

# CLINICAL PATHOLOGY

## Biopsy Considerations in the Diagnosis of Hepatic Masses

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**Diagnosis of hepatic masses requires clinical correlation with imaging and pathologic information. In this review we will discuss the differential diagnosis of hepatic masses and present the relevant pathologic and radiologic issues in different clinical situations. We address the challenges in obtaining, handling, and interpreting biopsy of a liver mass.**

### Clinical Scenario 1

A 42-year-old woman presents with right upper quadrant discomfort that radiates to the right shoulder blade. The discomfort can be intense and has been affecting her daily activities. Laboratory studies are within normal limits. After consulting with her primary care physician, she undergoes an ultrasound that reveals gallstones and a hepatic lesion. She is referred to you for consultation. The hepatic lesion had a slightly heterogeneous echogenicity thought to represent foci of hemorrhage. Magnetic resonance imaging (MRI) showed the typical appearance of a hepatic adenoma, slight increased signal on T1 images and increased signal on T2 images. A biopsy was performed to further characterize the mass. Biopsies were obtained from the mass and non-mass liver. The biopsy report states the mass is composed of cords of hepatocytes and unequivocally diagnoses a hepatic adenoma, whereas the non-mass liver showed no significant inflammation or fibrosis.

### Chronic Liver Disease and Hepatic Tumors

Evaluation of patients with a hepatic mass begins with determination of possible chronic liver disease. Chronic liver disease, particularly infection with hepatitis B or C viruses, is a risk factor for the development of hepatocellular carcinoma (HCC).<sup>1</sup> Figure 1 describes the diagnostic approach to hepatic masses in patients at low or high risk for chronic liver disease. In either case, the radiologic characteristics of hepatic masses influence the diagnostic algorithm.

### Initial Characterization of a Hepatic Mass

Initial characterization of a hepatic lesion with ultrasonography has many advantages including safety, availability, versatility, and low cost. The main disadvantage of ultrasound is operator dependency. Ultrasound has superior performance in the detection of the sharp margins of a cyst. Uniloculated cysts have imperceptible walls around anechoic fluid. Most simple cysts do not present a diagnostic challenge and, if large, are often followed at 6- to 12-month intervals to see whether there is a change in size or risk of rupture that might require

surgery. Multiloculated cysts have a thick wall or internal papillary infoldings that might be better visualized on computed tomography (CT) or MRI. Biopsy is of little use in the diagnosis of complex cystic lesions because of sampling error or contraindicated such as in echinococcal cyst. Additional work-up is often aimed at surgical resection.

### Approach to Solid Hepatic Mass in a Patient Without Chronic Liver Disease

The most common solid hepatic lesion, hemangioma, occurs in 5%–20% of the population. Hemangiomas are usually asymptomatic, subcapsular in the right lobe, and affect women more than men. With proper CT technique and interpretive criteria, confident diagnosis should be achieved in the majority of cases.<sup>2</sup> More sophisticated dynamic CT or MRI can help separate the 2 differential diagnostic considerations for hemangioma, a cyst and a hypervascular hepatic metastasis.<sup>3</sup>

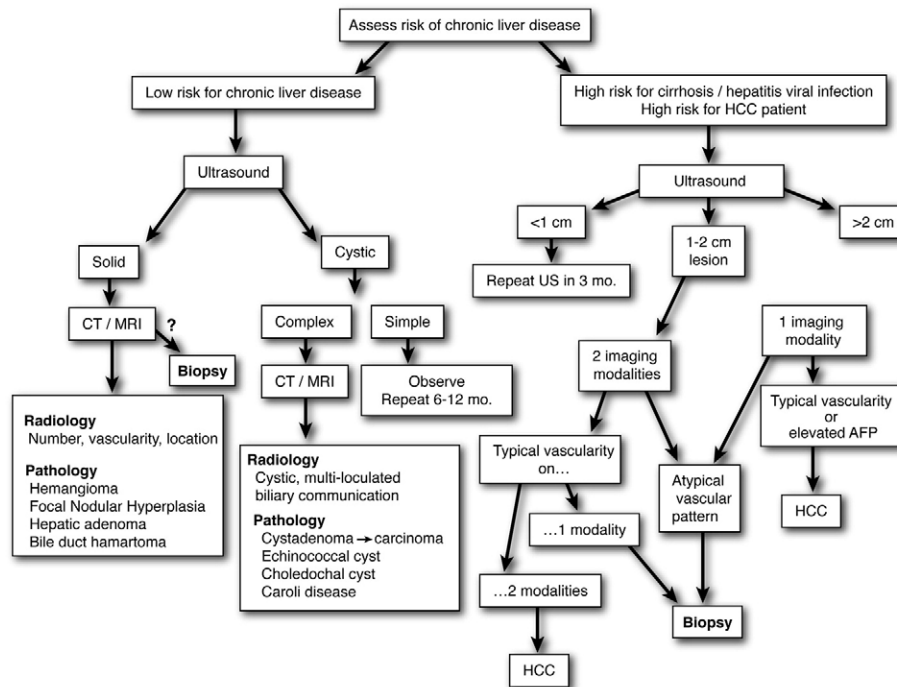
Biopsy of a hemangioma can be a frustrating endeavor; often abundant blood with little tissue is obtained. Microscopic examination of a hemangioma reveals abundant blood with thin fibrotic walls and a single layer of endothelial cells.

A common solid lesion seen more often in female patients is focal nodular hyperplasia (FNH). It is thought to arise as a hyperplastic response to altered arterial blood flow. The characteristics of FNH on helical CT have been well-described.<sup>4</sup> The lesion typically has a smooth surface with ill-defined margins, is homogeneously hyperattenuating in the hepatic arterial phase, and is isoattenuating to the surrounding liver parenchyma on unenhanced, portal venous, and delayed phases. A fibrous central scar has been reported in 35% of lesions 3 cm or smaller and in 65% of larger lesions and can be seen in 50% of cases on MRI scan.<sup>5,6</sup>

Biopsy of an FNH shows hyperplastic hepatocytes divided into nodules formed by a radiating central scar containing thick-walled arteries. Biopsy of FNH might be misinterpreted as cirrhotic liver. Finding hepatic arteries without accompanying bile duct in fibrous septa and hyperplastic hepatic cords 1–2 hepatocytes thick are key diagnostic features. Biopsies obtained

**Abbreviations used in this paper:** AASLD, American Association for the Study of Liver Diseases; CK, cytokeratin; CT, computed tomography; EASL, European Association for the Study of the Liver; FNH, focal nodular hyperplasia; HCC, hepatocellular carcinoma; MRI, magnetic resonance imaging.

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**Figure 1.** The diagnostic approach to hepatic lesions depends on the clinical context in which the lesion is discovered. Patients at low risk for chronic liver disease and cirrhosis often have initial characterization of a mass with ultrasound. Patients with a cystic lesion will either be observed for interval change or undergo formal resection. Complex cysts can be further defined by imaging characteristics to correlate with the pathologic differential diagnosis. Solid lesions might be characterized on imaging or will require biopsy. Imaging characteristics, particularly vascularity, can help narrow the pathologic differential diagnosis. Patients at high risk for viral hepatitis or cirrhosis are at significant risk for HCC. The size of the lesion has been used as a decision point in AASLD guidelines. Patients with lesions <1 cm should be followed at 3- to 4-month intervals. Stable lesions can return to standard screening. If the lesion enlarges, proceed according to the size of the lesion. Lesions 1–2 cm should be evaluated with 2 dynamic imaging modalities (typically CT and MRI in the USA). If the vascularity typical of HCC is seen (arterial enhancement and portal venous washout) by both modalities, the lesion is treated as HCC. A typical HCC vascular pattern on only one imaging modality or an atypical vascular pattern suggests the lesion should be biopsied. Lesions larger than 2 cm at initial diagnosis that have the typical features of HCC on a dynamic imaging modality might not require biopsy. Patients with a lesion >2 cm and serum alpha fetoprotein >200 ng/mL might also not require biopsy.

from both lesional and non-lesional tissue by using multiple needle passes can add to the diagnostic yield.

A hepatic adenoma is a rare hepatic neoplasm that is associated with abnormal metabolism caused by exogenous steroids or congenital abnormalities of carbohydrate metabolism. More than 90% of cases occur in women of reproductive age, and lesions might regress with withdrawal of oral contraceptives or other steroids.<sup>7</sup>

As in the patient in scenario 1, an adenoma is usually heterogeneous as a result of the presence of hemorrhage, necrosis, and/or fat (Figure 2A). CT is less definitive in detection of these specific features than MRI. The typical adenoma on MRI demonstrates slight increased signal on T1 images (probably as a result of fat content) and moderate increased signal on T2 images.

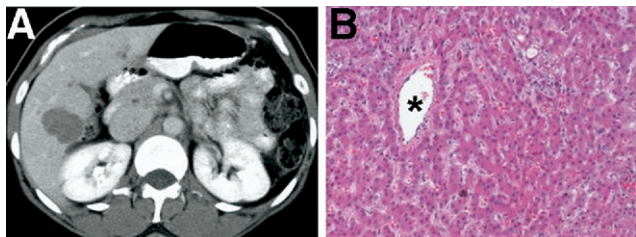
Biopsy of an adenoma shows hepatocytes arranged in cords that recapitulate the normal sinusoidal architecture. However, adenomas lack bile ducts, portal tracts, and hepatic veins, which make up the normal hepatic lobule. Excessive glycogen and lipid accumulation in hepatocytes is also characteristic. Arteries separate from portal tracts are another diagnostic feature. As with most hepatic lesions, biopsy of non-lesional liver tissue is strongly recommended. Comparison between hepatocytes within and outside the mass can aid the pathologist.

## Clinical Scenario 2

A 47-year-old man with a history of chronic hepatitis C viral infection is undergoing screening liver ultrasound for HCC. A 1.8-cm distinct nodule is seen on ultrasound. The serum alpha fetoprotein level is not elevated. The patient presents to a gastroenterologist for recommendation on diagnosis of the newly discovered hepatic lesion. CT evaluation shows the lesion is 1.8 cm, and a biopsy is done. The biopsy shows a moderately differentiated HCC without evidence of vascular invasion (Figure 3A). The hepatic parenchyma shows cirrhosis.

### *Approach to a Hepatic Mass in a Patient With Chronic Liver Disease*

The incidence of HCC is increasing in the United States and many other countries.<sup>8</sup> The increase is predominantly an effect of a peak incidence of hepatitis B and C infection during the 1970s and 1980s and accounts for nearly 75% of HCC. The annual incidence of HCC in the setting of viral hepatitis increases with age and is further amplified in patients with cirrhosis. Consequently, patients known to have cirrhosis or chronic viral hepatitis are often screened for the development of HCC.



**Figure 2.** (A) A CT image showing a mass of low attenuation caused by the presence of fat. (B) Biopsy of an adenoma demonstrates cords of hepatocytes and a rich arterial supply (\*), which can lead to spontaneous hemorrhage. Original magnification, 20 $\times$ .

### Hepatocellular Carcinoma Screening

Most masses that present a diagnostic challenge occur in cirrhotic patients as part of an HCC surveillance program. To date, there is no adequate screening serologic test for HCC. Screening of cirrhotic patients with cirrhosis and/or chronic viral hepatitis relies on liver ultrasound at 6-month intervals. Recommendations from the 2005 Guideline from the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) use size and vascularity to determine the diagnostic algorithm<sup>9,10</sup> (Figure 1). A recent study, however, prospectively examined the vascularity of 72 small (1–3 cm) nodules in cirrhosis as determined by contrast-enhanced ultrasonography and helical CT. Imaging techniques, on the basis of the EASL criteria, would have missed 38% of nodules that were subsequently diagnosed as HCC.<sup>11</sup> This underscores the need for improved screening techniques and the conundrum that small lesions create.

### Biopsy Considerations

Biopsy of hepatic nodules can be challenging and requires image guidance into the lesion.<sup>12</sup> With this technique the accuracy for the diagnosis of HCC ranges from 80%–95%.<sup>13</sup> Fine-needle aspirations are best interpreted by a pathologist who practices cytology. Cytologic examination focuses on the size and shape of cells and cell groups. True-cut or core biopsies result in a cylinder of tissue that allows a pathologist to examine cytologic and tissue structural features. These 2 complementary techniques increase diagnostic yield when material from the same biopsy is sent for both examinations.

There are situations when hepatic nodule biopsy might be avoided. For instance, if a patient has a hepatic mass that appears on imaging to be HCC, but the patient is a liver transplant candidate, some centers might avoid biopsy because of the small but real risk of tumor seeding. Other nodules might not be amenable to percutaneous biopsy if imaging localization is not clear, or a lesion is small. Biopsy of small lesions suffers from the risk of needle misplacement and difficulties in separating a well-differentiated HCC from benign liver.

### Specimen Adequacy

If possible, having a pathologist familiar with cytology present at the time of biopsy can be useful. This is particularly critical if a patient is undergoing CT-guided biopsy. The pathologist can rapidly stain and microscopically examine the fine-needle aspiration material to determine specimen ade-

quacy. If the biopsy is inadequate, additional biopsies can be immediately performed while the needle is still in position. Thus, patients are limited to a single procedure while maximizing diagnostic yield.

### Biopsy Size

The caliber of the needle and number of biopsies are critical in assessing the presence of cirrhosis. The diameter or gauge of the biopsy is often left to the physician performing the procedure. Histologic evaluation is enhanced with larger bore biopsies because the architectural features that define HCC are better represented. However, the diameter of a liver mass biopsy is probably not as critical as it is in the evaluation of medical liver disease. There is no minimum length of a liver mass biopsy, as long as the biopsy is representative of the mass. At least 1.0 cm is desirable for most tissue core biopsies.

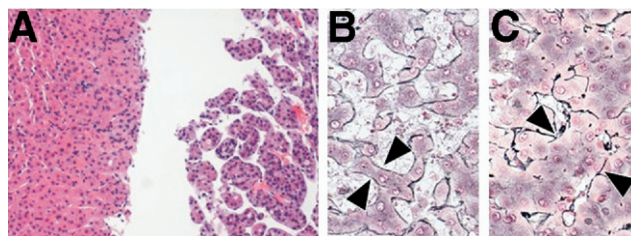
### Biopsy of Non-Lesional Tissue

At the time of guided biopsy, much attention is focused on the lesion. Success is often determined by images that capture the needle in the lesion of interest. However, attention should also be focused on the non-lesional liver. Needle biopsy of non-lesion liver tissue is strongly recommended. These biopsies should be placed into separate containers and clearly labeled. Comparing the lesional and non-lesional liver is tremendously helpful to the interpreting pathologist. In addition, knowledge of the degree of fibrosis can be vital in surgical planning and listing for possible liver transplant.

### Biopsy Handling

Care should be taken to avoid excessive fragmentation of needle core biopsies. Fragmentation of the needle biopsy can cause loss of diagnostic yield. Interpreting fragmented biopsies is usually not a problem for the pathologist; however, tiny tissue fragments are more difficult to process. Placing multiple tissue fragments into a flat plane that can be cut and put onto a glass slide is a challenge for histology technicians.

Once the biopsy is performed, the tissue should be quickly placed into an appropriate fixative-filled (typically buffered formalin) biopsy container. If possible, the biopsy specimen should be directly expelled into the container. Manipulation with forceps distorts the architecture and might rupture the malignant cells, which, paradoxically, are more delicate than the surrounding nonmalignant cells. Dried biopsy specimens can



**Figure 3.** (A) Biopsy of non-lesional liver (left) and HCC (right) from a patient with a liver mass. HCC shows an abnormal architecture of thickened hepatic cords, which is easily recognized when compared with the non-lesional liver tissue on the left. Reticulin stain highlights in black the edges (arrowheads) of the hepatic cords 1-cell wide in the normal liver (B) and several hepatocytes thick in HCC (C). Original magnification, 40 $\times$ .

result in complete loss of diagnostic information. Dried biopsy specimens cannot be salvaged in the histology lab. Tissue cores that are exposed to air or placed onto cotton gauze dry rapidly. If the biopsy specimen cannot be immediately placed into liquid, a saline-soaked pad could be used for transport (across the room) to the biopsy container. If a fixative-filled biopsy container is not available, a biopsy specimen can be temporarily placed into a saline-filled container. Biopsy specimens can also dry out during transport if the lid is not tightly sealed, or the core is stuck to the container side or lid.

### **Pathologic Evaluation**

Each pathologist has a basic set of stains in the evaluation of hepatic masses. The routine stain consists of hematoxylin-eosin and is the most important in formulating the diagnosis. Additional stains to demonstrate the structure of the liver and mass, such as trichrome and reticulin, are also very helpful. The trichrome stains collagen and aids in determining the amount of fibrosis or presence of cirrhosis. The reticulin stain highlights the hepatic sinusoids and hepatic plates that demonstrate the altered architecture seen in HCC (Figure 3B and C). Immunohistochemical stains directed at specific tumor types might also be necessary to determine whether a mass is a primary hepatic mass or a metastatic lesion. The immunohistochemical stain HepPar1 is useful in determining whether the tumor is a primary liver tumor because this stain is rather specific for hepatocytes. Immunohistochemical stains for cytokeratins such as CK7 and CK20 might help narrow down a differential diagnosis for a metastatic adenocarcinoma of unknown origin. For instance, tumor cells that stain with CK20 but not CK7 strongly support the diagnosis of metastatic colorectal adenocarcinoma because this pattern would be very unusual in HCC.

### **Pathology Report**

If it is possible to render a diagnosis of HCC, some additional comments in the report can benefit the patient. Poorly differentiated tumors or the presence of vascular invasion are likely poor prognostic factors and should be noted.<sup>14</sup> If the biopsy report indicates no malignancy is identified, there should be a comment whether the histologic findings can account for a hepatic mass, and if the specimen was adequate. If the histologic findings cannot account for a mass, additional studies/biopsies should be considered. Finally, the degree of fibrosis and inflammation in the non-lesional liver should be reported if an additional non-tumor biopsy was performed.

### **Recommendations: Hepatic Masses in Patients at Low Risk for Chronic Liver Disease**

Ultrasound can classify masses as solid simple or complex cysts. Simple and complex cysts are not typically biopsied. Biopsy aids in differentiation of solid lesions not well-characterized with imaging techniques. Biopsy of a hepatic mass and non-hepatic mass tissue greatly aids pathologic interpretation.

### **Recommendations: Hepatic Masses in Patients With Chronic Liver Disease**

The screening process for HCC in patients with chronic hepatitis is controversial, and guided needle biopsies should be performed if imaging cannot characterize a lesion.

Assessing specimen adequacy at the time of biopsy benefits the patient and increases diagnostic yield.

Biopsy of hepatic mass and non-hepatic mass tissue aids diagnosis, surgical planning, and listing for possible transplantation.

The pathology report should communicate whether the biopsy explains the radiologic impression of a hepatic mass.

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