Original Article

Interpretation Pitfalls in Immunohistochemistry of Primary Liver Carcinoma: A Retrospective Analysis of Liver Biopsy Specimens

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Background: The diagnosis of primary liver carcinoma (PLC) primarily relies on routine histopathology with hematoxylin and eosin (H&E) and the supportive evidence of immunohistochemistry (IHC). Inappropriate use of immunostains without either careful histomorphological evaluation or clinical correlation may lead to misdiagnosis and inappropriate patient management.

Objective: To retrospectively analyze the interpretation pitfalls in IHC as an adjunct in assessing PLCs in liver biopsy specimens at a tertiary hospital in Thailand.

Materials and Methods: The archives of the Division of Pathology, Thammasat University Hospital, were searched between 2015 and 2020 using a search tool from the pathology software system and a combination of codewords, including PLC, hepatocellular carcinoma (HCC), and intrahepatic cholangiocarcinoma (iCCA). Histomorphology of PLCs in liver biopsy specimens and the corresponding immunophenotypes were retrospectively analyzed. Clinical data for each patient was retrieved from the electronic medical record.

Results: One hundred fifty-three liver biopsy specimens were retrieved. There were 128 (83.7%) iCCA, 23 (15%) HCC, and 2 (1.3%) combined hepatocellular-cholangiocarcinoma (cHCC-CCA). Six cases (3.9%) with interpretation pitfalls in IHC were analyzed. These pitfalls included incorrect subtyping of PLCs in two cases (1.3%) and PLCs misdiagnosed as metastatic cancers in four cases (2.6%).

Conclusion: Interpretation pitfalls in IHC of PLCs were noted in 3.9% of the present study cohorts. Pathologists should be familiar with the histomorphology of PLCs together with their rare variants. Appropriate use of IHC as adjuncts for evaluating PLCs and correlation with clinical details were essential for rendering the correct diagnoses of PLCs.

Keywords: Immunohistochemistry; Primary liver carcinoma; Interpretation

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Historically, primary liver carcinoma (PLC) was dichotomously classified into two types, hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA). This binomial categorization has been challenged by increasing recognition of PLCs with biphenotypic differentiation, such as carcinoma with both hepatocytic and cholangiocytic differentiation[1,2]. Thus, PLCs should be considered a spectrum of diseases with diverse differentiation resulting in variable histo-morphology and immunophenotype. Such a spectrum includes HCC (classic HCC and classic HCC with cholangiocyte immunophenotype), combined hepatocellular-cholangiocarcinoma (cHCC-CCA), and iCCA (classic iCCA and classic iCCA with hepatocyte immunophenotype)[3]. These differentiative and histological relationships among PLCs could lead to difficulties in the pathological diagnosis of PLCs, particularly those in the setting of small biopsy samples.

According to pathological aspects, the diagnosis of PLC is primarily based on routine histopathology with hematoxylin and eosin (H&E) with the supportive evidence of immunohistochemistry

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Table 1. Cases of PLCs with pitfalls in IHC interpretation

Histomorphology	Immunophenotype	Potential IHC pitfall	Pathological diagnosis	Treatment	Survival (months), status
Glandular	Hepatocytic and cholangiocytic	HCC, cHCC-CCA	iCCA	Chemotherapy	41, alive with disease
Hepatocellular	Hepatocytic and cholangiocytic	iCCA, cHCC-CCA	HCC	Yttrium-90	24, alive with disease
Glandular	Lower GI	Metastatic CRC	iCCA	Supportive treatment	1, dead
Glandular	Lower GI	Metastatic CRC	iCCA	Supportive treatment	3, dead
Glandular with excessive mucin	Lower GI	Metastatic ADC	iCCA with mucinous feature	Palliative chemotherapy	30, alive with disease
Glandular with excessive mucin	Inconclusive (hepatocytic and cholangiocytic, upper GI)	Metastatic ADC	iCCA with mucinous feature	Supportive treatment	26, alive with disease

IHC=immunohistochemistry; HCC=hepatocellular carcinoma; cHCC-CCA=combined hepatocellular-cholangiocarcinoma; iCCA=intrahepatic cholangiocarcinoma; GI=gastrointestinal; CRC=colorectal carcinoma; ADC=adenocarcinoma

(IHC). Inappropriate use of immunostains without either careful histomorphological evaluation or clinical correlation may lead to misdiagnosis and inappropriate patient management. The present study aimed to retrospectively analyze the interpretation pitfalls in IHC as an adjunct in assessing PLCs in liver biopsy specimens at a tertiary hospital in Thailand.

Materials and methods

Study population

The archives of the Division of Pathology, Thammasat University Hospital, were searched between 2015 and 2020 using a search tool from the pathology software system and a combination of codewords, including PLC, HCC, and iCCA. All percutaneous liver biopsy specimens in which the clinical queries were to evaluate whether the patient had PLC were retrieved. Each patient's clinical data, including age, gender, clinical presentation, underlying hepatobiliary diseases, pre-biopsy diagnosis, treatment, and prognosis, was retrieved from the electronic medical record.

All procedures performed in the current study were approved by the Human Ethics Committee of the Faculty of Medicine, Thammasat University (COA No. 128/2020, on 16 June 2020), and in accordance with the 1964 Helsinki Declaration and its later amendments. Formal written informed consent was waived due to its retrospective nature.

Histomorphology and immunophenotype

Histomorphology and immunophenotypes of PLCs in the retrieved liver biopsy specimens were blindly evaluated by a hepatopathologist (TL) using previously stained slides. The revised pathological diagnosis was primarily based on histomorphology using IHC as an adjunct was compared with the pre-biopsy and initial pathology diagnoses. If there were any discordances with the previous diagnoses such as interpretation pitfalls in IHC, two hepatopathologists (NW and NL) and a general pathologist (SA) would further analyze those liver biopsy specimens.

Results

One hundred fifty-three liver biopsy specimens were retrieved. There were 128 (83.7%) iCCA, 23 (15%) HCC, and two (1.3%) cHCC-CCA. The pre-biopsy diagnosis could distinguish these PLCs from metastatic cancers. Six cases (3.9%) with interpretation pitfalls in IHC are summarized in Table 1. These pitfalls included incorrect subtyping of PLCs in two cases (1.3%) and PLCs misdiagnosed as metastatic cancers in four cases (2.6%).

Those six cases were elderly patients in which the pre-biopsy diagnoses were PLCs with either HCC or iCCA. Clinical presentations were variable, including abdominal pain, abdominal mass, weight loss, and jaundice. Most patients had no underlying hepatobiliary disease. One patient had HBV cirrhosis. Another had a history of cholecystectomy due to calculous cholecystitis years ago.

Interpretation pitfalls in IHC of the first two cases (Case #1 and #2) were that of incorrect subtyping of PLCs due to the immunoreactivity of both hepatocyte (HepPar-1) and cholangiocyte (CK7 or CK19) markers. Although the immunostaining revealed biphenotypic differentiation, the appropriate pathological diagnosis should rely on histomorphology, such as glandular or hepatocellular pattern, rather than immunoreactivity. Hence, the pathological diagnosis for Case #1 and #2 was iCCA with hepatocyte immunophenotype (Figure 1) and HCC with cholangiocyte immunophenotype, respectively. Of note, iCCA with hepatocyte immunophenotype and HCC with cholangiocyte immunophenotype were relatively common. They accounted for 0.7% and 4.3% of iCCA and HCC in the present study series, respectively.

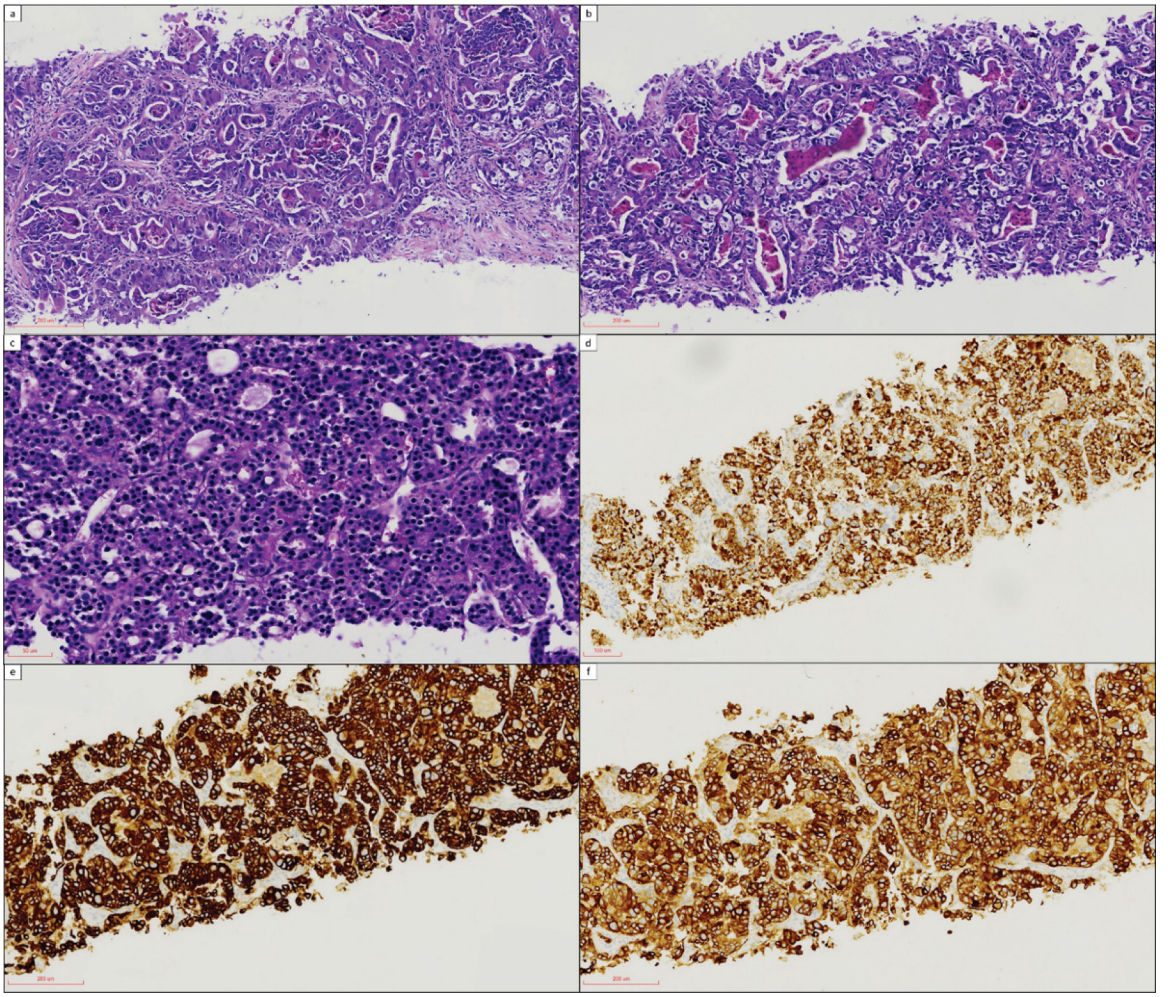

Figure 1. iCCA with HCC immunophenotype. Adenocarcinoma, characterized by cancer cells arranged in glandular and cribriform patterns with luminal necrotic debris and associated stromal desmoplasia, is observed (a). Closely packed glands are occasionally present (b). This histomorphological pattern could resemble that of the pseudoglandular pattern of HCC (another case) (c). The cancer cells are highlighted by HepPar1 (d), CK7 (e), and CK19 (f) immunostains.

PLCs misdiagnosed as metastatic cancers were considered as another pitfall in the present study series. Diagnosing adenocarcinoma with either lower or upper gastrointestinal (GI) immunophenotype (Case #3 to #6) in liver biopsy specimens was a challenging issue in pathology. Case #3 was adenocarcinoma with lower GI immunophenotype, whose morphology could not be distinguished from that of adenocarcinoma of other organs. The presence of pancreaticobiliary epithelium, a feature of iCCA (Case #4) (Figure 2), helped avoid such an error. The iCCA with mucinous appearance, also known as mucinous iCCA if the mucinous content accounted for more than 50% of total tumor volume, could be misdiagnosed as metastatic adenocarcinoma of GI primary due to expression of GI immunoprofile (Case #5 and #6) (Figure 3). Furthermore, distinguishing iCCA from metastatic GI carcinoma on a morphological basis could be challenging since former cancer could have intestinal-type epithelium (Figure 3b). Recognizing such a rare variant of iCCA and correlation with radiologic (i.e., computed tomography (CT) of the whole abdomen) and endoscopic findings were essential to arrive at the correct diagnosis.

Discussion

Although radiological studies usually diagnose most PLCs, a liver biopsy still plays a crucial role in managing patients with focal liver lesions, particularly those with inconclusive imaging findings[4]. According to the clinical practice guidelines, a liver biopsy is

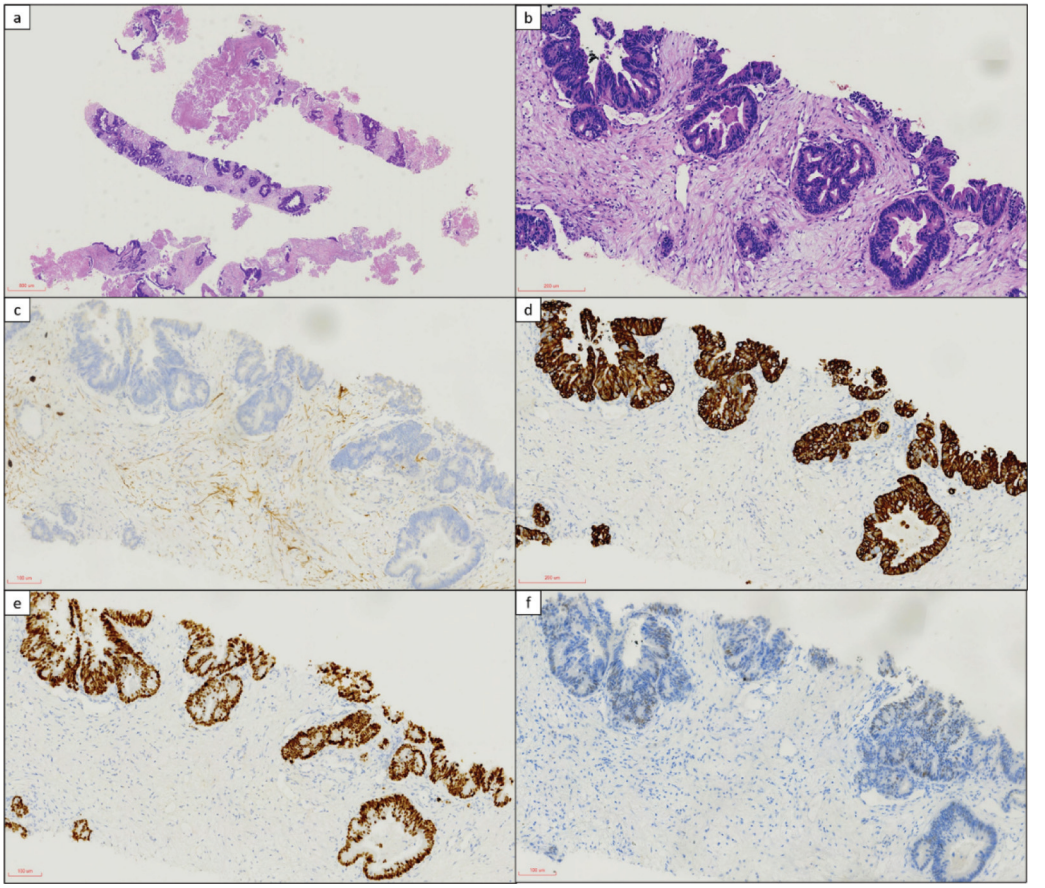

Figure 2. Large-duct iCCA with lower GI immunophenotype. Scanning magnification of a liver biopsy specimen shows adenocarcinoma (a). At higher magnification, the cancer cells are arranged in glands and papillae. Note the pancreaticobiliary epithelium (b). These malignant cells show lower GI immunophenotype, characterized by CK7–/CK20+/CDX2+ immunoprofile (c-e). They had patchy and weak nuclear SATB2 immunoreactivity (f).

indicated in two clinical settings. First, a liver biopsy should be considered in patients with suspected HCC having atypical radiologic findings[5-8]. A systematic review concluded that neither CT nor magnetic resonance imaging (MRI) could be used to diagnose HCC in patients with cirrhosis[9] confidently. Second, patients with non-operable cholangiocarcinoma (CCA) have warranted a liver biopsy. The iCCA may be challenging to distinguish from metastatic cancer on a radiological basis[10]. A diagnosis of CCA cannot be confidently made with radiological findings alone; therefore, histologic confirmation is required to establish CCA diagnosis[5].

Interpretation of liver biopsy specimens is critical in the clinical setting of patients with liver masses. The diagnosis of PLC is mostly based on routine histopathology with H&E with supportive evidence of IHC. A pattern-based approach to the cytohistological diagnosis of focal liver lesions has been proposed[11]. HCC and iCCA are fitted into hepatocellular (hepatoid/epithelioid) and glandular (ducts, glands, or mucin) patterns, respectively. Nevertheless, complex differentiative and histological relationships between PLCs influence the interpretation of biopsies for solid focal liver lesions in terms of complex histomorphology and variability in the immunophenotype.

Diagnostic pitfalls in the evaluation of histopathology of PLCs are common, especially when pathologists are not familiar with diverse morphological spectrums and variable immunoreactivity of PLCs. It is critical to correctly diagnose PLCs in liver biopsy specimens because of the differences in patient management among HCC, iCCA, and metastatic cancers. Morphological variants of HCC, particularly acinar/pseudoglandular pattern, could resemble that of iCCA. Such a pattern is common, accounting for almost one-third of HCC in a reported series[12].

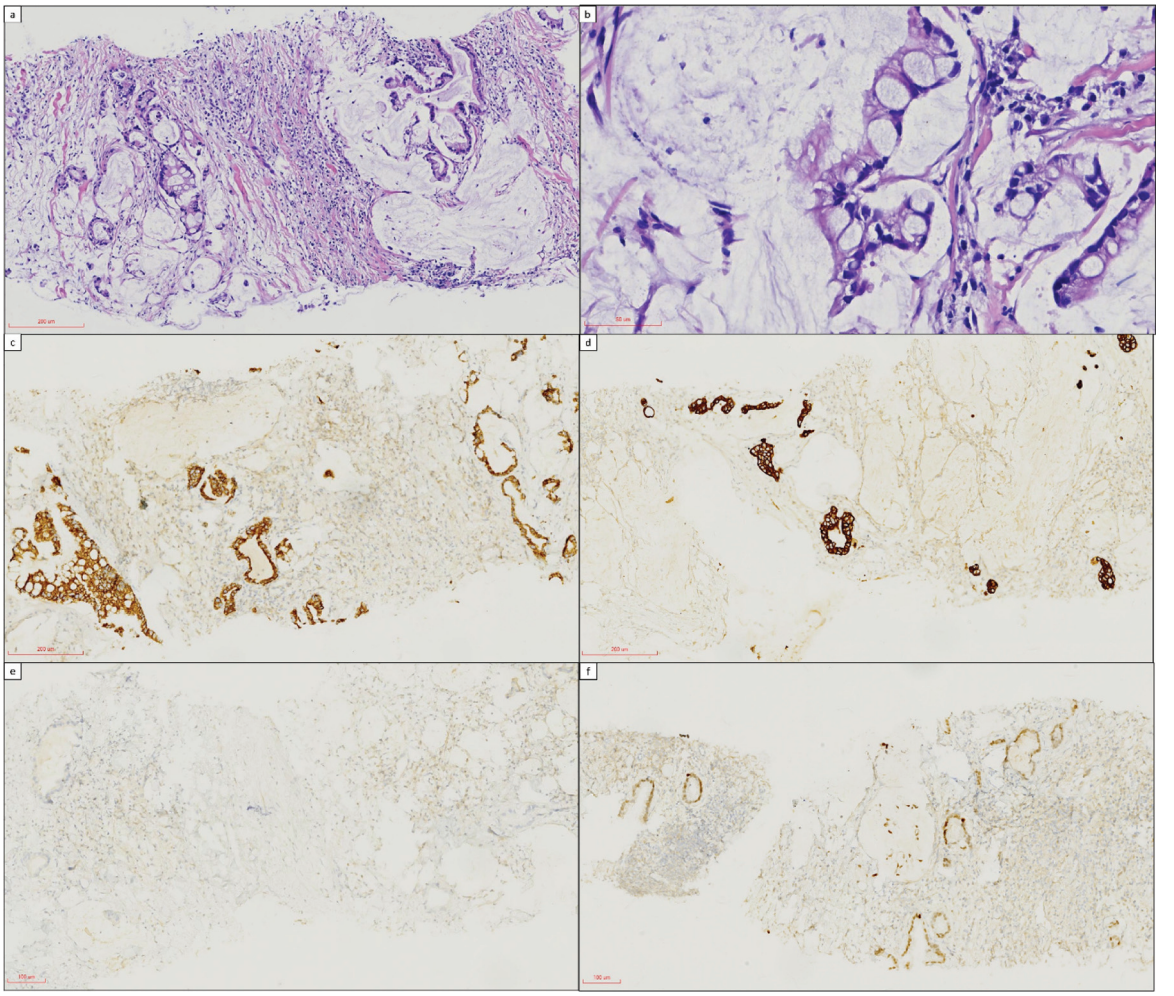

Figure 3. iCCA with mucinous feature showing unusual immunophenotype. Adenocarcinomatous cells floating in an abundant extracellular mucin pool are observed (a). Presence of intestinal-type epithelium cannot be used to distinguish iCCA from metastatic GI cancer (b). The tumor cells show HepPar1+/CK7+/CK20−/CDX2+ immunophenotype (c-f).

Besides, radiological features of pseudoglandular HCC may mimic that of metastatic gastric cancer, hemangioma, and mucinous cystic neoplasm[13]. A comparative analysis of immunohistochemical markers for differential diagnosis of HCC and CCA showed that the expression rates of CK7 and CK19 in HCC were 31% and 10%, respectively[14]. Although arginase-1 is a more sensitive and specific marker of hepatic differentiation than HepPar-1, iCCA and metastatic carcinoma sometimes revealed positive immunoreactivity for arginase-1[15,16]. These immunostains are essential for definite subtyping of poorly differentiated carcinoma as poorly differentiated HCC or poorly differentiated CCA. Nevertheless, interpretation is difficult in the setting of focal and weak immunoreactivity. Regardless of their immunophenotypes, PLCs with the obvious mixed epithelioid-glandular pattern should be diagnosed as cHCC-CCA[3].

The liver is one of the most common sites for metastatic cancers, accounting for almost one-fourth of all cases[17]. Solid tumors, such as GI, breast, and lung cancers, frequently metastasize to the liver[18]. The liver is the most common site of distant spread of colorectal cancer (CRC). Approximately 20% of CRC patients will have distant metastases at the first diagnosis[19]. Rarely, hepatic metastasis from CRC can mimic CCA[20,21].

Histologically, iCCA consists of cancer cells arranged in a glandular pattern. In liver biopsy specimens, iCCA is an adenocarcinoma, in which the morphology is similar to that of metastatic adenocarcinoma from other primaries, including GI, pancreas, lung, and breast. Immunohistochemistry

has been used to distinguish iCCA from other metastatic cancers. The immunohistochemical approach to a variety of adenocarcinoma in the liver includes CK20/CDX2 homogeneous as lower GI immunophenotype, CK20/CDX2 heterogeneous as upper GI/pancreaticobiliary immunophenotype, TTF-1-positive as carcinoma of lung origin, GATA-3+ as carcinoma of breast origin, and other non-specific patterns such as CK7+ only and CK7–/CK20–[22]. Of note, these immunostains are sometimes not helpful to discriminate iCCA from metastatic cancer. The expression of GI differentiation markers that may play a role in developing extrahepatic, such as perihilar and distal, CCA was reported[23]. On rare occasions, CCA could be highlighted by TTF-1 and napsin A[24]. GATA3 immunoreactivity can also occur in CCA and pancreatic ductal adenocarcinoma[25]. Further investigation, such as radiological study and endoscopy, is needed to exclude metastatic cancers and arrive at the correct diagnosis of PLCs.

The limitation of the present study is that the series may not represent the entire PLCs at the given tertiary hospital or adult population in central Thailand. The indications for performing a liver biopsy are different between HCC and iCCA. A liver biopsy is not required to diagnose HCC with classic radiologic findings. Nevertheless, a liver biopsy is needed to confirm the diagnosis of iCCA. As a result of these differences, the number of iCCA cases was far greater than that of HCC cases in the present study cohorts. Of note, it is impossible to calculate the exact proportion of HCC expressing cholangiocyte immunoprofile and vice versa. However, diagnosing these PLCs in liver resection specimens is not problematic since the histomorphology is relatively apparent.

The present study suggested that it is unnecessary to perform IHC to diagnose PLCs in most liver biopsy specimens, especially those with obvious histomorphology. Besides, IHC may lead to an incorrect diagnosis of PLCs due to these cancers' variable immunoreactivity. Of note, PLC diagnosis is primarily based on routine histopathology with H&E using IHC as an adjunct[3]. Pathologists should be familiar with diverse histomorphologic patterns of PLCs and always look up the clinical information before rendering the diagnoses of PLCs in any given liver biopsy samples.

A liver biopsy still plays an essential role in diagnosing focal liver lesions, particularly in those with indeterminate radiological findings. PLCs are now considered as a spectrum of disease rather than the historically dichotomous classification. Interpretation pitfalls in IHC of PLCs were noted in 3.9% of the present study cohorts. Pathologists should be familiar with the diverse histomorphology of PLCs together with their rare variants. Furthermore, they should be aware of possibility of metastatic carcinoma in patients with a pre-biopsy diagnosis of iCCA. Therefore, a thorough evaluation of clinical data is essential. Appropriate use of IHC as adjuncts for evaluating PLCs and correlation with clinical details are essential for rendering the correct diagnosis of PLCs.

What is already known on this topic?

The diagnosis of PLC primarily relies on routine histopathology with H&E and the supportive evidence of IHC. Inappropriate use of immunostains without either careful histomorphological evaluation or clinical correlation may lead to misdiagnosis and inappropriate patient management.

What this study adds?

Interpretation pitfalls in IHC of PLCs were common, accounting for 3.9% in the present study series. Pathologists should be familiar with the histomorphology of PLCs together with their rare variants. Appropriate use of IHC as adjuncts for evaluating PLCs and correlation with clinical details are essential for rendering the correct diagnosis of PLCs.

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Conflicts of interest

The authors declare no conflict of interest.

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