architectural changes are termed low grade, intermediate grade, and high grade dysplasia. As with the BilINs, IPNBs are associated with risk of invasive carcinoma. Mucinous cystic neoplasm (MCN) of the biliary tract, like its counterpart in the pancreas, is characterized by a cystic lesion without communication with the ductal system, along with ovarian type stroma surrounding the epithelial lining of the cyst. Epithelial proliferation along the cyst is likewise categorized into low, intermediate, and high grade dysplasia. Similar predilection for women ages 40 to 50 years is seen. A recent large case series identified invasive carcinoma in 2 of 30 cases (6%), both of which were associated with an overall good prognosis [67].

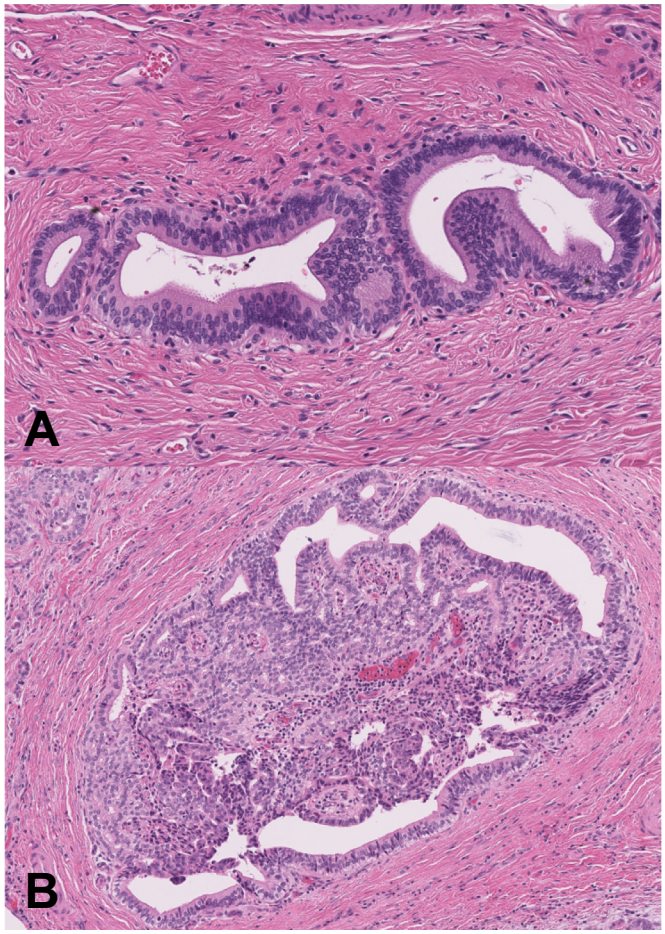

Fig. 7. Precursor lesions of intrahepatic cholangiocarcinoma. (A) This example of biliary intraepithelial neoplasia, grade 1, (BilIN-1) demonstrates a single layer of epithelial cells lining the duct with mild nuclear crowding, mild nuclear enlargement, and focal pseudopapillary architecture. Higher grade BilINs demonstrate increased nuclear enlargement, more pronounced nuclear hyperchromasia, mitotic activity, and loss of nuclear polarity. (B) Intraductal papillary neoplasm of bile duct (IPNB) is characterized by a luminal proliferation of neoplastic biliary epithelium resulting in localized ductal dilatation. The neoplastic epithelial proliferation grows along fibrovascular cores which extend into the duct lumen, giving it a papillary and architecturally complex appearance.

3.4. AJCC staging of intrahepatic CCA

As mentioned previously, intrahepatic CCA is one of the few tumors in which lymphovascular invasion included in the T component of its

TNM classification. Multifocal disease (including multiple separate tumors, satellitosis, and intrahepatic metastasis) is also recognized as an important negative prognostic factor and similarly distinguishes pT1 from pT2 (and correspondingly clinical stage I from clinical stage II). This is supported by outcome data which demonstrate a marked decrease in overall survival based on the presence of microvascular invasion or multifocal disease [50].

Approximately 30% of resected intrahepatic CCAs demonstrate multifocal disease [68-70]. Microvascular invasion is seen with similar frequency, being present in approximately one-third of cases [68,69,71]. As such, ample sampling near the interface of the tumor and non-neoplastic liver (i.e., area of highest yield) is recommended. Additionally, sampling of the larger bile ducts at the margin as well as within the parenchyma is important to evaluate for BilIN.

As highlighted previously, the AJCC definition of major vascular invasion for intrahepatic CCA differs from that of HCC and includes: (1) first and second order branches of the portal vein, (2) first and second order branches of the hepatic artery, and (3) first order branches of the hepatic vein. Additionally, surgical staging of intrahepatic CCA requires lymphadenectomy, and the AJCC now requires a minimum of 6 lymph nodes from the appropriate lymph node stations for complete pathologic staging, necessitating additional pathologist attentiveness in regard to lymph node examination and count [50]. Furthermore, relevant lymph node stations depend on whether the tumor is centered in the right or left liver due to the distinct lymphatic drainage of the intrahepatic bile ducts in each hemisphere - with the contralateral nodes being considered distant metastasis (if the tumor is confined to a single hemiliver). Of note, lymph node metastasis is common, with approximately 25% of cases of surgically resected CCA with lymphadenectomy demonstrating positive lymph nodes [69,72]. Perineural invasion is also seen in approximately 25% of cases [69,71] and when present is more commonly identified near the hilum [73].

3.5. Combined hepatocellular carcinoma-cholangiocarcinoma (cHCC-CCA)

In its classic form, combined HCC-CCA is a biphenotypic tumor with clear HCC in one area and clear adenocarcinoma (CCA) in another area (Fig. 8). These two components are typically adjacent but spatially distinct and show a morphologic transition from one phenotype to the other. Despite having been described in the literature > 100 years ago, this entity continues to be challenging to diagnose. A few reasons for this include: (1) they are relatively uncommon, comprising approximately 2% of primary liver tumors, (2) when either the hepatocellular or biliary component of the tumor is poorly differentiated, the distinction between the two components can be quite subtle, (3) descriptions of its subtypes as defined in the World Health Organization Tumours of the Digestive Tract (4th edition) [74] were limited, and (4) the literature is plagued by usage of multiple terms and various definitions for this tumor. To address the last issue, an international panel of pathologists, radiologists, and clinicians recently convened to consolidate terminology and definitions for this set of primary liver tumors [75]. This has led to re-classification of some of the entities formerly designated as combined HCC-CCA as well as clarification of their morphologic features. In particular, the concept of “stem cell features” was defined as uniform small cells with high nucleus:cytoplasm ratio present as a component of any adult primary liver carcinoma. Such cells, if present, typically reside at the interface of the tumor and its surrounding stroma and show no significant mitotic activity. They often demonstrate expression of stem cell markers, such as CD56/NCAM, CD117/KIT, EpCAM/MOC-31, and cytokeratin 19. Importantly, immunopositivity for stem cell markers without corresponding stem cell morphology is insufficient for this designation. An additional important point of clarification was that immunohistochemical evidence of biphenotypic differentiation in the absence of corresponding morphologic features is of no consequence for classification of primary liver tumors. Thus, a CCA focally expressing arginase should not be classified as a combined HCC-CCA.

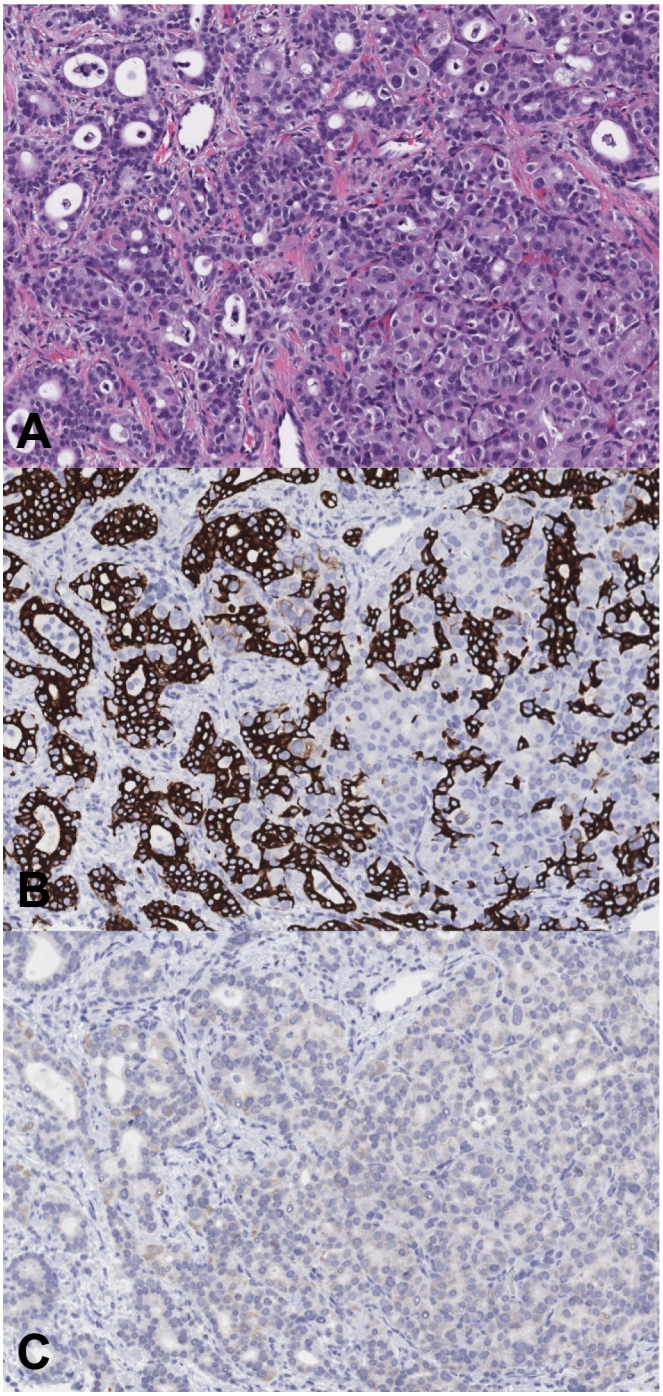

Fig. 8. Combined hepatocellular carcinoma-cholangiocarcinoma (HCC-CCA). (A) Combined HCC-CCA is a biphenotypic tumor that demonstrates clear hepatocellular differentiation in some areas and true glandular formation in other areas. In this example, the two tumor morphologies are intimately comingled. (B) CK7 immunostain highlights the glandular (CCA) component. (C) TTF-1 stain demonstrates granular cytoplasmic staining in the hepatocellular areas (a known immunohistochemical pattern of hepatocytic differentiation) but not in the glandular component.

As described above the term combined HCC-CCA refers to those tumors showing unequivocal evidence of both HCC and CCA. Currently, no minimum percentage of either component has been established for the diagnosis. One key diagnostic pitfall for this diagnosis is misinterpreting ductular reaction at the edge of a HCC as representing

malignant glands. Absence of cytologic features of malignancy is the best support for a benign process. Additionally, presence of chronic inflammation within the glandular focus is more characteristic of ductular reaction. Additionally, pseudoglandular architecture in a HCC should not be confused with CCA [76]. Features in favor of pseudoglands include evidence of bile production and absence of mucin. Combined HCC-CCA can be seen in association with stem cell features, as defined above. If so, it is recommended that the phrase "stem/progenitor cell features present" be included in the diagnostic comment. This tumor can arise in both a background of cirrhosis (more frequently noted in case series reported in Asian countries, [77]) as well as in non-cirrhotic livers (more frequently reported in case series in the United States [78,79]).

3.6. Transitional cell carcinoma

Although this tumor was originally classified as a "combined HCC-CCA with stem cell features, intermediate type" [74], it is now proposed as a distinct primary liver carcinoma. This tumor is characterized by monomorphic, small to medium-sized round cells with centrally placed nuclei and moderate amounts of clear to lightly eosinophilic cytoplasm. The cells are arranged in small nests, tight tubules, and trabeculae and lie within a prominent fibrous stroma. Although by definition the neoplastic cells do not bear a strong resemblance to hepatocytes or cholangiocytes, they express both hepatocellular and biliary markers - a feature that can be useful for its recognition.

3.7. Cholangiolocellular carcinoma (CLC)

In the past this term has been used to denote various neoplasms with morphologic features of cholangioles. Per international consensus [75], it now replaces "combined HCC-CCA with stem cell features, cholangiolocellular type." This tumor is composed of cuboidal cells with small round nuclei arranged in thin, angular glands, without mucin production. Similar to transitional cell carcinoma, the neoplastic proliferation is invested by a dense stromal fibrosis.

3.8. Additional conceptual changes

In addition to the separation of combined HCC-CCA into the above three distinct tumor categories, the international consensus group acknowledged the existence of combinations of all these tumors and encouraged use of combined terminology, such as HCC-CCA-CLC, as appropriate. While the adoption of these new categories of primary liver carcinoma will require re-interpretation of previous observations, going forward it is anticipated to allow for clarification of the incidence, prognosis, and molecular features of these rare tumors.

4. Conclusion

In this review we have discussed the major primary liver neoplasms from the point of view of the practicing pathologist, with a stepwise approach to the most common diagnostic challenges. While many tried and true stains (H&E, reticulin) remain the backbone of diagnosis, a new menu of immunostains (e.g., glutamine synthetase, CRP, LFABP) is becoming increasingly important for prognostication. Nonetheless, none of these operates in a vacuum, particularly when the findings of the ancillary tests are in conflict with the clinical history. Additionally, these newer stains have many caveats to their interpretation, making integration of all data, clinical and otherwise, even more important for preventing diagnostic error. Marked progress in our understanding of the morphologic categories and molecular basis of CCA has been made recently, and the pathologist, at the intersection of clinical and investigative endeavors, plays an important role not only by recognizing the established microscopic features essential for TNM prognostication but also by aiding in morphologic classification of these tumors. Finally, by adopting and adhering to consistent terminology for rare neoplasms such as combined HCC-CCA, the pathologist can pave the way for collective study and understanding of these uncommon entities.

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