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Review Article

A Diagnostic Approach to Combined Hepatocellular-Cholangocarcinomas (cHCC-CCA)

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Abstract

Combined Hepatocellular-Cholangiocarcinomas (cHCC-CCA) are rare tumours, accounting for 2% to 5% of all primary liver cancers. cHCC-CCA is defined by the unequivocal presence of both hepatocytic and cholangiocytic differentiation within the same tumour. The nomenclature of these tumours is confusing but there have been recent efforts by experts in the liver community to clarify the terminology and diagnostic criteria. We aim to reinforce the diagnostic criteria for cHCC-CCA, illustrated by two case reports; and share our approach to evading common pitfalls in the work-up of such tumours.

Keywords: Hepatocellular carcinoma; Cholangiocarcinoma; Liver cancer; Diagnostic pathology

Introduction

Primary liver cancer is the second most common cancer in Asia [1]. Hepatocellular Carcinoma (HCC) is the most common primary liver cancer, accounting for 75% to 85% of primary liver cancers. Intrahepatic Cholangiocarcinoma (iCCA) is the second most common primary liver malignancy with an incidence of 10% to 15%. In comparison, combined Hepatocellular-Cholangiocarcinomas (cHCC-CCA) are rare tumours, accounting for 2% to 5% of all primary liver cancers [2]. A clinical audit of our institution's cases over the last 4 years showed an even lower incidence of 1.3%, likely a result of under-recognition of this rare beast [3]. If one considers HCC and iCCA as two polar ends of the spectrum of primary liver cancers, then cHCC-CCA lies squarely in between. This tumour is defined by the unequivocal presence of both hepatocytic and cholangiocytic differentiation within the same tumour. In essence, the pathologist must be able to discern phenotypic features of both HCC and iCCA within the same tumour. This definition also removes collision tumours from consideration [2]. The previous World Health Organization's (WHO) Classification of Tumors of the Digestive System, 4th edition described cHCC-CCA with three variants that contained stem cell features. The confusing nomenclature resulted in the mistaken perception that cHCC-CCA must have stem cell features [1]. Recent work has shown that stem cell features can be demonstrated in the many forms of primary liver cancers, including HCC and iCCA; and is not limited to only cHCC-CCA [4,5]. Lifting the fog, Brunt et al. [6] released a consensus document from an international community of pathologists, radiologists, and clinicians; that recommends a working terminology for such tumours. The key takeaway point from the article is that the diagnosis of cHCC-CCA relies on the morphological recognition of classical HCC and iCCA components in the tumour based on routine histochemical stains. Immunohistochemistry (IHC) is used as a supplemental diagnostic tool, rightly so as there are potential pitfalls in the over-utilization of these techniques. In our limited single institution experience, we have encountered some diagnostic challenges when attempting to make such diagnoses. In this article, we will reinforce the diagnostic criteria for cHCC-CCA and share our approach to the common pitfalls in the interpretation of IHC in the work-up of such tumours, with illustrations from two case reports.

Making the Diagnosis of cHCC-CCA

As previously emphasized, the diagnosis of cHCC-CCA rests upon the recognition of classical HCC and iCCA components in the tumour. Therefore, we wish to underscore the importance of adequate sampling of all heterogenous areas within the tumour during prosection. In our lab, we also sample at least 1 section per 1cm^3 of tumour. HCCs usually have a fleshy tan-to-green gross appearance, contributed by the vascular and bile-producing nature of these tumours. In contrast, iCCA tends to appear firmer and whiter, afforded by the desmoplastic stroma that frequently accompanies the malignant glands. By sampling more generously, one is also liable to pick up a separate second component, should it be present. Under the objective lenses of the light microscope, the tumour should show a distinct biphenotypic morphology. HCCs show four principal histological patterns: trabecular, solid, pseudoglandular and macrotrabecular. For the uninitiated, the pseudoglandular pattern may be mistaken as cholangiocytic differentiation. Most cases of iCCAs show a ductal or tubular pattern, accompanied by variable but frequently abundant fibrous stroma. Mucin is commonly seen but may be absent in the small duct variant of iCCA. It is important to be familiar with the myriad of patterns and subtypes of each entity as the backbone of a cHCC-CCA diagnosis rests on accurate histomorphological identification.

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The Role of IHC

In a well-equipped lab, it is often very tempting to want to confirm histomorphological impressions with IHC stains. However, IHC stains require expertise in interpretation and may result in potential pitfalls in difficult diagnoses, especially in rare tumours. The key to a definite diagnosis should always be grounded on a strong histomorphological impression from Haematoxylin & Eosin (H&E)-stained sections. The myriad of IHC markers can be divided into three main categories. IHC markers that characterize hepatocytic differentiation are hepatocyte paraffin 1 (HepPar-1), Alpha Fetoprotein (AFP), arginase-1 and glypican-3. Canalicular staining patterns by Polyclonal Carcinoembryonic Antigen (pCEA) and CD10 are also indicative of hepatocytic lineage. Cholangiocytic IHC markers for biliary cells include CK7, CK19, EpCAM (BerEP4) and cytoplasmic staining patterns by pCEA and CD10. Stem cell IHC markers such as CK7, EpCAM (BerEP4), CD117, CD133 and CD56 have been reported in the literature. It is important to note the overlap of CK7 and EpCAM (BerEP4) in highlighting cholangiocytes and stem cells.

Case Study 1

A 57-year-oldchinese male with history of chronic hepatitis B, presented with a liver mass measuring 2x1.5x1.3 cm. On microscopy, the tumor showed a predominantly hepatocytic morphology with polygonal cells containing eosinophilic cytoplasm, and arranged in trabecular, solid and pseudoglandular architectures. However, there was a distinctly different area within the tumor that showed a glandular architecture set within desmoplastic background. The adjacent non-tumoural liver showed bridging fibrosis. The hepatocytic component showed immunopositivity for HepPar-1 and glypican-3. In contrast, the cholangiocytic component showed immunonegativity for HepPar-1 and glypican-3, while being positive for CK7, CK19, BerEP4 and CD56. The final diagnosis was that of cHCC-CCA on a background of chronic hepatitis B with bridging fibrosis (Figure 1).

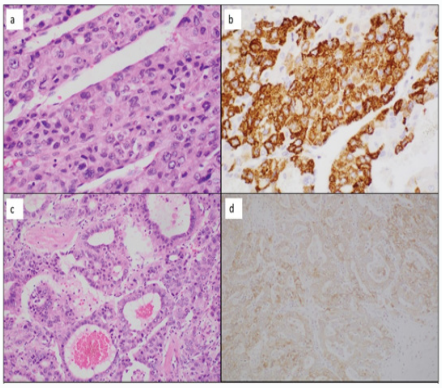

Figure 1: Case study 1 of combined hepatocellular-cholangiocarcinoma (cHCC-CCA). The tumour shows classical areas of (a) hepatocytic and (c) cholangiocytic morphologies on haematoxylin & eosin-stained sections. (b) shows HepPar-1 positive staining in the hepatocytic regions and (d) shows BerEP4 positive staining in the cholangiocytic regions.

High Grade HCCs can Acquire CK7 and/or CK19 Immunopositivity

Many studies have shown the prognostic ability of CK19 when expressed in hepatocellular carcinomas [7-10]. CK7 has also been shown to be positive in some poorly differentiated HCCs, which is similar to our experience [11]. If one is unaware of this fact, it is easy to interpret a positive CK19 or CK7 stain and mistakenly arrive at the conclusion that there is a combined cholangiocytic component. It is at this juncture that accurate histomorphological identification is critical. In our case study 1, we illustrate how both hepatocytic and cholangiocytic components should be histomorphologically distinct and express the appropriate IHC markers in each region. In the case study 2 below, there is only a hepatocytic component that stains with both hepatocytic markers, CK19 andCK7 markers. As such this is best interpreted as an HCC rather than a cHCC-CCA. In this situation, we advocate caution when using CK7 and CK19 as the only cholangiocytic markers in your panel. We suggest the utilization of other cholangiocytic markers such as EpCAM (BerEP4) and/or a histochemical stain that highlights mucin such as mucicarmine.

High Grade HCCs can lose HepPar-1 Positivity

Another lesser known fact to pathologists not familiar with in the diagnosis of HCCs is that poorly differentiated HCCs may lose expression of HepPar1. Although HepPar1 is extremely sensitive in picking up HCCs (92%), it has been shown to be lost in cases with higher nuclear grade, sarcomatoid or compact growth patterns [12]. Hence utilizing HepPar1 as the only hepatocytic marker is a potential pitfall. Pathologists should consider a panel of at least 2 markers for this purpose. S. Kakar and his team compared the efficacy of 5 hepatocellular markers for the diagnosis of hepatocellular carcinoma across various levels of differentiations [13]. They found that arginase-1 and HepPar-1 had the highest sensitivity for well-differentiated HCCs, whereas arginase-1 and glypican-3 had the highest sensitivity for poorly differentiated HCCs. We recommend a combination of these markers in the immunohistochemical workup of cHCC-CCAs, particularly HepPar-1 and arginase-1 to cover the spectrum of differentiation.

Case Study 2

A 67-year-old Chinese female with chronic liver disease of unknown aetiology, presented with a liver nodule measuring 1.3 cm × 1 cm × 0.8 cm. On microscopy, the tumor showed a predominantly hepatocytic appearance with poorly differentiated tumour cells arranged in trabecular, pseudoglandular and solid architectures. In addition, there were scattered areas with tumour cells that exhibited a more primitive morphology with high nuclear-cytoplasmic ratios, brisk mitoses and spindled forms. There was no definite cholangiocytic component seen. The adjacent non-tumoural liver was cirrhotic. The hepatocytic component showed immunopositivity for HepPar-1 and glypican-3. The tumour cells with primitive morphology were negative for HepPar-1 and glypican-3 but positive for CK7, CK19, CK20, BerEP4, synaptophysin and CD117. The final diagnosis was that of a poorly differentiated HCC with stem cell features (Figure 2).

Stem Cells can Arise in all forms of Primary Liver Cancers

Another important point brought up by the consensus article by Brunt et al is that stem cells can arise in all forms of primary liver cancers [6]. One must avoid making the diagnosis of cHCC-CCA just because of the presence of stem cells. Hepatic cancer stem cells are small uniform tumour cells with scant cytoplasm and inconspicuous nucleoli. They are often seen at the transitional zone between the hepatocytic and cholangiocytic components, at the periphery of HCC trabeculae or scattered small nests without specific localization Figure 3.They can be seen in either pure HCC or iCCA, and when present their presence and percentage can be mentioned in the histopathology report as there is prognostic significance [14]. We highlighted a few stem cell IHC markers such as CK19, EpCAM (BerEP4), CD117,

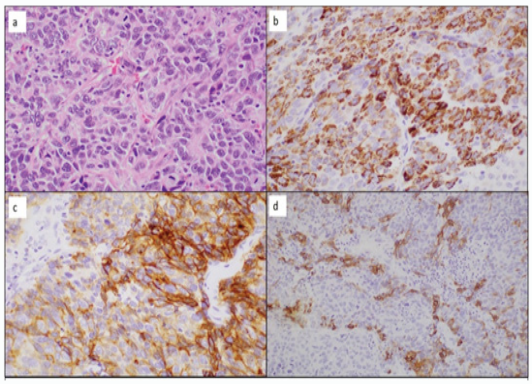

Figure 2: Case study 2 of hepatocellular carcinoma (HCC) with stem cell features. the tumour shows classical areas of (a) hepatocytic morphology. (b) shows HepPar-1 positive staining, with (c) showing BerEP4 positive staining. (D) Note how CK7 highlights the distribution of stem cells at the periphery of the tumour trabeculae.

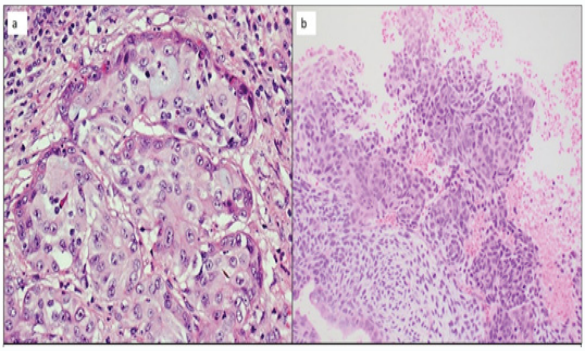

Figure 3: Hepatic cancer stem cells are small uniform tumour cells scant cytoplasm and inconspicuous nucleoli. They can be found (a) at the periphery of tumour trabeculae/nests or (b) as scattered small nests without specific localization.

CD133 and CD56; and have highlighted the cross reactivity with hepatocytic and cholangiocytic components. Once again, it is important to stress upon the importance of interpreting IHC markers with the histomorphological impression on H&E-stained sections.

Intermediate Cell Carcinomas

An even rarer beast is the intermediate cell carcinoma of the liver. Of note, this tumour must be homogenous, comprising monotonous tumour cells that not only shows intermediate morphological features between hepatocytes and cholangiocytes but also dual expression of hepatocytic and cholangiocytic IHC markers. The tumour cells are smaller than hepatocytes but larger than stem cells, often arranged in cords, strands, trabeculae and the occasional gland; within abundant hyalinized stroma. Mitoses are uncommon and the tumor cells are not overtly atypical. Mucin production is not seen in these tumors. Focal presence of such intermediate tumour cells in a cHCC-CCA does not qualify for diagnosis of intermediate cell carcinoma. Due to the rarity of such tumours, there is limited data on their characteristics, but recent work has uncovered new IHC markers for these intermediate cells [15-21].

Conclusion

Molecular studies show a variable mutation spectrum that supports the biphasic nature of cHCC-CCA. Liver stem cells show marked plasticity and can trans differentiate between both hepatocytic and cholangiocytic ends of the liver cell spectrum. This very nature of the hepatic stem cells fuels intense basic science research into a variety of liver diseases. To support studies into the pathogenesis of cHCC-CCA, we must first be able to diagnose and categories these tumours accurately. As illustrated, there are many pitfalls that the pathologist can encounter when undertaking this endeavour, especially in the interpretation of IHC markers.

By evading such pitfalls and embracing the evolution of immunohistochemical and molecular techniques, we can only hope to understand such rare liver tumours better, in order to discover effective treatment regimens. It is with this hope that we share our humble single institution experience in the diagnosis and workup of cHCC-CCA.

Author Contributions

Conceptualization, WQ.L.; data collection, WQ.L., S.A.S., LLY.T.; writing, WQ.L., S.A.S., LLY.T. All authors have read and agreed to the published version of the manuscript.

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