Primary liver cancer (including hepatocellular carcinoma and tumors of the intrahepatic biliary tract) represents the fifth most common form of neoplastic disease world-wide (accounting for >5% of all human cancers) and the third most common cause of cancer-related death (1-4). The majority of primary liver tumors are hepatocellular carcinomas. The world regions of highest incidence of hepatocellular carcinoma include Eastern Asia and sub-Saharan Africa (3, 4). In contrast, North and South America, Northern Europe, and Australia have very low incidence of hepatocellular carcinoma (3, 4). The world-wide variation in incidence of hepatocellular carcinoma largely reflects variations in risk factors for the resident populations, particularly exposure to hepatitis viruses (5, 6). Other risk factors associated with the development of hepatocellular carcinoma include gender, dietary factors (including alcohol consumption and exposure to Aflatoxin B1), other environmental exposures, and various genetic (metabolic) diseases (6, 7). All of these risk factors contribute to chronic liver disease, which may be the most important determinant of liver cancer risk. Numerous recent studies have documented changes in the world-wide incidence of hepatocellular carcinoma, including increasing numbers of cases in historically low prevalence areas. These studies suggest that hepatocellular carcinoma will emerge as a major form of malignant cancer in the United States in the coming decades if current trends persist (8-11). The dramatic recent increase in hepatocellular carcinoma in the United States is attributed primarily to HCV infection, and less so to HBV infection (9). The continuing high incidence of hepatocellular carcinoma in Eastern Asia and sub-Saharan Africa, combined with the increasing incidence of this disease in other parts of the world, suggests that this disease will continue to represent a global health problem into the future. Thus, additional investigation of hepatocellular carcinoma is required to elucidate the molecular and cellular basis of the disease, and to uncover opportunities for development of new strategies for prevention, diagnosis, and treatment.

The adult mammalian liver is proliferatively quiescent under normal conditions, with minimal rates of cellular division and apoptosis (12). However, the regenerative capability of the mammalian liver is well known (13-15). The extensive proliferative capacity of the mature (fully differentiated) hepatocyte is clearly demonstrated following surgical partial hepatectomy (16, 17), and it has been suggested that the hepatocyte be unrestricted in its ability to give rise to new cells (18). Various forms of liver injury and certain disease states are characterized by reactivation of cellular proliferation. Important among these from the perspective of risk for development of hepatocellular carcinoma are chronic hepatitis and cirrhosis. In both chronic hepatitis (either viral or alcohol-related) and cirrhosis there are significant levels of hepatocyte cell death (necrotic injury) and cellular proliferation (regeneration). The majority of human hepatocellular carcinomas occur in the setting of the cirrhotic liver (19, 20). Since cirrhosis is a
Unusual Liver tumors including pediatric liver tumors
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The more usual of the unusual liver tumors include pediatric liver tumors (hepatoblastoma, mesenchymal hamartoma, infantile hemangioendothelioma, embryonal sarcoma and rhabdomyosarcoma), angiomyolipoma, inflammatory myofibroblastic pseudotumor, epithelioid hemangioendothelioma and angiosarcoma: these are the subjects of this brief summary. Other tumors that occur commonly in other body systems may occur rarely in the liver, and are easily diagnosed by knowledge of their pathology in other sites.

Pediatric liver tumors
Tumors and pseudotumors of the liver comprise < 2% of tumors in children; approximately a third of these are benign and vascular in nature. Malignant liver tumors make up 1% of pediatric malignancies; the majority of these are hepatoblastomas and hepatocellular carcinomas. Most liver tumors in children under 5 years of age are comprised of hepatoblastoma, infantile hemangioendothelioma and mesenchymal hamartoma, while hepatocellular carcinoma, focal nodular hyperplasia and embryonal sarcoma occur in older children.

Hepatoblastoma
Hepatoblastoma is the third most common intra-abdominal malignancy in children following neuroblastoma and Wilm’s tumor. The rarity of this tumor has led to the formation of multi-institutional study groups in an effort to obtain meaningful data regarding biology and prognosis, and pursue advances in its treatment. Such co-operative groups include the Children’s Cancer Group (CCG), Pediatric Oncology Group (POG) and Children’s Oncology Group (COG) in the US, SIOPEL (Société Internationale D’Oncologie Pediatrique) in France and the German Pediatric Liver Tumor Study group.

Incidence and presentation
The incidence of hepatoblastoma has increased over the past two to three decades, paralleling the increased survival of low birth weight infants. In an analysis of the SEER (Surveillance, Epidemiology and End Results) database, the incidence of hepatoblastoma in 1993-1997 doubled over that in 1973-1977 from 0.6 to 1.2 per 1,000,000. The strong association of hepatoblastoma with prematurity and birth weight was first reported in a study from Japan, which found that hepatoblastoma accounted for 58% of all malignancies in children with birth weights < 1000g. The relative risk of hepatoblastoma in children with birth weights <1000g is 15.64 vs 2.53 for infants with birth weights of 1000 -1500g and 1.21 for those with birth weights of 2000 -2499 g. The underlying etiopathogenesis is not clear; therapeutic agents used to maintain low birth weight infants possibly play a role. Two-thirds of tumors occur before 2 years of age, and 90% before age 5. A significant number of tumors are congenital. There is a strong association with Beckwith-Wiedemann syndrome (BWS), hemihypertrophy and familial adenomatous polyposis.
Patients present with abdominal distension, abdominal pain, gastrointestinal symptoms, and failure to thrive. Paraneoplastic syndromes like virilization are not uncommon.

**Pathology and prognostic subtypes**

Macroscopically, hepatoblastoma consists of a large mass with a variegated cut surface that may show extensive areas of necrosis and hemorrhage. Tumors that are resected following chemotherapy are usually firm to hard depending due to presence of fibrosis and osteoid.

Microscopically, the tumor may be composed entirely of epithelial elements or an admixture of epithelial and mesenchymal elements. Epithelial hepatoblastoma is composed of varying proportion of cells that resemble early embryonal hepatocytes, fetal hepatocytes or small undifferentiated cells. Some tumors show macrotrabecular areas where the cells are arranged as 5-20 cells thick trabecula. These observations form the basis of the current classification of hepatoblastoma:

**Epithelial:**
- Fetal (pure fetal)
  - Well-differentiated (<2 mitoses per 10 HPF)
  - Mitotically active (>2 mitoses per 10 HPF)
- Embryonal, or mixed embryonal and fetal
- Small cell undifferentiated (SCUD, formerly anaplastic)
- Macrotrabecular

**Mixed epithelial and mesenchymal type:**
- Without teratoid features
- With teratoid features

The presence of the *small undifferentiated phenotype* (SCUD) (previously called “anaplastic” type), even in small amounts, confers a bad prognosis in the form of increased risk of recurrence, which may be resistant to chemotherapy and decrease in disease-free survival. The small undifferentiated cell hepatoblastoma is a “small blue cell tumor” consisting of a monomorphous population of small round to ovoid cells with a high nucleo-cytoplasmic ratio and scant cytoplasm. Thus, all tumors should be thoroughly evaluated for the presence of small cells and a minimum of 1 section per cm of largest tumor dimension should be examined.

A second unfavorable variant of hepatoblastoma is the *macrotrabecular* variant, although this has not been borne out in all studies. “Macrotrabecular” describes a growth pattern of cells in trabecula that are 5 to 20 cells thick. The nature of the cells making up this trabecula however is a source of confusion: some authors refer to this subtype when fetal or embryonal cells are arranged in macrotrabecula, whereas others refer to this pattern only when the macrotrabecula are comprised of adult hepatocytes with an appearance not dissimilar to hepatocellular carcinoma. The original reports ascribing bad prognosis to the macrotrabecular pattern referred to the second situation. However, in view of the confusion and the rarity of this phenotype, there is an attempt to further study this group
from the SIOPEL-3 database by breaking it down into MT-1 composed of adult hepatocyte/ HCC like cells and MT-2 composed of embryonal and/or fetal cells. When made up of adult-type hepatocytes, this pattern may be indistinguishable from hepatocellular carcinoma, and is recognized as a macrotrabecular subtype of hepatoblastoma due to the presence of more recognizable elements of this tumor.

Favorable prognosis is conferred by the well-differentiated pure fetal hepatoblastoma, which is strictly defined as a completely resectable tumor of pure fetal phenotype with < 2 mitoses per 10 high power fields. The favorable prognosis of this subtype is restricted to stage I tumors.

A host of unusual histological patterns are encountered by pathologists at reference centers for the study groups, and are the basis of a recently proposed classification (Zimmermann 2005). These tumors are too rare for the scope of this summary. For practical purposes, when encountered, all morphologically rare subtypes are best forwarded to a reference center.

It has been postulated that the various morphological subtypes of hepatoblastoma reflect various stages of hepatic development. Thus epithelial-mesenchymal hepatoblastoma may reflect the stage of epithelial-mesenchymal transition during hepatic development. The various epithelial components may represent various stages of hepatocyte development. The small cell component has been postulated to represent a tumor of hepatic stem cells but stem cell markers are not consistently demonstrable.

**Genetic and molecular findings**

The most frequently encountered chromosomal abnormalities are triploidy of chromosomes 2 and 20; chromosomal gains of 20 and 8q have been reported to adversely affect prognosis.

The most frequent findings in about 90% of hepatoblastomas are alterations in the β-catenin signaling pathway. β-catenin has two roles: in the nucleus, it acts as a transcription co-factor, which along with TCF/LEF (T cell factor/ lymphoid enhancer factor) leads to transcription of genes like c-myc and cyclin D1. This function is positively regulated through the Wnt signaling pathway, which is activated by numerous growth factors and inhibited by other proteins, an example of the latter is Dickkopf-1. Normally, signaling or cytoplasmic β-catenin is kept in check by cytoplasmic sequestration in the APC-Axin- GSK3β complex where it is targeted for proteosomal degradation. Wnt signaling inhibits degradation of β-catenin, stabilizing it and allowing it to exert its nuclear effects. Stabilized β-catenin also inhibits TNF-α induced apoptosis. The second role of β-catenin is at the cell membrane where it is involved in intercellular adhesion by acting as an adaptor protein linking β-cadherin to the actin cytoskeleton of the cell. Thus the protein is normally expressed on cell membranes. Since β-catenin is central to both the Wnt and cadherin pathways, it is affected by changes in either of these pathways, or by changes in the various proteins involved in its sequestration and degradation. Any mutation or molecular change that leads to increased Wnt signaling, decreased degradation, increased stabilization or decrease in membrane function of β-
Catenin leads to increased availability of the protein for nuclear signaling. Mutations of β-catenin are associated with the SCUD phenotype, which imparts a negative prognosis even when present focally in a tumor. It has been observed that foci of SCUD express β-catenin in a nuclear pattern, whereas surrounding more differentiated cells do not show this nuclear localization. Nuclear localization of β-catenin is also found in the invasive front of tumors as opposed to more central portions. These findings suggest that presence of nuclear β-catenin is an indicator of an aggressive clone of cells.

**Imprinting errors** in the 11p15 gene cluster are linked to the Beckwith-Wiedemann Syndrome (BWS). This gene cluster has two domains: Domain 1 has a telomeric location and contains genes for IGFl (insulin growth factor 2) and H19 (a putative tumor suppressor). Domain 2 has a centromeric location and contains three genes that have been implicated in BWS: KCNQ1, KCNQ1OT1 and CDKN1C, the latter encodes for p57/KIP2 (negative regulator of cell proliferation). The reported abnormalities include uniparental disomy (UPD) with two paternal copies of 11p15, translocations, inversions and loss or gain of methylation.

Analysis of gene expression in hepatoblastoma showed differential expression of genes that were distinct from those seen in hepatocellular carcinoma. These genes included IGFl (insulin growth factor 2), fibronectin, DLK1 (delta-like 1 homolog: inactivates a gene associated with growth arrest), TGFβ1 (a growth factor associated with many human tumors), MALAT1 (metastasis associated lung adenocarcinoma transcript 1) and MIG6 (mitogen inducible gene 6: unexpected finding as it seems to inhibit the effects of both EGF and HGF). A separate study showed higher expression of **PLK1** oncogene in hepatoblastoma and correlated its higher expression with poor outcome: 5-year survival rate of 55.9% for high expression vs 87.0% for low expression (p=0.042). PLK1 is involved in various stages of mitotic progression including centrosome maturation, spindle function, activation of cyclin B/Cdc2 and regulation of anaphase-promoting complex.

**Prognosis and treatment**

The prognosis of hepatoblastoma has improved dramatically over the last three decades since the advent of cisplatin-based chemotherapy. The single most important prognostic determinant of hepatoblastoma is complete resectability. However, most children present with large or multifocal tumors that cannot be safely resected either due to their size or proximity to and/or involvement of major vascular structures. Preoperative chemotherapy causes the tumor to shrink, allowing successful and complete resection. The success of chemotherapy is complemented by progress in knowledge and techniques of imaging, anesthesia and surgery.

Clinical stage obviously imparts prognostic significance. Various staging systems have been employed by various study groups. To facilitate comparison, all groups use the **PRETEXT (pretreatment extent of disease)** system formulated by SIOPEN, which has been shown to have high correlation with overall survival and event-free survival. The PRETEXT system is also useful in assessing the efficacy of neoadjuvant chemotherapy and predicting surgical resectability. The system defines four stages depending upon the
number of sectors involved by the tumor. Extrahepatic growth is indicated by alphabetical modifiers, the VPEM system: V for involvement of hepatic or caval veins, P for involvement of portal vein, E for presence of extra-hepatic tumor and M for distant metastases.

Post-operative staging systems include the TNM staging system of the International Union against Cancer, or a simplified staging system adopted by COG:
Stage 1: complete resection of tumor
Stage 2: microscopic residual tumor
Stage 3: unresectable tumor (or macroscopic residual tumor); lymph node metastases
Stage 4: metastatic disease

As mentioned above, histological patterns with distinct prognostic implications are SCUD, macrotrabecular and well-differentiated pure fetal hepatoblastoma. The emergence of these histological prognostic patterns has led to some controversy on the role of presurgical chemotherapy for every hepatoblastoma, even when it is completely resectable. The argument against chemotherapy in completely resectable tumors is dictated by two situations: first, surgical resection is curative in the well-differentiated pure fetal phenotype when the defining criteria are strictly adhered to; harmful adjuvant therapy is therefore unnecessary in these patients. Secondly, foci of small cell undifferentiated hepatoblastoma may be ablated and their prognostic implication missed by presurgical chemotherapy.

With the ongoing revolution in molecular and genetic research, we can look forward to further definition of prognostic subtypes based on the presence or absence of genetic and molecular markers. Nuclear localization of β-catenin and presence of PLK1 have already been shown to have negative prognostic impact. In fact, the emphasis of current is to define histological, molecular and genetic factors that will allow precise tailoring of therapy to individual cases.

**Role of transplantation**
Liver transplantation offers a viable therapeutic option for patients with unresectable disease limited to the liver after chemotherapy; this includes tumors invading all four sectors or tumors in close proximity to major vascular structures. In such cases, liver transplantation offers 80% long-term disease-free survival compared to 30% long-term survival following rescue transplantation for incomplete resection. The persistence of viable extrahepatic deposits after chemotherapy, not amenable to surgical resection, is considered to be the only absolute contraindication to liver transplantation. The 1-, 5- and 10-year patient survival of 135 pediatric patients with hepatoblastoma in the UNOS database from 1987-2004 was 79%, 69% and 66% respectively. The primary cause of death was metastatic or recurrent disease, accounting for 54% of deaths. The main parameters that correlated with graft and patient survival on univariate and multivariate analysis were pretransplant medical condition and transplant era.

**Mesenchymal hamartoma**
Mesenchymal hamartoma is a tumor of infancy, the average patient being 15 months of age. The tumor may not uncommonly be present prenatally and is more common in male
children. Patients present with an enlarging abdomen. Imaging studies show a well-delineated heterogeneous cystic mass in the liver. Macroscopically, the tumor is well-circumscribed, large, smooth and fluctuant. Sectioning reveals a soft myxoid, cystic surface that exudes thin fluid or soft gelatinous material. Microscopically, the main component is loose, edematous connective tissue containing acid mucopolysaccharides and bile ducts, vessels and liver cell nodules. Microscopically, the lesion extends out into the surrounding liver belying the macroscopic demarcation. Surgical resection is curative. Rare instances of malignant transformation are present in literature. Controversy exists over whether this lesion represents a developmental anomaly or a true neoplastic process. Proponents of the former idea believe the lesion arises as a result of ductal plate malformation. However, the demonstration of a translocation consistently involving 19q13.4 in a handful of cases seems to argue to the contrary.

**Infantile hemangioendothelioma**

Infantile hemangioendothelioma is the most common mesenchymal tumor in infancy, occurring in children younger than 6 months of age. This tumor is more common in female children. Patients present with abdominal distention, hepatomegaly and failure to thrive; high output cardiac failure due to AV shunting and Kassabach-Merritt syndrome with consumptive coagulopathy and thrombocytopenia has been reported. The tumor tends to be multifocal or diffuse. The cut surface is red-brown and spongy and may show areas of scarring. Microscopically, the tumor consists of numerous interconnecting vascular channels lined by a single layer of endothelial cells. Bile ducts are seen throughout the tumor. Thrombosis, scarring and extramedullary hematopoeisis are common. The tumor tends to show microscopic extension into the surrounding hepatic parenchyma. These tumors have been classified into Type I and Type II, distinguished by atypia, papillary projections, multilayering, mitoses or solid areas in the latter. These tumors may involute after several months but the cardiac function needs to be supported. Surgical resection is curative, Type II is more likely to recur and there are anecdotal reports of transformation to a more malignant phenotype.

**Embryonal sarcoma**

Embryonal sarcoma occurs in children between 6 and 10 years of age with an approximately equal gender distribution. The tumor presents with abdominal distension, abdominal mass and weight loss. The tumor appears solid on ultrasound, but may show a misleading cystic appearance on CT and MRI, leading to misguided attempts at aspiration or delay in treatment. Grossly, the tumor has a variegated, soft cut surface that may be cystic or solid with areas of necrosis and hemorrhage. Microscopically, the tumor is an undifferentiated sarcoma consisting of stellate, spindle or pleomorphic cells. The cells are either arranged loosely in a myxoid stroma or as more compact fascicles and bundles of spindle cells. Abundant matrix of acid mucopolysaccharides, extramedullary hematopoeisis, PAS positive diastase resistant globules and benign bile ducts are prominently present in most tumors. p53 mutations have been reported in these tumors and may represent the major carcinogenic pathway. This is a very aggressive tumor with poor prognosis, although the Soft Tissue Sarcoma Italian and German Co-operative groups have recently shown almost 60% 5-year survival following multimodal therapy.
Rhabdomyosarcoma (sarcoma botyroides)
This tumor occurs in children under 5 years of age and has also been reported in adults. Patients present with obstructive jaundice, fever and weight loss. This tumor arises in large hilar bile ducts, and like sarcoma botyroides in other parts of the body, appears as soft intraluminal grape-like masses. The tumor is covered by biliary epithelium and has an abundant myxoid stroma. There is a submucosal cambium layer that contains dark, round to oval tumor cells. Immunocytochemistry and electron microscopy show evidence of rhabdomyoblastic differentiation: actin, myosin, myoglobin, thick and thin myofilaments and Z bands. The treatment and prognosis parallels that of embryonal sarcoma.

Epithelioid hemangioendothelioma
Epithelioid hemangioendothelioma occurs over a wide age range, but the average patient is a woman in her fifties. Patients present with an abdominal mass, pain or nonspecific symptoms such as weight loss and malaise. Some patients may present with features of veno-occlusive disease or Budd-Chiari syndrome due to vascular occlusion. Imaging shows either a single mass, but more commonly, multifocal masses that are avascular and calcified. Grossly, the tumors are white, firm with central calcification and/or ossification. The microscopic appearance is distinctive with a centrally fibrotic, calcified or ossified tumor. Tumor cells are more abundant at the periphery and consist of epithelioid cells, many of which have a signet ring appearance with an intracytoplasmic lumen; pathognomic cells contain red blood cells within these lumina. The tumor infiltrates the liver along the sinusoids, using them as scaffolding. Typically, adjacent portal tracts show invasion of vascular structures by papillary or glomeruloid tufts of malignant cells. The tumor demonstrates evidence of endothelial differentiation by immunohistochemistry and electron microscopy. Epithelioid hemangioendothelioma is a low-grade malignancy with 5-year survival of almost 50%. Complete surgical resection is the treatment of choice. In patients with multifocal disease, liver transplantation offers long-term disease-free survival. Metastases occur in 30-45% of cases but are also conducive to long-term survival if the lesions are resectable. There are anecdotal instances of recurrence of a more malignant, angiosarcoma-like tumor in the transplanted liver. A translocation, t(1;3)(p36.3;q25) has been described in 2 cases.

Angiosarcoma
A rare tumor, angiosarcoma is nonetheless the most common sarcoma arising in the liver. The peak incidence is in the 6th and 7th decades of life, with a M:F ratio of 3:1. Although the etiology of angiosarcoma is unknown in the majority of cases, the most common associations are exposure to vinyl chloride monomer, arsenic, pesticides and thorium dioxide; the last mentioned was a radioactive contrast medium used from the 1920s to the 1950s. The tumor presents with abdominal enlargement, pain, hepatomegaly, jaundice, hemorrhage and portal hypertension. Grossly, the liver shows multifocal or diffuse spongy, hemorrhagic, ill-defined lesions. The tumor infiltrates extensively along the hepatic sinusoids causing gradual atrophy and effacement of the liver cell plates. The tumor cells may be spindle-shaped or epithelioid, and are pleomorphic with malignant nuclear features. Diagnosis is made by immunohistochemical or electron microscopic demonstration of endothelial differentiation. This is a highly aggressive tumor with no
genuinely effective therapy. Metastases occur frequently and rapidly to the spleen, lymph nodes, lungs, bone and adrenals.

**Angiomyolipoma**
Angiomyolipoma in the liver resembles its counterpart in the kidney. When present in the context of tuberous sclerosis, liver tumors are always associated with renal tumors. Patients are usually middle-aged females. The tumors are usually solitary and contain a variable amount of fat, vessels and smooth muscle cells. Diagnostic confusion arises when the fat cell component is inconspicuous and the smooth muscle component assumes an unusual morphologic phenotype; smooth muscle cells may be epithelioid, clear cell, oncocytic, or spindle cell in nature. The pathognomic feature is positivity for HMB-45 along with positivity for muscle markers; the expression of melanocytic and muscle markers places this tumor in the category of PEComas, or tumors of perivascular epithelioid cells. CD117 is also positive in angiomyolipomas. These tumors are benign and surgical resection is curative.

**Inflammatory (myofibroblastic) tumor**
This lesion is characterized by the presence of infection-like symptoms that include fever, abdominal pain, vomiting, diarrhea and jaundice. Laboratory work-up may show leucocytosis, eosinophilia, high ESR and hyperglobulinemia. A history of travel or gallstones may be elicited. Most patients are young and there is a male predominance. Imaging may show a single mass or multiple lesions, the latter may arouse suspicion of malignancy. The lesion can assume massive size. Microscopically, there are chronic inflammatory cells that include numerous polyclonal plasma cells, lymphocytes and eosinophils. A spindle cell component that shows evidence of fibroblastic or myofibroblastic differentiation is present in variable proportion. No microorganisms are found in the lesion. The most frequent association is with an autoimmune pancreatitis with or without sclerosing cholangitis; rare cases have been reported with Crohn’s disease and primary biliary cirrhosis. The lesion may regress spontaneously or reduce in size over a relatively short time of observation along with the clinical symptoms; thus surgery is not uniformly recommended for these lesions.

**References**

**Pediatric tumors**


**Hepatoblastoma**


\textbf{Mesenchymal hamartoma}


\textbf{Infantile hemangioendothelioma}

\textbf{Embryonal sarcoma}


\textbf{Epithelioid hemangioendothelioma}


**Angiosarcoma**


**Angiomyolipoma**
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INTRODUCTION

Primary liver cancer may derive from hepatocytes or bile duct cells. Recently it has been suggested the progenitor cell compartment is also a target cell population for carcinogenesis (1). Hepatic progenitor cells (HPC) are located in the most peripheral branches of the biliary tree, i.e., the ductules and canals of Hering. Biliary epithelium is normally CK7 and CK19 positive. HPC are also immunoreactive to CK7, CK19, oval cell markers OV6 and OV1, neural cell adhesion molecules (NCAM), chromogranin-A, etc. Bile ductules, irrespective of being derived from HPC or from metaplasia of the hepatocytes, express cytokeratins of biliary pattern including CK7, CK8, CK18, and CK19, whereas mature hepatocytes express CK8, CK18, Hepar-1 and albumin (2,3).

The term “ductular reaction” was first coined by Popper et al. in 1957 (4) and is now the preferred term to describe “a reaction of ductular phenomenon, possibly but not necessarily of ductular origin” by the consensus opinions from a group of expert liver pathologists (5). In normal liver, ductules are barely visible. Ductular reaction is characterized by increased ductules in and around the periphery of the portal tracts with accompanying inflammatory cells and stromal changes, which can be seen in various liver diseases, including biliary obstruction, chronic cholestasis, submassive liver necrosis with regeneration, focal nodular hyperplasia, etc. Ductular reaction is an important phenomenon that careful consideration is needed to arrive at a diagnosis (6).

BENIGN TUMORS OR TUMOR-LIKE LESIONS

Benign cysts exist in the liver, including solitary bile duct cysts, ciliated hepatic foregut cysts, and multiple intrahepatic cysts as in polycystic disease. Bile duct cysts are unilocular and lined by a single layer of columnar or cuboidal epithelium that are immunoreactive to CK8, CK18, CK7, and CK19, reflecting their cell lineages (7). Ciliated foregut cysts are usually unilocular and in part lined by ciliated pseudostratified columnar epithelium, although in part may be lined by cuboidal or even squamous epithelium. Bundles of smooth muscle are usually present, surrounding the cysts. They are thought to derive from the embryonic foregut and to differentiate towards bronchial structures in the liver. The multiple cysts in polycystic disease are lined by cuboidal, columnar, or flat epithelium. Most solitary bile duct cysts and ciliated foregut cysts are small and asymptomatic, whereas polycystic liver are most likely symptomatic (2,8).

Bile duct hamartoma (aka. von Meyenburg complex or biliary microhamartoma) is usually small (up to several mm in diameter), multiple, and located adjacent to a portal region (9). It arises as a result of developmental anomaly and is often associated with polycystic liver or kidney disease. Histologically it is composed of a variable number of
ductal structures embedded in a hyalinized stroma (2). The ductal structures are variably dilated and may have microcystic dilatation with bile in the ductal lumens. The ductal lumens are lined by a flattened or cuboidal epithelium. Bile duct hamartoma has been associated with neoplastic transformation in reported cases (10). Peribiliary gland hamartoma (bile duct adenoma) is a different entity but it can be at times challenging to discern peribiliary gland hamartoma from bile duct hamartoma. Peribiliary glands are small accessory glands of the major bile ducts in the liver. Grossly peribiliary gland hamartoma is round or ovoid, well-demarcated, but not encapsulated. The lesion is usually single, in contrast to the multiplicity of bile duct hamartoma. Histologically peribiliary gland hamartoma is composed of tubules and acini, which are lined by a single layer of cuboidal to columnar cells. They do not show cystic changes or contain bile, but intracytoplasmic mucin may be highlighted by special stains such as the Alcian blue stain. The lining epithelial cells of both bile duct hamartoma and adenoma are immunoreactive to CK7, CK8, CK18, and CK19 (11).

Hepatobiliary cystadenoma is a solitary and multilocular cystic neoplasm that predominantly arises within the liver but may arise in the extrahepatic biliary tree including the gallbladder (8). It has striking similarities to mucinous cystic neoplasm of the pancreas and both are almost exclusively a tumor of women. Microscopically it is almost always mucinous and the locules are lined by a columnar epithelium that focally may become cuboidal, flattened or even papillary. The epithelial cells resemble biliary epithelium with cytoplasmic mucin which can be highlighted by alcian blue or mucicarmine. Intestinal metaplasia with goblet cells can be present. The typical densely cellular “ovarian-like” stroma is a feature that hepatobiliary cystadenoma shares with its counterpart in the pancreas (12,13). The ovarian-like stroma is exclusively present in women and has been shown to be immunoreactive to estrogen and progesterone receptors and inhibin (14). The epithelial cells of hepatobiliary cystadenoma and cystadenocarcinoma are immunoreactive to CK8, CK18, CK7, and CK19, a profile consistent with their respective cell lineages (7). Varying degrees of dysplasia in the epithelium may be seen in that it can develop into invasive cystadenocarcinoma (12).

Biliary papillomatosis, or the so called intraductal papillary neoplasms of the biliary tract are rare lesions that may be solitary or may spread extensively along the biliary tree within or outside of the liver including the gallbladder. Similar to intraductal papillary mucinous neoplasm of the pancreas, the involved bile ducts are dilated, containing papillary growth of columnar epithelial cells overlying fibrovascular stalks. The neoplastic epithelial cells may mimic biliary epithelium or show gastric or intestinal metaplasia. Mucin production may be present. The epithelial cells may show varying degrees of dysplasia and may progress to invasive carcinoma (8).

Focal nodular hyperplasia (FNH) is a tumor-like lesion characterized by hyperplastic nodules of hepatocytes, surrounded by fibrous septa radiating from a central stellate scar. Thick-walled arteries are present in the scar and septa, and bile ductules occur at the interface of parenchyma and septa. Hepatocytes near the fibrous septa commonly show chronic cholestasis with pseudoxanthomatous change (cholate stasis), which can be readily demonstrated by a copper stain. FNH is most common in adult women, although
it can occur in both sexes and any ages. While not fully understood, the notion that abnormal vascularization is the major cause for FNH has been gradually recognized (2,15) and recent studies have suggested the ductular reaction in FNH may result in part from activation of the hepatic progenitor cells (16).

MALIGNANT TUMORS

Although its incidence is far less common than hepatocellular carcinoma (intrahepatic cholangiocarcinoma accounts 10-20% of all primary liver malignancies), cholangiocarcinoma (CC) is gaining increasing recognition especially as the incidence and mortality rates of intrahepatic cholangiocarcinoma (ICC) have been increasing in several regions around the world. In the United States, the age-adjusted incidence rates of ICC increased by 165% from 0.32 per 100,000 in 1975 to 1979 to 0.85 per 100,000 in 1995 to 1999. At the same time, there has been very little improvement in long-term survival, which remains dismal (17). There are few well known risk factors for CC, including primary sclerosing cholangitis, hepatolithiasis, liver fluke infections, Thorotrast exposure, and choledochal cysts. None of these risk factors can explain the recent increasing trends of ICC in the United States. Some data, including population-based study, however, point to a potential role for chronic liver disease, liver cirrhosis, hepatitis C, and probably hepatitis B infections in the development of ICC (18,19). These observations suggest that chronic inflammation may be the common pathogenic pathway for CC. The clinical presentation of CC may vary depending on the site of the tumor. Generally speaking, peripheral ICC may reach to a large mass before detection, whereas CC occurring near the hilum is usually small but may cause obstructive jaundice that causes clinical attention (8). The tumors are generally firm, white to tan.

Microscopically they may mimic adenocarcinoma of any sites. The typical structures of the neoplastic cells are glandular, although solid nests, cords or papillary structures can also be seen. The neoplastic cells are those of columnar to cuboidal epithelial cells. A desmoplastic stroma surrounding the carcinoma cells is common. While not always present, the presence of mucin can help distinguish CC from HCC. CC is immunoreactive for CK7 and CK19, reflecting its biliary phenotype, although it is worth mentioning that the expression of CK7 and CK19 is not uncommon from several series (20,21,22). The positive CA 19-9 is helpful in separating CC from HCC but not from metastatic carcinoma.

Combined hepatocellular-cholangiocarcinoma (HCC-CC) is a rare but intriguing entity in that it has both HCC and CC components. In fact a diagnosis of combined HCC-CC requires unequivocal features of HCC such as trabecular growth, bile production, or bile canaliculi, and unequivocal elements of CC including glandular structures with biliary epithelial cells, mucin production, or in a desmoplastic stroma (8). Cautions should be exercised not to mistake the “pseudoglands” in HCC as the glandular structures in CC. Being able to identify mucin in the lumens or bile in the canaliculi may be helpful in distinguishing one or the other. Some studies show they behave similarly to HCC (2), while other authors suggest these tumors in fact are more comparable to CC in some aspects (23,24,25). Whether combined HCC-CC is biologically closer to HCC or CC is still a debatable issue, but the readily demonstrated biphenotypic differentiation in these
tumors raises an attractive hypothesis that hepatic progenitor cells may be the cells of origin of this unique tumor (25,26). Further studies are needed to address this issue.

Most of the hepatobiliary cystadenocarcinoma arise from preexisting cystadenoma, but some may represent cholangiocarcinoma with cystic formation or rare adenocarcinoma arising in the bile duct cysts (2,8). For this reason cystadenocarcinoma in women often show ovarian-type stroma, but not in men. Features that can help to distinguish from cystadenoma include cytological atypia, mitosis, and invasion of underlying stroma.

**DIAGNOSTIC PITFALLS AND CONCLUSIONS**

The challenging issues in diagnosing cholangiocellular and ductular lesions are to distinguish them from metastatic carcinoma or hepatocellular carcinoma, and to distinguish malignant from benign lesions, especially if the diagnosis relies on a core needle biopsy or during intraoperative consultation. At times it may be difficult to distinguish between the neoplastic cholangiocellular or ductular epithelium and the benign ductular reaction or reactive epithelium in the background of inflammation. Likewise, bile duct hamartoma, peribiliary gland hamartoma, or reactive peribiliary glands may be mistaken for CC or metastatic carcinoma. Typical features of CC are the complex cribriform glands, nuclear pleomorphism, hyperchromasia, mitotic figures, desmoplastic stroma, and lymphovascular space invasion that are lacking in the benign lesions. In addition, epithelioid hemangioendothelioma can be misdiagnosed as ICC because its cyttoplasmic vascular lumina may mimic gland formation or mucin. When ductular reaction with associated inflammation is present in a liver biopsy performed for a suspicious neoplasm, an adjacent unsampled mass has to be considered.

Immunohistochemical analysis may be of value when diagnostic difficulty occurs. It has been shown the expression of p53, bcl-2, and Ki-67 was greater in CC or metastatic carcinoma than in peribiliary gland hamartoma, bile duct hamartoma, and nonneoplastic inflammatory lesions (11,27). Furthermore, although to some extent immunohistochemistry may help to distinguish CC from metastatic carcinoma from other sites, the immunophenotypic profiles of CC and pancreatic adenocarcinoma are virtually identical and the ultimate distinction relies on the clinical and image correlation. Finally, phenotypic variability is common in liver neoplasms, which may be explained by different histogenesis, or alternatively, by a phenotypic alteration of the mature elements during neoplastic transformation. The role of HPC as the cell of origin in some liver tumors is an interesting subject and ongoing studies may shade more light in the near future.

**REFERENCES**


Hepatocellular lesions can be neoplastic or non-neoplastic. The common non-neoplasms include regenerative hyperplasia, focal accumulation of fat, focal nodular hyperplasia (FNH), macroregenerative nodules (MRN), and nodular regenerative hyperplasia (NRH). The neoplasms include hepatic adenomas, hepatocellular carcinomas (HCC) and their variants. Accurate diagnosis of these lesions can be sometimes difficult, particularly in liver biopsy specimens. I will briefly discuss the main characteristics of each of these lesions.

Non-Neoplastic Hepatocellular Lesions:

Focal Accumulation of Fat in Liver

Fat accumulation in the liver is common in the liver. However, focal or multifocal fat accumulation with unusual imaging patterns may cause diagnostic difficulty. Sometimes, these lesions are biopsied under CT guidance. Histologically, the liver tissue just exhibits steatosis and non-specific reactive changes. Radiological information is crucial for accurate diagnosis. The major differential diagnosis includes focal nodular hyperplasia, hepatic adenoma, macroregenerative nodules, hepatocellular carcinoma or metastatic tumors.

Macregenerative Nodules

MRN is often associated with cirrhotic liver. The prevalence of these nodules is approximately 25% in cirrhotic livers. The size of these nodules is usually 0.5 cm to 1.5 cm, but they can be several centimeters. It has been proposed that MRN may be a precursor for HCC. The supporting evidence came from the fact that such nodules are common in HCC patients and dysplastic changes (i.e., dysplastic nodules) are frequently identified in MRN. When these lesions are biopsied, it may be difficult to distinguish from well differentiated HCC. A good reticulin stain, which should highlight the hepatic architecture, is helpful. The other differential diagnosis includes FNH or hepatic adenoma. Examination of the histology of surrounding liver tissue is critical for making accurate diagnosis.

Nodular Regenerative Hyperplasia

NRH is characterized by the presence of small regenerative nodules, the adjacent hepatic acinar atrophy, and occluded portal vessels. It usually occurs in non-cirrhotic livers. NRH is associated with numerous variable systemic diseases, including immunological diseases, vascular disorders, infection, drug toxicity, and cancers. The clinical manifestation of NRH is variable, ranging from asymptomatic, mild liver function abnormality to symptoms related to portal hypertension.
The nodules are usually smaller than 0.3 cm. There is no fibrosis between the nodules. The key histological features are: nodules with regenerative hepatocytes (two cells thick hepatic plates), compressing adjacent hepatic tissue (atrophy), occluded portal veins, dilated sinusoids, presence of bile ducts, and absence of fibrosis or inflammation. NRH should be considered in any liver biopsies taken from patients with non-cirrhotic portal hypertension. A reticulin stain is essential to aid histological evaluation. The key differential diagnoses are: hepatic adenoma, cirrhotic nodules, FNH, and congenital hepatic fibrosis.

NRH is believed to be caused by abnormal blood flow in the liver. The nodules are compensatory liver cell regeneration. Interestingly, Notch1 gene knockout mice show NRH-like lesions in the liver. The natural history of the disease is not clear. It is possible that this disorder is reversible.

**Focal Nodular Hyperplasia**

FNH is a benign non-neoplastic lesion; it occurs in both men and women; and it is most commonly seen in young women. It is thought that the lesion represents local compensatory hepatocyte regeneration in response to a vascular abnormality. It has been proposed that hormonal use is associated with the development of FNH, but the role of oral contraceptives in FNH remains controversial.

The characteristic gross pathology finding is presence of stellate scars. Microscopically, the nodule is composed of benign hepatocytes arranged in two cells thick hepatic plates. The fibrous septa contain proliferating bile ductules with inflammatory cells. Abnormal, thickened arteries are present. The key differential diagnosis is hepatic adenoma. Presence of bile duct differentiation and abnormal arteries aids FNH diagnosis. Ruling out cirrhotic nodules is critical for accurate diagnosis. Distinction between FNH and adenoma in a small biopsy tissue may not be possible in some instances. Occasionally, both FNH and adenoma are present in the same liver.

**Neoplastic Hepatocellular Lesions:**

**Hepatic Adenoma:**

Hepatic adenoma is a benign liver neoplasm. It usually occurs in a normal liver, but it can also occur in glycogen storage disease. Its development is associated with hormonal or metabolic disorders, particularly the use of oral contraceptives. Therefore, hepatic adenoma occurs frequently in young women. When there are multiple adenomas, it is referred as "hepatic adenomatosis". Genetic mutations in hepatocyte nuclear factor 1alpha (HNF1alpha) gene has been recently identified in hepatic adenomas.

Histologically, hepatic adenoma is comprised of sheets of hepatocytes without forming normal acinar architecture. No bile duct differentiation is present. Steatosis in the neoplastic hepatocytes is common. Abnormal small arteries can be identified.
The major differential diagnosis includes well-differentiated HCC and FNH. It can be very challenging in liver biopsy specimens.

Hepatocellular Carcinoma

Hepatocellular carcinoma is the most common primary malignancy in the liver. It usually develops in the setting of chronic liver diseases, in particular, chronic viral hepatitis. The incidence of HCC is increasing. Unfortunately, many cases of HCC are diagnosed in later stages, which tend to have poor clinical outcome.

HCC can be accurately diagnosed in the majority of the liver biopsy cases, although some clinical practices are against the idea of liver biopsy to diagnose HCC for concerns about tumor spreading along the needle track. Classical HCC is not difficult to diagnose, although care must be taken to distinguish dysplastic nodules from definitive HCC. The dual differentiation potential of liver progenitor cells (presumably involved in liver carcinogenesis) may impose a diagnostic dilemma when both biliary and hepatocellular cells are present. The histological variants can be established with the assistance of immunohistochemical staining. The most important histological criterion is the architectural changes (more than three-layers of hepatic plates). Identification of bile production or bile canaliculi is very helpful for diagnosis.

Several histological variants are identified in HCC. Fibrolamellar variant appears to have better clinical outcome than classical HCC. Although there are still no uniformly accepted histological features that predict the tumor behavior, vascular invasion and nuclear features seem to be associated with patient overall survival. The well-accepted system of grading HCC is the one proposed by Edmondson and Steiner in their 1954, which grades HCC with 1-4 based on the overall architectural and cytological evaluation. Identification of HCC biomarkers is currently an active research field. It is expected that novel markers will be available in coming years.

Distinction between HCC and other metastatic cancers can be challenging in some cases. Immunostains are helpful. Most HCCs are reactive to Hep Par1 antibody. We have recently identified the liver specific antigen for this antibody. Polyclonal CEA antibody stains bile canaliculi, which is a very specific for HCC. Detection of AFP can also be helpful for differential diagnosis. Recently, glypican-3 expression has been used to differential HCC from benign hepatic tumors.

References


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 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 results have 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. Benign liver will produce a microabscess 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 hypocellular 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. Lipofuscin 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.

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. 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. These can be broken down into an evaluation of architectural features (cell group patterns and vascular patterns), 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, and is one of the most important patterns for separating reactive non-neoplastic and benign neoplastic proliferations from WDHCC. 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 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, 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. 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, macroesinophilic 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 in 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. 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% . A positive test also does not prove it is HCC, since tumors with hepatoid differentiation will also be positive. Immunohistochemistry for CD and Factor VIII demonstrate the vascular pattern. However, these tests do not offer any significant advantage over reticulin stain.

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<thead>
<tr>
<th>Criteria</th>
<th>Benign</th>
<th>HCC</th>
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<tr>
<td>Gross Appearance</td>
<td>Microbiopsy</td>
<td>Granular</td>
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<tr>
<td>Architecture Of Groups</td>
<td>Loosely Cohesive Groups With A Jagged Edge</td>
<td>Groups With A Smooth, Rounded Border</td>
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<td>Widened Trabeculae And Pseudoacini</td>
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<td>Peripheral Endothelium</td>
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<td>Reticulin</td>
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**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. 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.

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. 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. Hepatocellular carcinoma has a specific expression pattern for pCEA and CD 10 in which they are expressed in the bile canaliculi. Adenocarcinomas show a cytoplasmic expression pattern for CEA. MOC 31 is reported to be very sensitive for the diagnosis of adenocarcinoma. HepPar antigen is limited by occasional expression in other tumors and decreased expression in poorly differentiated HCC.

**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 cytoplasmatic 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. 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 metastasis 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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Liver Imaging

Patricia Lipford Abbitt, MD
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Department of Radiology, College of Medicine
University of Florida

Liver Imaging/Cirrhosis

- Number of patients with hepatitis / hepatocellular carcinoma increasing & interfacing w/ health care
- May contract hepatitis by occupational exposure, sexual partners
- Infection with hepatitis for 20 years before signs of liver failure

Signs of Liver Failure and Portal Hypertension

- Small, cirrhotic liver
- Splenomegaly
- Ascites
- Collaterals

Methods of Imaging the Liver

- Ultrasound
- CT
- MR
- Nuclear Medicine

CT – Workhorse of Imaging

- Three phase technique
  - Arterial, venous and delayed
  - Shows enhancement pattern and vascular involvement
Importance of Three Phase Imaging

Lesions may not be conspicuous in certain phases

State-of-the-Art Imaging

- Evaluating patients with liver disease or liver masses requires careful preparation and technique for imaging
- State-of-the-art imaging requires quality imaging equipment, specially trained technologists with precise protocols

Hepatocellular Carcinoma

- Avidly enhances in the arterial phase
- May be hard to see in venous phase
- Look for size of lesion, satellites, and vascular invasion for prognosis
- Look for distant disease in lungs and bones

Hepatocellular Carcinoma with Portal Vein Involvement
Portal Vein Thrombosis Secondary to Tumor Thrombus

• A feature of hepatocellular carcinoma
• Poor prognostic feature, often eliminates surgical options
• Neovasularity within tumor thrombus can sometimes be seen

Role of Liver Biopsy for Focal Lesion

• Increasingly, the histology of a liver lesion can be reliably predicted by non-invasive imaging
• Biopsy is often unnecessary and often, harmful to the patient

Role of Liver Biopsy for Focal Liver Lesion

• Can cause significant bleeding, especially with hepatocellular carcinoma
• Can cause intraperitoneal spread of disease, especially with hepatocellular carcinoma
Lesions of the Liver that Bleed

- Hepatocellular Carcinoma
- Hepatic Adenomas

Lesions with Kupffer Cells (RES Cells)

- Take up Ferridex (iron particles) – MR
- Take up sulfur colloid - nuclear medicine
- Help distinguish regenerating nodule or focal nodular hyperplasia which will take up Ferridex or sulfur colloid
- Mets, HCC, adenomas usually do not take up Ferridex or sulfur colloid

Liver Mass Imaging

- Must have very precise criteria for imaging diagnosis
- For example, hemangioma of the liver has a characteristic enhancement pattern...globular, peripheral, discontinuous enhancement which fills in centrally
**Solitary Hyperechoic Lesion in Liver—Hemangioma**

**Hemangioma**

- Ultrasound of hemangioma can be identical to metastatic disease
- MR, tagged red blood cell nuclear medicine or dynamic CT can definitively diagnose, making biopsy unnecessary

**Liver Mass Imaging**

- The enhancement pattern of hemangioma is not to be confused with the peripheral, ringlike enhancement pattern of intrahepatic cholangiocarcinoma
**Intrahepatic Cholangiocarcinoma**
- Second most common primary hepatobiliary malignancy
- Usually large because rarely symptomatic early
- Presents with ruq pain, weight loss, hepatomegaly, weakness
- May have elevated bilirubin, CA-19 markers, and LFTs
- Adenocarcinoma on path that may not be easy to differentiate from mets on path alone
- Surgery is best treatment option

**Ring-enhancing Liver Lesions**
- Intrahepatic cholangiocarcinoma and metastatic disease may look very similar radiographically
- Ring-enhancement needs to be differentiated by hemangioma

**Metastatic Disease of the Liver**
- Can be variable in sonographic or CT appearance
- Will reflect properties of primary tumor

**Cystic Lesions of the Liver**
- Differential consideration include simple epithelial cyst, liver abscess, biliary cystadenoma or biliary cystadenocarcinoma
- With increasing complexity of a cystic liver mass, more likely a malignancy
- Aspiration of the mass may allow cytologic evaluation, mucin strain, CEA & CA 19-9
Cystadenocarcinoma

Focal Fat/Focal Sparing in the Liver

- Many causes of fatty liver: alcohol, steroids, TPN, obesity, diabetes
- Can cause focal lesions in characteristic places: anterior to portal vein, near GB, near falciform ligament
- No vessels will be displaced or show mass effect
- MR can be definitive

Liver Imaging

Patients with liver disease / liver masses are more frequently requiring evaluation prior to therapy (resection, chemotherapy, transplantation)

State-of-the-art three phase imaging required

Multi-modality (US, CT, MR) evaluation helpful

Biopsy less often needed for focal lesions

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- "Imaging the Liver" The Oncologist – July 2004 – Volume 9, #4 Dushyant V. Sahani & Sanjeeva P. Kalva
consequence of persistent hepatitis, the association of cirrhotic liver with preneoplastic lesions (such as liver cell dysplasia) may reflect the culmination of long-term injury to the liver. Likewise, persistent proliferation of various cell types in chronic liver injury increases their susceptibility to carcinogenesis.

While the molecular mechanisms of hepatocarcinogenesis have been well studied (21, 22), the cells of origin of the various primary liver tumors (including hepatocellular carcinoma and cholangiocarcinoma) have not been definitively identified. Nonetheless, there are several obvious candidate cell types that might give rise to the major hepatocellular neoplasms. Hepatocellular carcinoma is a tumor composed of aberrant hepatocytes of varying degree of differentiation (from well differentiated to poorly differentiated). One possible cell of origin for this tumor is the mature parenchymal hepatocyte. Experimental studies show clearly that proliferative cells are much more susceptible to carcinogenic insult when they are proliferative (or contain a high S-phase fraction within the population) (23). Thus, the hepatocytes that constitute regenerative nodules in liver cirrhosis may be the targets of neoplastic transformation through endogenous mechanisms or exogenous exposures. In world regions that have a high prevalence of hepatocellular carcinoma, the at-risk population is HBV-positive and exposed to dietary Aflatoxin B1 (24-27). Thus, among these people, chronic liver injury related to HBV occurs in the presence of the potent and direct-acting hepatocarcinogen AFB1 (28, 29). This mechanism seems plausible for the origin and generation of well differentiated hepatocellular carcinoma. However, there is great debate over the origin of less well differentiated tumors. The concept of “de-differentiation” of hepatocytes has never been very popular, but remains a possibility. The alternative hypothesis invokes the possibility that undifferentiated (or less differentiated) stem cells might be the target cells for hepatocarcinogenesis (30-33). Clearly, a stem cell origin of liver tumors could account for all histological forms of these neoplasms, as well as the less differentiated and well differentiated tumor types. However, the existence of liver stem cells has been the subject of some controversy, particularly in humans (34, 35). Thus, the debate remains open to several possibilities. It is likely that with further investigation, we will discover that there are multiple cell types that can function as precursors for hepatocellular tumors, including differentiated cell types and liver progenitor cells.

In this presentation, the cell types and kinetics of cell proliferation in normal and pathological liver will be described with the objective of reviewing the evidence related to the possible cells of origin of the primary liver neoplasms. The literature cited in this overview represents a limited selection of major reviews and/or primary contributions to the field. Interested persons should consult these papers and the citations contained therein for a more complete treatment of the cell biology of normal and neoplastic liver.

Literature Cited