Cytohistology of hepatocellular carcinoma

Aileen Wee

Pichet Sampatanukul

Abstract

Hepatocellular carcinoma (HCC) is a heterogeneous tumor that is often but not invariably associated with cirrhosis. Pre-operative diagnosis by fine needle aspiration or core biopsy is not mandated if dynamic imaging criteria concur with clinical expectations. Classic HCC can be well-, moderately or poorly differentiated. Variants and special types of HCCs exist. The cytohistological diagnosis of HCC can be challenging on small tissue samples. At the highly well-differentiated end, the hepatocytic histogenesis is obvious but the malignant status may not be evident. At the poorly differentiated end, malignancy is obvious but the histogenesis may be obscure. Tumor size, location and heterogeneity, radiological guidance, operator skill, type of needle, on-site cytology service with optimal sample preparation, availability of ancillary tests, and interpreter experience all contribute to the accuracy of the final diagnosis. A problem-based clinicopathological approach is recommended. Key cytohistological features, differential diagnoses, diagnostic pitfalls, and utility of ancillary tests are presented.

Keywords core biopsy; cytohistology; fine needle aspiration; hepatocellular carcinoma; liver

Introduction

Hepatocellular carcinoma (HCC) is a heterogeneous tumor that is often but not invariably associated with cirrhosis. Classic HCC can be well-, moderately or poorly differentiated, and variants and special types exist.[1–3] Pre-operative tissue confirmation by fine needle aspiration biopsy (FNAB) or core biopsy is not mandated if dynamic imaging features concur.[4–7] Significant serum α-fetoprotein (AFP) elevation is encountered in less than half of the cases.[8,9] The future, however, may see a paradigm shift in that pre-operative tissue confirmation and cytohistological tumor mapping become best practice for prognostication and therapeutic regimens such as extended (recipient) criteria-liver transplantation and personalized molecular targeted therapy.[10–12]

The cytohistological diagnosis of HCC can be very challenging on small tissue samples. A problem-based clinicopathological approach with radiological input is recommended.[13,14] Key cytohistological features with ancillary tests, differential diagnoses and diagnostic pitfalls are discussed.

Aileen Wee *MB BS FRCPath FRCPA Professor of Pathology and Senior Consultant, Department of Pathology, Yong Loo Lin School of Medicine, National University of Singapore, National University Hospital, National University Health System, Singapore. Conflicts of interest: none declared.*

Pichet Sampatanukul *MD MSc Professor of Pathology, Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Conflicts of interest: none declared.*

Clinical perspective

Some cases of HCC have no underlying chronic liver disease and not all types of cirrhosis carry the same risk for HCC. Patients can present with any of the following clinical settings:

(i) *Symptomatic patients who complain of abdominal symptoms or who present with metastatic disease or manifestations of decompensated cirrhosis and/or portal hypertension*.

(ii) *Significant elevation of serum AFP levels or rising trends*. Its low sensitivity of around 40% renders it a poor screening tool.[8,9]

(iii) *Detection of liver nodule(s) on regular ultrasound surveillance of high-risk patients with chronic liver disease*. Subcentimeter nodules showing interval growth, especially in a cirrhotic background, are highly suspect.[6,7]

(iv) *Incidental discovery of liver nodule(s) in asymptomatic patients on routine health screening*. Liver function test results, tumor markers or imaging findings may be abnormal. This scenario is uncommon and requires distinction from benign hepatocellular nodules such as focal nodular hyperplasia.

Radiological perspective

The evolution of HCC from a benign regenerative nodule through a dysplastic phase is associated with progressive loss of portal venous blood supply and development of de novo arterialization. Dynamic contrast enhanced imaging studies of classic HCCs display a characteristic pattern of "wash in" (contrast enhancement) during arterial phase and "washout" (contrast dispersion) during portal venous and delayed phases, with respect to surrounding nontumor-bearing parenchyma.[6,7] These "noninvasive" imaging criteria on computed tomography and magnetic resonance imaging for the establishment of a firm diagnosis of malignancy are endorsed by both the European Association for the Study of Liver-European Organization Research and Treatment of Cancer (EASL-EORTC) and the American Association for the Study of Liver Diseases (AASLD).[5] However, a biopsy is required in all cases regardless of size when imaging findings are inconclusive or atypical.

Pathological perspective

Hepatocellular carcinomas exhibit solitary, multifocal or diffuse growth patterns in a likely cirrhotic background. The tumors may have well-defined or pushing infiltrative borders with a pale or variegated cut appearance due to bile staining, fatty change, hemorrhage and necrosis. Satellite nodules and portal vein invasion are common. The diversity and complexity of the histological patterns, cell morphology and differentiation of HCC pose diagnostic challenges in small tissue samples and affect accuracy of grading.[15–17] The majority of HCCs have recognizable malignant hepatocytic phenotypes with trabecular-sinusoidal, pseudoacinar, and/or solid compact patterns.

Major issues and pitfalls in the diagnosis of HCC are highlighted:

(i) Cirrhosis can give rise to the development of a host of well-differentiated hepatocellular nodular lesions (WDHNLs) ranging from large regenerative nodule, low- and high-grade dysplastic nodules characterized by large

Problem-based approach to the clinicopathological diagnosis of hepatocellular carcinoma

Clinical and radiological setting	Task	Major differential diagnoses	Tips and pitfalls
Cirrhosis with small (by definition, $\leq$2 cm size) and/or atypical nodule(s)	To establish HCC arising in cirrhosis	Large regenerative nodule Dysplastic nodules, low-grade (with large cell change) and high-grade (with small cell change)	Small HCCs can be early or progressed; most early HCCs are highly well-differentiated while progressed lesions are at least moderately differentiated "Nodule-in-nodule" lesion
Liver mass with clinical history of chronic hepatitis and/or rising serum α-fetoprotein levels	To confirm HCC *No biopsy if imaging concurs or mass is resectable*	Combined hepatocellular-cholangiocarcinoma (CHCC-CC) Adenocarcinoma	HCCs can be well-, moderately and poorly differentiated Poorly differentiated HCC diagnosis can be challenging Consider variants of HCC in the presence of atypical cell profiles
Liver mass without history of chronic hepatitis/cirrhosis or elevated serum α-fetoprotein levels	To rule out HCC *Surgical extirpation without pre-operative biopsy confirmation if mass is resectable*	Focal nodular hyperplasia Hepatocellular adenoma Fibrolamellar HCC CHCC-CC Intrahepatic cholangiocarcinoma Angiomyolipoma Others (benign or malignant, including metastases)	HCC variants and CHCC-CC may be more likely than the classic HCC Fibrolamellar HCC has typical clinical features
Multiple nodules with or without acknowledged liver primary	To suggest and/or to confirm primary tumor	Adenocarcinoma	Malignant hepatocytic cells should display trabecular-sinusoidal pattern with corroborative immunoprofiles Immunohistochemistry is helpful for profiling adenocarcinomas (e.g. CK7/CK20) Rarely, metastases from extrahepatic hepatoid/nonhepatoid carcinomas $\pm$ α-fetoprotein production can confound the situation
Liver mass in infants and children	To distinguish from hepatoblastoma *Surgical extirpation without pre-operative biopsy confirmation if mass is resectable*	HCC Teratoma Others	Recommend multiple representative biopsy samples, according to institutional practice, as hepatoblastoma is heterogeneous and may have an HCC component

Table 1

cell and small cell change, respectively, to HCC. Small HCCs (by definition, $\leq$2 cm in size) may be early (highly well-differentiated) or progressed (at least moderately differentiated).[1]

(ii) In noncirrhotic livers, other WDHNLs such as focal fatty change, focal nodular hyperplasia (FNH), and hepatocellular adenoma have to be considered in addition to HCC. Fatty metamorphosis can occur in all types of WDHNLs.

(iii) A "nodule-in-nodule" lesion in which a malignant daughter subnodule develops within a larger parent dysplastic/large regenerative nodule may be under-diagnosed due to its incipient small size or sampling error.[18]

(iv) At the highly well-differentiated end of the HCC spectrum, the histogenesis is obvious but the malignant status may be abstruse. Early HCCs have to be distinguished from the other WDHNLs, especially high-grade dysplastic nodule.[16,17]

(v) At the poorly differentiated end, the cancer is obvious but the cell of origin remains undecipherable. It may even be difficult to separate the two most common primary hepatic carcinomas, namely HCC and intrahepatic cholangiocarcinoma, from each other and from metastases.

(vi) The liver is a common depository for metastases from virtually any part of the body. Metastases with hepatoid and epithelioid appearances can mimic HCC and its

