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FINE NEEDLE ASPIRATION CYTOLOGY IN THE WORK-UP OF LIVER LESIONS

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GENERAL OVERVIEW [1-4]

Introduction

Any of a number of entities may present as focal liver lesions, including abscesses, cysts, benign liver nodules and primary and metastatic malignancies. The main focus is most often the diagnosis of hepatocellular carcinoma (HCC) and its differentiation from morphologically similar benign or malignant entities. Fine needle aspiration (FNA) is the procedure of choice at many institutions for the work-up of focal liver lesions. In the hands of an experienced radiologist and pathologist, the procedure is considered safe, efficacious, accurate and cost-effective.

Fine Needle Aspiration vs. Core biopsy

A misconception among some is that needle core biopsy (NCB) is preferable to FNA because it procures more tissue. However, most studies in the literature and personal experience indicate that both are complementary. One of the earliest studies in 1981 comparing FNA to NCB showed that NCB had a much a higher false negative rate. In a more recent study of 141 patients with abdominal lesions sampled with both core biopsy and FNA, FNA proved more sensitive than NCB at diagnosing malignancy (86.1% vs. 80.6%). In other studies evaluating FNA and NCB of abdominal organs, similar result shave been shown by other authors. All of these studies showed that FNA had sensitivity 2-24% times greater than that of NCB. The combination of FNA and NCB increased the overall sensitivity. A few studies have provided results contradicting these findings but none of these studies used on site immediate assessment of FNA samples by a cytologist, which has been shown to maximize diagnostic yield and accuracy.

An advantage of FNA is that smears made from the FNA provide a rapid means of evaluating the specimen on-site for adequacy, provisional diagnosis and triage for ancillary studies. At our institution, the radiologists prefer to do both FNA and NCB for this reason. If a NCB cannot be obtained, cell blocks made from needle rinsing and visible tissue fragments make a microhistology specimen that can provide sufficient material to evaluate for architectural features and to perform immunohistochemical studies.

Diagnostic accuracy

In institutions with considerable experience, the sensitivity of FNA ranges from 67-100%, averaging about 85%, with specificity approaching 100%. As with any other form of biopsy, sampling error can occur. Sampling error is most often because of inexact needle localization, sometimes reflecting the fact that the sought after lesion is less than 1 cm, or, in larger lesions, that there are large areas of necrosis, fibrosis, or a prominent inflammatory rim. For these reasons, the supplemental nature of fine needle aspiration and concomitant core biopsy improves accuracy, specificity, and sensitivity.

On-Site Cytological Evaluation

On-site cytological evaluation has been shown to improve the overall accuracy and to reduce the need for repeat procedures, with obvious benefit to the patient. If only a NCB is going to be obtained, then cytological evaluation of touch preparations of the cores can provide similar rapid assessments of specimen adequacy. An additional benefit of evaluating the specimen at the time of the procedure is that the cytologist can determine whether additional studies, such as flow cytometry or microbiology are needed, and triage the sample accordingly.

KEY DIAGNOSTIC ISSUES

I. Distinction of well-differentiated HCC from benign liver nodules and reactive hepatocytes
II. Distinction of poorly differentiated HCC from cholangiocarcinoma and metastatic malignancies
III. Determination of primary site of origin for metastatic adenocarcinomas
IV. Determination of histogenesis of poorly differentiated malignancies

APPROACH TO WORK UP OF FOCAL LIVER LESIONS

The work-up of focal liver lesions begins with an evaluation of clinical information. Important factors are age, gender, history of contraceptive use, and history with risk factors for liver disease, serum tumor markers, and history of previous carcinoma. Next, knowledge of the radiological findings is key. Some entities, such as focal nodular hyperplasia, have characteristic features, which are crucial in making the diagnosis. The next step is evaluation of the cytohistologic findings with the use of ancillary studies. The most accurate interpretations are rendered when all of the information, clinical, radiological and cytological are correlated.

Evaluation of FNA

Gross or naked eye evaluation

Much information can be obtained by naked eye examination of the smears[1, 5]. Benign liver will produce a microbiopsy pattern, with intact tissue fragments, theoretically because the intact reticulin prevents the tissue from breaking down. HCC will from a granular pattern, in which tissue fragments are distributed in rows and tend to be equidistant. This has been called the uniformly granular pattern. A non-uniformly granular pattern in which the tissue fragments are of variable size and shape is associated with both benign and malignant entities. A hypcellular aspirate is equally nonspecific. A fluid pattern, which is a smear with an amorphous look, is typically the result an abscess or cyst, but lymphomas may produce a similar pattern.

Cytological findings

Normal liver

Components of normal liver that may be found on aspirates include hepatocytes, bile duct cells, endothelial cells and Kupffer cells. The normal hepatocyte is a large polygonal cell with abundant granular cytoplasm, one or two round to oval, centrally placed nuclei with an even chromatin pattern and occasional prominent nucleoli. They may be present singly, in small clusters, or in larger flat sheets, which have an irregular jagged edge. Normal liver may also present with associated stripped benign, hepatocyte nuclei. Steatosis presents in cytology specimens as it does in histology specimens as either large or small clear vacuoles in the cytoplasm. Lipofucsin is a non-refractile, fine, pigment concentrated around the nucleus, which is golden black on Romanowski stains and golden brown on Papanicolaou. It stains with Fontana Masson, so caution is needed when using this stain to differentiate it from melanin pigment. Bile pigment is also non-refractile. It is golden-green on Papanicolaou and green black on Romanowski stains and is variable in appearance. It may be seen in canaliculi forming plugs or in the cytoplasm. Hemosiderin pigment is a refractile, coarse pigment seen on Papanicolaou stains as a golden brown and black on Romanowski stains. Normal bile duct epithelial cells are smaller than hepatocytes and may have a varied appearance. They are most often present as flat monolayered sheets of glandular epithelial cells. Other presentations include an on-edge picket-fence arrangement and small acinar structures. Endothelial cells and Kupffer cells are rarely appreciated in the normal aspirate and are only sporadically present in benign non-neoplastic and neoplastic entities.

Large and small cell change

Large cell change shows cells with cellular and nuclear enlargement, nuclear atypia, and normal nuclear to cytoplasmic ratio (N/C) or less than or equal to one-third. This change is not premalignant and may be seen in reactive processes. Small and monotonous cells characterize small cell change with subtle increase in the N/C, and nuclear crowding. These cells may originate from dysplastic foci or nodules and are therefore more significant when identified on an aspirate [1].

Benign Liver and liver Nodules

Benign processes or nodules that may be aspirated include cirrhosis, regenerative nodules, focal nodular hyperplasia and adenomas. All are characterized by the presence of benign hepatocytes and bile duct cells and other elements, except for adenomas, which lack bile duct cells. The hepatocytes in these processes may occur singly or as clusters with irregularly shaped jagged edges. Cirrhosis may produce clusters with smoother edges. Smears from a reactive process will show hepatocytes with pleomorphic atypia and large cell change punctuated among other more typically reactive appearing hepatocytes and an increased number of binucleated cells.

Hepatocellular carcinoma

The ease with which HCC is recognized on cytology smears depends on its grade of differentiation. [6]. Typically, moderately differentiated hepatocellular carcinomas are the easiest to recognize, since they retain their resemblance to hepatocytes while demonstrating more obvious features of malignancy. Well-differentiated hepatocellular carcinoma is difficult to differentiate from normal liver. Poorly differentiated hepatocellular carcinoma (PDHCC) has poor hepatic preservation and therefore is difficult to differentiate from other carcinoma types.

Many studies have evaluated cytological criteria of hepatocellular carcinoma to identify the most sensitive and specific [7-15]. These can be broken down into an evaluation of architectural features (cell group patterns and vascular patterns) [16, 17], background (presence or absence of bile duct epithelium, stripped atypical nuclei) and cytological features (N/C, nuclear features, macronucleoli)

Architectural patterns: Microscopic review of smears from an FNA of focal liver nodules begins with a low power assessment of the architectural patterns. The hepatocytes are arranged in one of three types of patterns:

-Complex branching trabeculae or trabecular arrangements, both with sharp, smooth borders due to peripheral endothelial cell wrapping

-Loosely cohesive groups with transgressing endothelium

-Dispersed single cells

The most specific pattern for the recognition of HCC is the presence of widened trabeculae surrounded by peripherally wrapping endothelium [8, 12, 17-19], and is one of the most important patterns for separating reactive non-neoplastic and benign neoplastic proliferations from WDHCC [17]. Benign reactive and neoplastic processes share the presence of benign appearing hepatocytes present in irregularly shaped jagged edge clusters without associated peripheral endothelium. Nodules of cirrhosis may produce smooth edged appearing clusters but there will not be any endothelial wrapping. Adrenal carcinoma uncommonly metastasizes to the liver, but is the other tumor that has a smear pattern in which peripherally wrapping endothelium may be seen around trabeculae of tumor cells, especially in cell block preparations.

The other pattern of endothelial proliferation has been termed transgressing, arborizing or central. This pattern is not as specific for HCC as peripheral endothelium but is highly associated with the presence of HCC, although it can occasionally be seen in cases of cirrhosis and hepatitis [17] and some metastatic malignancies. In fact, the transgressing endothelial pattern is the most common pattern seen in both primary and metastatic tumors, especially renal cell carcinoma [20], and therefore is not specific. It is, however, very useful for differentiating HCC from adenocarcinoma.

The pseudoacinar pattern of HCC mimics adenocarcinoma. Features to differentiate HCC from adenocarcinoma are the presence of bile in some of the lumens and the hepatocytic appearance of the epithelial cells. Sometimes this pattern is best appreciated on the cellblock. Peripheral endothelium occasionally found around these groups will also aid with the interpretation.

Variations include the microtrabecular and the microacinar patterns. Microtrabeculae show narrow trabeculae composed of one or two cells. Microacini will small acini composed of 5-6 cells. CD 34 will demonstrate endothelium around the groups that is not easily identified on routine stain [21]. This pattern is associated with the solid pattern of HCC on histology.

Dispersed single cells are the least specific feature, but these may be seen in association with some of the other patterns.

Background: HCC produces numerous stripped atypical nuclei. Their presence is very suggestive of HCC, but not specific, since other malignancies such as renal cell carcinoma produce a similar pattern.. Bile duct cells should be absent or scarce.

Cytological features: The individual cells may be smaller, larger, or the same size as normal hepatocytes. The cells of HCC are characterized by abundant, granular, cytoplasm with sharply defined cell

border and centrally placed nuclei. The cytoplasm will contain all of the normal pigments and inclusion seen in benign cells, except for iron.

A uniformly increased nuclear to cytoplasmic ratio is pivotal to diagnosing malignancy, particularly when other features are missing. The pleomorphic atypia of HCC has a monotonous appearance to it, termed "regularly irregular", in that the atypical hepatocytes appear to have the same degree of atypia. This is in contrast to reactive processes, which demonstrate a more varied degree of atypia, or "irregularly irregular" atypia. Binucleation is less frequent. Uniform, macroeosinophilic nucleoli are very helpful to separate benign from malignant hepatocytes. Other features include multiple, irregular nucleoli, and intranuclear inclusions. Tumor giant cells are seen with all grades of HCC. Mitotic activity increases with loss of differentiation.

Variants

Fibrolamellar hepatocellular carcinoma is characterized by abundant dense oxyphilic type cytoplasm and numerous intranuclear inclusions. Cells also typically have prominent nucleoli. Pale bodies may be seen smears. Due to the dense fibrous stromal component of this tumor, smears may be paucicellular and malignant hepatocytes may be individual and single, and widely scattered. Peripherally wrapping endothelium has not been a feature of this tumor, but transgressing endothelium has been observed.

Clear cell HCC is one in which the cytoplasm of the hepatocellular carcinoma is clear and vacuolated raising the differential diagnosis of metastatic clear cell tumors such as from the ovary or the kidney.A small cell variant is composed of cells with scant cytoplasm and a high N/C. Nucleoli will distinguish this from small cell carcinoma. Both clear cell and small cell variants will have more typical patterns of HCC on the smears.

Ancillary studies for the Diagnosis of WDHCC vs. nonneoplastic liver

Ancillary studies are relatively limited in the diagnosis of well-differentiated hepatocellular carcinoma. One of the most helpful includes the use of the reticulin stain [22]. The reticulin stain can be used on either smears or cellblock preparations. An abnormal reticulin staining pattern, usually the absence of reticulin staining, is highly associated with the presence of hepatocellular carcinoma. A pitfall in interpretation is that reticulin is decreased in steatosis.

Immunocytochemistry is of much more limited use. Alpha-fetoprotein (AFP) staining is helpful if it is positive, but a negative stain in no way rules out the presence of a tumor, since the sensitivity averages about 50% [1]. A positive test also does not prove it is HCC, since tumors with hepatoid differentiation will also be positive. Immunohistochemistry for CD 34[23] and Factor VIII [24] demonstrate the vascular pattern. However, these tests do not offer any significant advantage over reticulin stain.

Table 1: Criteria for the distinction of benign liver from well-differentiated HCC

Criteria	Benign	HCC
Gross Appearance	Microbiopsy	Granular
Architecture Of Groups	Loosely Cohesive Groups With A Jagged Edge	Groups With A Smooth, Rounded Border
Widened Trabeculae And Pseudoacini	Absent	Present
Peripheral Endothelium	Absent	Present
Atypia	Irregular	Regular
N/C	Retained	Increased
Binucleation	Frequent	Decreased To Absent
Reticulin	Normal	Variable Or Absent

Cholangiocarcinoma

Cholangiocarcinoma is an adenocarcinoma and therefore shows the cytomorphological features of adenocarcinoma. Low power shows glandular cells in flat, angulated sheets or in a drunken honeycomb pattern. The degree of atypia will depend on the degree of differentiation. The cells demonstrate standard cytological criteria of malignancy. CC typically cannot be readily distinguished from other adenocarcinomas metastatic to the liver. Features that are suggestive of primary CC include a range of atypia in the malignant cells, and dense stroma and dysplastic glands in corresponding microhistology samples.

Mixed HCC and Cholangiocarcinoma

This is a rare tumor containing unequivocal elements of HCC and CC with transitional cells. This diagnosis may be difficult to make on cytology alone and will probably require ancillary studies, which shows cells with specific features of HCC or CC, or hybrid cells, with equivocal immunohistochemical profiles.

Adenocarcinoma vs. Hepatocellular carcinoma

Morphologic features that distinguish HCC from adenocarcinoma include cells with characteristic abundant, granular cytoplasm; atypical stripped, nuclei (often with prominent macronucleoli), trabeculae and the peripheral endothelial wrapping vascular patterns. HCC may be misclassified as adenocarcinoma when the smears do not demonstrate peripheral endothelial wrapping vascular pattern or trabeculae [25]. Atypical stripped nuclei are a very characteristic feature of HCC, and when present, should lead the observer to consider this over adenocarcinoma. Adenocarcinoma may be mistaken for HCC when the cytoplasm is focally granular, it shows a few stripped nuclei, or has a focal trabecular or peripheral endothelial-wrapping pattern [26].

Confirmation of the diagnosis of HCC using special stains and immunohistochemistry is recommended. Bile pigment is pathognomonic for hepatocellular differentiation. A Hall's stain can be used to confirm the nature of the pigment as bile. Mucin positive vacuoles will establish the carcinoma as an adenocarcinoma.

Markers that may be included for this distinction include high and low molecular weight cytokeratins, AFP, HepPar, pCEA, MOC 31, CD 10 and CK 19. Adenocarcinoma will express both high and low molecular weight cytokeratin, whereas the cytokeratin expression of hepatocellular carcinoma is usually limited to low molecular weight cytokeratin [2]. More specifically, hepatocytes and hepatocellular carcinoma do not express cytokeratins 1,5,10,11, and 19. Immunohistochemical evaluation for CK 19 is particularly useful, since adenocarcinomas but not hepatocellular carcinomas express this antigen [27]. Hepatocellular carcinoma has a specific expression pattern for pCEA and CD 10 in which they are expressed in the bile canaliculi [1]. Adenocarcinomas show a cytoplasmic expression pattern for CEA. MOC 31 is reported to be very sensitive for the diagnosis of adenocarcinoma [28, 29]. HepPar antigen is limited by occasional expression in other tumors and decreased expression in poorly differentiated HCC [1].

Metastatic adenocarcinoma vs. Cholangiocarcinoma

The majority of malignancies in the liver are metastases. In most of these cases, the patients have a known history of a primary carcinoma. This history is of vital importance in evaluating the morphology and in determining whether it is compatible with that primary site. Adenocarcinomas are the most common metastatic tumors to the liver. Colon carcinoma is by far the most frequent, followed by pancreas, lung and breast. The problem is differentiating these from Cholangiocarcinoma.

A problem is that most adenocarcinomas do not have specific morphologic features. One exception is colorectal carcinoma that has a characteristic smear pattern showing tall, columnar malignant cells with a dirty, necrotic background on cytology smears. Breast cancers occasionally show characteristic features. Ductal adenocarcinomas often appear as a low grade, monomorphic population on smears. The groups are flat and angulated. A characteristic feature is cells with a targetoid cytoplasmic lumen. Lobular carcinoma forms a dyshesive cell population composed of small cells with eccentric nuclei. Other adenocarcinomas do not have specific features, therefore, immunohistochemistry is key.

The immunohistochemical approach to the work-up of adenocarcinoma of unknown primary has been recently reviewed [4]., The primary question in the liver is whether it is primary cholangiocarcinoma or metastatic adenocarcinoma. The diagnosis of cholangiocarcinoma remains one of exclusion, because its phenotype overlaps with that of many other carcinomas. The most relevant phenotype to the discussion of metastatic adenocarcinoma to the liver is the CK 7-/CK 20 + phenotype because it is highly characteristic of colorectal primary. The predictive probability of this immunophenotype is 78%.

Other studies can help to further refine the differential diagnosis. Cytokeratin 17 is associated with ampullary and pancreatobiliary carcinomas more often that gastric or esophageal carcinomas. Additional markers help to further refine the identification such as TTF1 (nonmucinous pulmonary adenocarcinomas) estrogen receptor protein staining (breast, ovarian, endometrial), GCDFP15 (breast) WT1 (ovarian serous tumors), PSA and PAP (prostate), thrombomodulin and uroplakin (urothelial carcinoma). CDX 2 is used as markers of intestinal differentiation, and can help to differentiate colorectal, intestinal or gastric neoplasms from pancreatobiliary, biliary, ovarian, or pulmonary adenocarcinomas.

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