Update on Ancillary Testing in the Evaluation of High-Grade Liver Tumors

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KEYWORDS

• Liver • Hepatocellular carcinoma • Immunohistochemistry • Albumin in situ hybridization • Liver biopsy

Key points

- Liver biopsies for mass lesions may contain limited material, confounding hematoxylin-eosin diagnosis; a panel of immunostains, including hepatocyte in paraffin 1, arginase-1, CD10, polyclonal carcinoembryonic antigen, bile salt export pump, and glypican-3, may be helpful.
- In situ hybridization for albumin may also be helpful and is positive in tumors of liver origin, including hepatocellular carcinoma and intrahepatic cholangiocarcinoma.
- Scirrhous and fibrolamellar variants may present special challenges, because, unlike classic hepatocellular carcinoma, these variants can be positive for cytokeratin 7 and exhibit a fibrous stromal reaction, suggestive of metastatic adenocarcinoma or cholangiocarcinoma.

ABSTRACT

Tissue diagnosis is the gold standard for mass lesions of the liver, but needle core biopsies may sometimes prove challenging. Presented here is a review of a panel of immunohistochemical stains, including hepatocyte in paraffin 1, arginase-1, polyclonal carcinoembryonic antigen, CD10, bile salt export pump, glypican-3, as well as in situ hybridization for albumin RNA, to establish hepatocellular origin in cases in which hepatocellular carcinoma is suspected but the sample is limited or the morphology is challenging, as it may be with cases of scirrhous, fibrolamellar carcinoma, intrahepatic cholangiocarcinoma, and combined hepatocellular-cholangiocarcinoma.

OVERVIEW

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men, the ninth most common cancer in women, and the second leading cause of death due to cancer worldwide.[1] Because HCC most commonly arises in patients with underlying liver disease and cirrhosis, these patients are routinely screened by imaging at 6-month intervals for the detection of small (1–2 cm) lesions, in hopes of detecting cancer at an early and potentially curative stage.[2] Biopsy of such lesions for definitive histologic diagnosis is frequently performed. However, the liver is also a major site of metastatic disease. Therefore, although in some cases the evaluation of liver lesions may be accomplished by routine hematoxylin-eosin (H&E) staining, it is common to use ancillary tests to arrive at a definitive diagnosis. The situation in which the pathologist becomes most reliant on ancillary testing is when the lesion is at the extreme ends of the differentiation spectrum. In noncirrhotic liver, very well-differentiated or low-grade hepatocellular lesions evoke a differential diagnosis, which includes hepatocellular adenoma, focal nodular hyperplasia, cirrhotic/regenerative/dysplastic nodule, and well-differentiated HCC. This review deals with the opposite problem,

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wherein the pathologist encounters an obvious malignancy of uncertain histogenesis. The differential in this setting typically includes metastatic disease, poorly differentiated HCC, and intrahepatic cholangiocarcinoma (ChCa). Several ancillary tests have been added to the pathologists' armamentarium over the last several years. This review focuses on what is most novel and most useful in the authors' experience.

STAINS TO DETERMINE LIVER ORIGIN

Classic HCC most often occurs in patients with chronic liver disease and is composed of polygonal cells with abundant, eosinophilic, often granular cytoplasm and can be recognized on H&E stained sections (as well as on reticulin special stain) by its thickened hepatic plates (greater than 3 cells), formation of pseudoacinar structures, lack of portal tracts within nodules, and invasion of adjacent portal tracts at the leading edge of the tumor. Bile production by tumor cells may be noted and, if present, is highly specific for HCC. Mucin production, on the other hand, excludes classic HCC. Additional variants with distinctive histologic (and sometimes clinical) features include fibrolamellar, steatohepatitic (associated with underlying steatohepatitis and hepatitis C[3–5]), and clear cell variants. It is the authors' practice to use at least a minimal panel of immunostains in all but the most classic cases (in which it may be reasonable to rely strictly on H&E morphology and a reticulin stain). When encountering a case in which HCC is strongly suspected and a minimal workup is performed for confirmation, the authors typically use arginase-1 (ARG-1) immunostain, because of its high overall sensitivity and specificity.[6] Cases can become more challenging based on either clinical or pathologic characteristics. When the background liver is not cirrhotic, HCC is still an important differential, with 7% to 54% of hepatocellular carcinomas arising in noncirrhotic liver.[7] This cohort includes the fibrolamellar variant, which commonly arises in younger, noncirrhotic patients (see later discussion). In noncirrhotic patients, metastatic tumors are more common, however.[7] So, when either the histology or clinical setting are opaque, a panel of ancillary tests should be used. Although a discussion of all markers of carcinoma of unknown primary is beyond the scope of this review, the authors often use an immunohistochemical (IHC) panel consisting of cytokeratin 7 (CK7), CK20, TTF1, GATA3, CDX2, PAX8, CD31 (to exclude epithelioid hemangioendothelioma), and NKX3.1 (in male patients). If the tumor is not unequivocally epithelial by H&E, the authors include CD45, C-KIT, HMB45/Melan-A, and perhaps other markers based on morphology. The authors now discuss HCC markers in detail.

THE UREA CYCLE STAINS: ARGINASE-1 AND HEPATOCYTE IN PARAFFIN 1

Hepatocyte in paraffin 1 (HepPar-1) is an antibody that binds the urea cycle enzyme carbamoyl phosphate synthetase 1, expressed in hepatocellular mitochondria,[8] as well as small intestine[9] and Barrett metaplasia.[10,11] Staining is cytoplasmic and often granular in appearance. HepPar-1 is sensitive for well-differentiated tumors (91% to 100%)[12,13]; but its sensitivity drops with loss of differentiation (to 22%–81% in poorly differentiated HCC[13–16]), with an overall sensitivity of approximately 70%[12,17] to 85%.[18] HepPar-1 has also been reported to have less sensitivity in cases of scirrhous HCC.[19] In terms of specificity, as mentioned earlier, in addition to frequent staining of hepatoid tumors in other organs,[13,17,20,21] carbamoyl phosphate synthetase 1 is expressed in the small intestine; HepPar-1 staining has been reported in some small intestinal adenocarcinomas and intestinal-type ampullary adenocarcinomas.[22] Occasional strong HepPar-1 staining has also been reported in other tumor types, including melanomas and lung, gallbladder, pancreas, stomach, ovarian, and neuroendocrine tumors; but the overall specificity has still been reported as approximately 95%.[15,17] HepaPar-1 staining has also occasionally been reported in cholangiocarcinoma, at a rate from 0% to 16.7% in 3 different small series.[12,15,16] Furthermore, HepPar-1 staining in HCC can be patchy, which may be a pitfall in biopsy cases with limited tissue. Despite these caveats, HepPar1-is a relatively sensitive and specific marker with utility as a part of a panel of immunostains to characterize hepatocellular origin.

ARG-1 is an antibody that binds the urea cycle enzyme ARG-1, an isoform that is highly specific to the liver,[23] and exhibits cytoplasmic staining, generally with strong intensity. Several studies have shown it to be a highly sensitive and specific marker for liver tissue,[6,12,24,25] with sensitivities ranging from 83% to 100%, although Yan and colleagues[6] report that, similar to HepPar-1, the sensitivity may decrease somewhat as the degree of differentiation in the tumor decreases. Krings and colleagues[19] report that it is also sensitive for the scirrhous variant of HCC, for which HepPar-1 is significantly less likely to be positive.[26] In terms of specificity, ARG-1 has also been found to be positive in very rare cases of colonic, pancreatic, gastric, and prostatic