COLLEGE of AMERICAN PATHOLOGISTS

Protocol for the Examination of Biopsy Specimens From Patients With Hepatoblastoma

Version: Hepatoblastoma Biopsy 4.0.0.0 **Protocol Posting Date:** February 2019

Accreditation Requirements
The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.

This protocol should be used for the following procedures AND tumor types:

Procedure	Description
Biopsy	Includes specimens designated core biopsy, incisional biopsy, or other
Tumor Type	**Description**
Hepatoblastoma	Includes pediatric hepatoblastoma

The following should NOT be reported using this protocol:

Procedure
Resection (consider Hepatoblastoma Resection protocol)
Tumor Type
Other primary malignant hepatic tumors

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees
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Important Note

First priority should be given to formalin-fixed tissues for morphologic evaluation. The second priority for tissue processing is snap-freezing up to 1 g (minimum of 100 mg) of tumor from grossly different regions for molecular studies, as well as viable sterile tumor for cytogenetic studies (see Explanatory Note A). Samples from the same foci should be collected for histology, with appropriate identification. Samples of nontumoral liver should be collected for snap-freezing as well.

For more information, contact: The Children's Oncology Group Biopathology Center; Phone: (614) 722-2890 or (800) 347-2486.

Summary of Changes
v4.0.0.0 - Biopsy and resection procedures separated into individual protocols

Surgical Pathology Cancer Case Summary

Protocol posting date: February 2019

HEPATOBLASTOMA: Biopsy

Note: This case summary is recommended for reporting Hepatoblastoma but is NOT REQUIRED for accreditation purposes. Core data elements are bolded to help identify routinely reported elements.

Select a single response unless otherwise indicated.

Procedure (Note A)
___ Core biopsy
___ Incisional biopsy
___ Other (specify): _____
___ Not specified

Tumor Site
___ Right lobe
___ Left lobe
___ Right and left lobes
___ Other (specify): _____
___ Not specified

Tumor Focality (within liver)
___ Unifocal
___ Multifocal
___ Cannot be determined (explain): _____

Histologic Type (select all that apply) (Note B)
___ Hepatoblastoma, epithelial type, fetal pattern (mitotically inactive)
___ Hepatoblastoma, epithelial type, fetal pattern (mitotically active)
___ Hepatoblastoma, epithelial type, embryonal
___ Hepatoblastoma, epithelial type, pleomorphic (poorly differentiated)
___ Hepatoblastoma, epithelial type, macrotrabecular pattern
___ Hepatoblastoma, epithelial type, small cell undifferentiated pattern
Percentage of tumor with this histologic feature (if possible): _____%
___ Hepatoblastoma, mesenchymal type without teratoid features
___ Hepatoblastoma, mesenchymal type with teratoid features
___ Hepatoblastoma, other (specify subtypes if not included): _____
___ Hepatocellular neoplasm, not otherwise specified
Note: Ancillary studies (immunohistochemistry) may be performed to clarify histologic type.

Additional Pathologic Findings (select all that apply) (Note C)
___ No background liver available for evaluation (explain): _____
___ None identified
___ Cirrhosis/fibrosis (specify stage of fibrosis): _____
___ Iron overload
___ Hepatitis (specify type): _____
___ Other (specify): _____

Serum Alpha Fetoprotein (αFP) Level (Note D) _____
Note: Level at time of diagnosis may be prognostically important.
___ less than 100 ng/mL
___ 100 ng/mL – 1.2 million ng/mL

___ greater than 1.2 million ng/ml___ Not known

Ancillary Studies (select all that apply) (Note E)
___ INI1 immunohistochemistry performed
___ INI1 expression retained
___ INI1 expression lost
___ Glypican-3 immunohistochemistry performed
___ Negative
___ Positive
___ Pattern of staining (specify): _____
___ Beta-catenin immunohistochemistry performed
___ Negative (nuclear)
___ Positive (nuclear)

___ Other (specify): _____

Comment(s)

Explanatory Notes

A. Procedures

Fine-Needle Aspiration

Primary diagnosis by cytology (fine-needle aspiration) is not recommended as it may be misleading because of difficulties in distinguishing well-differentiated hepatocellular malignancy from regenerative changes and benign proliferations, and because of the variability of histologic features in hepatoblastoma. Hence, all attempts for fine-needle aspiration should be discouraged in favor of biopsy or resection.

Biopsy

The current recommendation for diagnosis of hepatoblastoma is a biopsy if upfront resection is not an option. This is the recommendation made in a recent consensus classification paper and will be followed in all future Children's Oncology Group (COG) and other international protocols for uniformity.[1] Hepatoblastomas are usually solitary lesions that occupy 1 or the other lobes of the liver, or may transgress more than 1 liver segment (the basis for pretreatment extent of disease [PRETEXT] staging). Multifocal lesions also occur, and multifocal tumors are the most likely cases to be diagnosed by biopsy. However, any tumor that is radiologically PRETEXT I or II *or* does not fit into stage I or II by the traditional COG staging system may be biopsied upfront, as primary resection may not be an option. Even with lower stage disease, large vessel invasion will be a contraindication to primary resection and will warrant preoperative chemotherapy.

The type of biopsy performed is entirely up to the discretion of the treating physicians and surgeons. Biopsy types include image guided needle biopsy (the more common scenario in the US) or open biopsy for cases that are difficult to access or in which there is potential for surgical resection. While it is much easier to get adequate tissue for studies with open biopsies, a needle biopsy done in interventional radiology is adequate as long as multiple (5-10) needle cores are obtained.[1] It is also recommended that the radiologist obtain needle cores from different portions of the tumor to maximize sampling of all areas of interest in the tumor. Calcified, bony, or hard tissue need not be sampled, however, and focus should be placed on obtaining adequate representation of the viable epithelial component. The region from which the biopsy is obtained should be noted if possible. If tumor involves more than 1 lobe, more than 1 lesion or area of the tumor should be sampled. These sites should be labeled separately, as different nodules in the same patient may have different histologies and biology. As most needle biopsy procedures are ultrasound guided, it may be easy to differentiate between tumor and uninvolved liver, and an attempt should be made to acquire adjacent nontumor liver tissue to understand underlying disease processes.

Upfront biopsy necessitates proper triage of the specimen for all pathologic and biologic studies, as required for COG trials of most pediatric tumors. The goal of the biopsy is tissue diagnosis to separate hepatoblastomas (the most common pediatric tumors) from other benign (especially mesenchymal hamartoma, adenomas, and focal nodular hyperplasia) or malignant (pediatric hepatocellular carcinoma and embryonal sarcoma) liver tumors, therapy for which are different. Regardless of the procedure type, every attempt should be made intraoperatively to assess if tissue obtained is viable and can be triaged for other studies. Imprint cytology may be used to assess tumor viability. No tissue diagnosis is needed at the time of frozen section, for that is the purpose of doing the biopsy, and the surgeon should be so educated. Tissue should instead be set aside for snap freezing (tumor and normal) as well as for cytogenetics (tumor only). While tissue may be set aside for electron microscopy, it is left to individual Institutions to make that decision. For further details, pathologists are referred to the consensus classification of hepatoblastoma published by Lopez-Terrada et al.[2]

References:

1. Finegold MJ. Hepatic Tumors in Childhood. In: Russo P RE, Piccoli D, eds. *Pathology of Pediatric Gastrointestinal and Liver Disease.* New York, NY: Springer-Verlag; 2004:300-346.
2. Lopez-Terrada D, Alaggio R, de Davila MT, et al. Towards an international pediatric liver tumor consensus classification: proceedings of the Los Angeles COG liver tumors symposium. *Mod Pathol.* 2014;27(3):472-491.

B. Histologic Type

Primary malignant tumors of the liver account for approximately 1% of all childhood cancer. The most common type is hepatoblastoma, which has an annual incidence of 0.9 per 1 million children.[1] Not only are

hepatoblastomas rare, but their diversity significantly limits the experience of any single center or pathologist. A classification scheme for hepatoblastoma that divides the more frequently or prognostically influential features from infrequent or inconsequential (minor) components is presented in Table 1.[2] The significance of a biopsy classification is that it reflects the true components of the tumor and is not limited by chemotherapy effects that alter the morphology of these tumors. It should, however, be noted that not all components may necessarily be sampled in a biopsy, and radiologic features, especially the presence of bone, need to be considered for subtyping.

Table 1. Pediatric Liver Tumors Consensus Classification

- Epithelial Tumors - Hepatocellular
  - Benign and tumor-like conditions
    - Hepatocellular adenoma (adenomatosis)
    - Focal nodular hyperplasia
    - Macroregenerative Nodule
  - Premalignant lesions
    - Dysplastic nodules
  - Malignant
    - Hepatoblastoma
      - Epithelial variants
        - Pure fetal with low mitotic activity
        - Fetal, mitotically active
        - Pleomorphic, poorly differentiated
        - Embryonal
        - Small-cell undifferentiated
          - INI1-negative
          - INI1-positive
        - Epithelial mixed (any/all above)
        - Cholangioblastic
        - Epithelial macrotrabecular pattern
      - Mixed epithelial and mesenchymal
        - Without teratoid features
        - With teratoid features
    - Hepatocellular carcinoma (HCC)
      - Classic HCC
      - Fibrolamellar HCC
    - Hepatocellular neoplasm, not otherwise specified (NOS)

Modified from Lopez-Terrada et al.[2]

There is no relationship between the age of the child and the predominant cell type in hepatoblastoma.[1,3] Of all cases at all ages, 85%-90% contain both fetal and embryonal derivatives in variable proportions; 20% have stromal derivatives. Because these histologic types tend to be randomly intermingled, both fine-needle aspiration and biopsies may capture a nonrepresentative sample of tumor.

The most significant component to identify in a biopsy of a low-stage tumor is well-differentiated fetal histology characterized by uniform-appearing round to polygonal cells with small central nuclei and clear or pale eosinophilic cytoplasm that may give the tumor a light-cell dark-cell pattern.[1,2] Nucleoli are usually inconspicuous and the mitotic rate is low (less than 2 mitoses per 10 high-power fields), the main criteria for this subtype. If the entire biopsy is composed only of this pattern, the possibility of primary resection should be advocated to minimize the need for chemotherapy if indeed the resected tumor appears histologically uniform. Again, this is only the case with low stage disease; higher stage diseases are likely to have other histologic components that are unsampled. It is important to realize that diagnosis of pure, well-differentiated fetal histology is to be made only on a completely resected tumor where adequate sampling excludes other areas and chemotherapy does not influence the morphology. The current Children's Oncology Group (COG) study is treating stage I well-differentiated fetal hepatoblastoma (with low mitotic rate) with surgery alone.[2-5]

Distinguishing well-differentiated (mitotically inactive) fetal hepatoblastoma tumor cells from normal liver in an infant can be difficult. The fetal tumor cells are larger than normal fetal hepatocytes and have a higher nuclear-to-

cytoplasmic ratio. The nuclei are regular and round with little discernible mitotic activity (less than 2 mitoses per 10 high-power [X40 objective] fields) in the well-differentiated variety.[2,5] Fetal tumor cells grow in cords, as in normal liver, or in nests or nodules. Clusters of normoblasts (extramedullary hematopoiesis) are seen, as in fetal liver. The cytoplasm of the fetal tumor cells varies from eosinophilic to clear, depending on the amount of glycogen content. Fetal tumor cells may also contain abundant lipid, producing vacuolization. In well-differentiated fetal tumors, bile secretion may be observed.

Histologically, the mitotically active fetal pattern shows greater than or equal to 2 mitoses per 10 high-power fields. Cells are arranged in trabeculae with abundant eosinophilic granular cytoplasm and round centrally placed nuclei with indistinct to occasional conspicuous nucleoli. Extramedullary hematopoiesis is frequently encountered in these areas. The embryonal pattern is composed of cells with high nuclear-to-cytoplasmic ratio with oval to angulated nuclei that are hyperchromatic with prominent single nucleoli and scant cytoplasm. Rosettes and tubular structures may be seen in this component. Purely embryonal tumors are almost never encountered and invariably show some fetal areas.

When tumor cells of either fetal or embryonal type show prominent nucleoli and more atypical morphology resembling hepatocellular carcinoma, the term *pleomorphic epithelial* is used. Most instances of these pleomorphic (also previously called *anaplastic fetal*) epithelial components are seen post resection, but one should be aware of this possibility in a biopsy. Arrangement of cells with fetal or embryonal morphology in areas in a trabecular arrangement where trabeculae are greater than 5 cells thick would warrant a description of macrotrabecular arrangement. This modification of cell thickness for plates was introduced in the new consensus classification, as the original 20-cell-thick plates were unusual and may represent hepatocellular carcinomas in a proportion of cases.

The other significant epithelial component that needs to be looked for is the small cell undifferentiated (SCU) pattern.[6] This is especially true if the entire biopsy or a significant portion of the biopsy shows this morphology. The more common scenario, however, is an epithelial hepatoblastoma with fetal and embryonal areas showing focal aggregates of small cells. These cells have uniform pale nuclei as compared to surrounding darker staining embryonal cells and are arranged in indistinct nests, which can be easily missed on histology. Immunohistochemistry may aid in this diagnosis, and care should be taken to differentiate a predominant SCU pattern from malignant rhabdoid tumor (MRT). Rhabdoid tumor cells have the characteristic, eccentric, pink cytoplasmic inclusions (periodic acid-Schiff/diastase positive, vimentin or cytokeratin positive) with vesicular nuclei and fibrillar inclusion bodies by electron microscopy. They may be associated with the small cell component in otherwise typical hepatoblastomas or as the exclusive cell type, in which case they occur in infancy and are associated with a poor prognosis. The classic rhabdoid tumors show loss of INI1 staining due to *INI1* gene mutation and are treated on the MRT protocol (see Note E).

When first distinguished from embryonal epithelium, small undifferentiated cells in hepatoblastoma were noted to resemble neuroblastoma, to have a low mitotic rate, and were called *anaplastic*, consistent with the dictionary definition, characterized by imperfect development. Because anaplastic was redefined by Faria et al[7] for Wilms tumor as nuclear enlargement to 3 times that of typical tumor cells, hyperchromasia, and atypical mitoses, the small cell undifferentiated component is no longer designated as anaplastic. Beckwith-type anaplasia does occur rarely in hepatoblastoma, and its significance is unknown. The small cells have been considered putative hepatic progenitor cells on the basis of immunohistochemical and electron microscopic studies. When present in a significant fraction of the hepatoblastoma (75%) or as the sole cell type, the small cell type is typically found in infants younger than 1 year; they have a poor prognosis, with poor response to current therapy. The prognostic significance of smaller proportions of the small cell undifferentiated type is still undetermined. The majority of tumors will show a mixed pattern of components, either epithelial alone or epithelial admixed with mesenchymal and even teratoid components. Even if mesenchymal components are not visualized histologically in a biopsy, radiologic documentation of bone or calcification may reflect a mixed epithelial-mesenchymal hepatoblastoma and help in the differential from other tumors. In some instances, biopsies may reveal primitive spindled cells at the edges of nodules of hepatoblastoma, mimicking small cells, but outside of nodules. These areas represent primitive mesenchyme, sometimes called “blastema” due to their ability to differentiate into epithelial or mesenchymal elements.

Often, mixed hepatoblastomas contain epithelial membrane antigen (EMA)-positive nests of squamous epithelium. The osteoid component of mixed hepatoblastomas is found to be a matrix of collagen surrounding cells expressing EMA and having ultrastructural features of epithelium, rather than osteoblasts. Hepatoblastomas may contain other stromal derivatives, including cartilage and rhabdomyoblasts. There is no prognostic significance to the presence of mixed histologic features.

Other unusual components that may be seen on a biopsy include the cholangioblastic pattern, neuroepithelium, glandular component (intestinal type), and even squamous elements.[2,8] Retinal pigment or immature neuroepithelial rosettes warrant a diagnosis of teratoid hepatoblastoma. These are usually intermingled with more classic morphology of hepatoblastomas. Teratoid hepatoblastoma was initially depicted as having intestinal, neural, and melanocytic elements. These are distinguished from true teratomas, which can also occur in the livers of children, on the basis of organoid differentiation and even greater diversity of tissue elements in the teratomas. Multinucleated tumor giant cells are found in rare hepatoblastomas, sometimes associated with human chorionic gonadotropin (HCG) production and clinical virilization.

References:

1. Finegold MJ. Hepatic Tumors in Childhood. In: Russo P RE, Piccoli D, eds. *Pathology of Pediatric Gastrointestinal and Liver Disease.* New York, NY: Springer-Verlag; 2004:300-346.
2. Lopez-Terrada D, Alaggio R, de Davila MT, et al. Towards an international pediatric liver tumor consensus classification: proceedings of the Los Angeles COG liver tumors symposium. *Mod Pathol.* 2014;27(3):472-491.
3. Czauderna P, Lopez-Terrada D, Hiyama E, Häberle B, Malogolowkin MH, Meyers RL. Hepatoblastoma state of the art: pathology, genetics, risk stratification, and chemotherapy. *Curr Opin Pediatr.* 2014;26(1):19-28.
4. Meyers RL, Tiao G, de Ville de Goyet J, Superina R, Aronson DC. Hepatoblastoma state of the art: pre-treatment extent of disease, surgical resection guidelines and the role of liver transplantation. *Curr Opin Pediatr.* 2014;26(1):29-36.
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C. Associated Clinical, Environmental, and Genetic Factors

Clinical Features and Differential Diagnosis

The presenting symptom of virtually all liver tumors in children is abdominal swelling secondary to hepatomegaly. When confronted with this symptom, it is useful to consider the age at which liver tumors tend to occur (see Table 2).[1] Exceptions are frequent, but age can serve as a guide when the presenting symptoms lack specificity. In the Pediatric Oncology Group series from 1986 to 2002,[2,3] 66% of hepatoblastomas were manifest by the second year, and 11% before 6 months of age. Approximately 50% of those in infants were congenital, given their size when discovered by 2-3 months of age; 6% of hepatoblastomas occurred after 5 years of age. Hepatocellular carcinomas have been observed as early as 6 months of age. Seven examples of mixed hepatoblastomas and hepatocellular carcinomas have been observed at a mean age of 8.5 years; perinatally acquired hepatitis B virus was responsible in 3 instances. Yolk sac tumors are more common in early childhood, but they also occur rarely in older adults. Systemic malignancies and metastatic disease must be considered at all ages because hepatomegaly due to megakaryoblastic leukemia, Langerhans cell histiocytosis, and neuroblastoma are important sources of confusion with hepatoblastoma in infancy, as are intraabdominal desmoplastic small round cell tumors later in childhood.

Table 2. Tumors of the Liver in Children: Usual Age of Presentation		
Age	**Benign**	**Malignant**
Infancy (0-1 y)	Hemangioendothelioma Mesenchymal hamartoma Teratoma	Hepatoblastoma, especially small cell undifferentiated Rhabdoid tumor Yolk sac tumor Langerhans cell histiocytosis Megakaryoblastic leukemia Disseminated neuroblastoma
Early childhood (1-3 y)	Hemangioendothelioma Mesenchymal hamartoma	Hepatoblastoma Rhabdomyosarcoma Inflammatory myofibroblastic (pseudo) tumor
Later childhood (3-10 y)	Perivascular epithelioid cell tumors (PE-Comas), including angiomyolipoma in liver and clear cell tumor of ligamentum teres / falciform ligament	Hepatocellular carcinoma Embryonal (undifferentiated) sarcoma Angiosarcoma Cholangiocarcinoma Endocrine (gastrin) carcinoma
Adolescence (10-16 y)	Adenoma Focal nodular hyperplasia Biliary cystadenoma	Fibrolamellar hepatocellular carcinoma Hodgkin lymphoma Leiomyosarcoma

Environmental Factors

Hepatoblastoma occurs in association with several well-described environmental factors and cancer genetic syndromes (see Table 3); however, not all of these associations are necessarily of statistical significance. Environmental factors and prenatal exposure to different agents have been implicated in hepatoblastoma.[4,5]

Data from the US National Cancer Institute Surveillance, Epidemiology, and End Result (SEER) program revealed an average annual increase of 5.2% in the incidence of hepatoblastoma from 1973 to 1992.[2] This change might be explained by hepatoblastoma occurring in surviving premature infants. Hepatoblastomas in Japan accounted for 58% of all malignancies in children who weighed less than 1000g at birth. Further analysis of the Japanese Children's Cancer Registry data revealed that 15 of 303 (5%) hepatoblastomas between 1985-1995 occurred in infants with a history of prematurity and weight less than 1500g at birth.[4] This rate was greater than 10 times that for all live births. The histologic features of hepatoblastoma after prematurity are indistinguishable from those of other hepatoblastomas.

The Children's Cancer Group has evaluated environmental or drug exposure. Seventy-five sets of parents of children with hepatoblastoma were compared with the parents of age-matched controls. In the group of children with hepatoblastoma, there was a significant excess of maternal exposure, before and during pregnancy, to metals used in welding and soldering, lubricating oils, and protective greases.[6] Paternal exposure to metals was also greater.

Table 3. Clinical Syndromes, Congenital Malformations, and Other Conditions Associated With Hepatoblastoma

Congenital Malformations
Absence of left adrenal gland
Bilateral talipes
Duplicated ureters
Dysplasia of ear lobes
Cleft palate
Fetal hydrops
Hemihypertrophy
Heterotopic lung tissue
Horseshoe kidney
Inguinal hernia
Intrathoracic kidney
Macroglossia
Meckel diverticulum
Persistent ductus arteriosus
Renal dysplasia
Right-sided diaphragmatic hernia
Single coronary artery
Umbilical hernia
Syndromes
Beckwith-Wiedemann syndrome
Beckwith-Wiedemann syndrome with opsoclonus, myoclonus
Budd-Chiari syndrome
Familial adenomatous polyposis syndrome
Li-Fraumeni cancer syndrome
Polyposis coli families
Schinzel-Geidion syndrome
Simpson-Golabi-Behmel syndrome
Trisomy 18
Metabolic / Pathophysiologic Abnormalities
Cystathioninuria
Glycogen storage disease types Ia, III, and IV
Hypoglycemia
Heterozygous α1-antitrypsin deficiency
Isosexual precocity
Prematurity
Total parenteral nutrition
Very low birth weight
Environmental / Other
Alcohol embryopathy
Human immunodeficiency virus or hepatitis B virus infection
Maternal clomiphene citrate or Pergonal
Oral contraceptive, mother
Oral contraceptive, patient
Osteoporosis
Synchronous Wilms tumor

Genetic Factors

Karyotyping of hepatoblastomas has revealed a recurrent pattern of chromosomal abnormalities.[7] The most common karyotypic changes are extra copies of entire chromosomes (trisomies), sometimes in conjunction with other complex structural changes and often in association with double-minute chromosomes. Trisomies of chromosomes 2 and 20 have each been reported most commonly, and each of these trisomies has been reported as a sole karyotypic event, suggesting that they may represent an early stage of tumor evolution. Trisomy of chromosome 20 and duplication of the long arm of chromosome 20 have also been observed in rhabdomyosarcoma, suggesting a link between these 2 embryonal tumors, both of which are also associated with losses at the Beckwith-Wiedemann syndrome locus.[8] Trisomy of chromosome 8 is also common; other trisomies are seen with lesser frequency. Occasional losses of entire chromosomes are seen, and these, too, are not random. The clinical significance of trisomies is unknown at present, although a recent study using comparative genomic hybridization has suggested that chromosomal gains at chromosomes 8 and 20 may be associated with an adverse prognosis.[9]

Numerous recent studies have documented molecular genetic abnormalities in hepatoblastomas (see Table 4) and other hepatic tumors. Several genetic changes are shared with other embryonal tumors, such as loss of heterozygosity at chromosome 11p15, also described in rhabdomyosarcomas and Wilms tumors. Acquired mutations of the *APC* gene and the *beta-catenin* gene, both members of the Wnt signaling pathway, have also been reported in hepatoblastoma.[7-11] The high frequency of *beta-catenin* mutations in hepatoblastomas and the increased incidence of hepatoblastomas in familial adenomatous polyposis families suggest the important role of an overactivation of wingless/Wnt pathway in the pathogenesis of hepatoblastoma. Collection of fresh or frozen hepatoblastoma tumor material as well as nontumoral liver tissue from these patients will be of great importance to the further investigation of the clinical relevance of these and other molecular genetic abnormalities in predicting the prognosis and clinical behavior of these tumors.

Table 4. Constitutional Genetic Disease Associated With Hepatoblastoma

Disease	Tumor Type	Chromosomal Locus	Gene
Familial adenomatous polyposis	Hepatoblastoma, hepatocellular carcinoma or adenoma, biliary adenoma	5q21.22	A*PC*
Beckwith-Wiedemann syndrome	Hepatoblastoma, hemangioendothelioma	11p15.5	*p57KIP2*, others
Li-Fraumeni syndrome	Hepatoblastoma, undifferentiated sarcoma	17p13	*TP53*
Trisomy 18	Hepatoblastoma	18	—
Glycogen storage disease types Ia, III, IV	Hepatocellular adenoma or carcinoma, hepatoblastoma	17	Glucose-6-phosphatase; debrancher and brancher enzymes

References:

1. Malogolowkin MH, Katzenstein HM, Meyers RL, et al. Complete surgical resection is curative for children with hepatoblastoma with pure fetal histology: a report from the Children's Oncology Group. *J Clin Oncol*. 2011;29(24):3301-3306.
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D. Tumor Markers

Serum α-fetoprotein (αFP) is the most useful indicator of hepatocellular neoplasia. Levels of serum αFP are markedly elevated in 80%-90% of hepatoblastomas and in 60%-70% of hepatocellular carcinomas.[1,2] Lesser degrees of elevation in infants can be due to variations in the rate of decline after birth or to secretion from regenerating hepatocytes adjacent to hemangioendotheliomas or mesenchymal hamartomas. Therefore, it is unacceptable practice to institute chemotherapy for mass lesions of the liver based solely on imaging studies and serum αFP levels. αFP also can be elevated in yolk sac tumors, which may occur as primary tumors in the liver or together with hepatoblastoma. On the contrary, αFP levels will not be increased when hepatoblastomas are primarily composed of the small cell undifferentiated type or in most fibrolamellar carcinomas, but even some typical fetal hepatoblastomas have failed to produce detectable increases in serum αFP levels. Low αFP levels below 100 ng/dL are therefore considered to be a poor prognostic indicator based on a large retrospective review of Children's Hepatic tumor International Collaboration (CHIC) database.[2-5] Following the αFP level in patients with unresectable hepatoblastoma after chemotherapy may have prognostic value.

References:

1. Finegold MJ. Hepatic Tumors in Childhood. In: Russo P RE, Piccoli D, eds. *Pathology of Pediatric Gastrointestinal and Liver Disease.* New York, NY: Springer-Verlag; 2004:300-346.
2. Czauderna P, Lopez-Terrada D, Hiyama E, Häberle B, Malogolowkin MH, Meyers RL. Hepatoblastoma state of the art: pathology, genetics, risk stratification, and chemotherapy. *Curr Opin Pediatr.* 2014;26(1):19-28.
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E. Ancillary Studies

Immunohistochemistry may help differentiate hepatoblastoma from normal liver or other hepatocellular tumors, or aid in accurate diagnosis of the various hepatoblastoma subtypes. Staining with glypican-3 has a distinctive pattern with a fine pericanalicular staining seen in cells of the well-differentiated fetal hepatoblastoma, while the

mitotically active fetal subtype and embryonal areas show similar patterns of coarse granular cytoplasmic staining. Small cell undifferentiated, cholangioblastic, and mesenchymal components are negative for glypican-3. Most teratoid components are also negative, except for an occasional glandular/yolk sac-like component that may show positive staining.

Beta-catenin staining is more variable. Rare pediatric hepatocellular carcinomas can show strong positive staining, as can nested epithelial-stromal tumors. The tumor currently considered under the rubric of hepatocellular neoplasms, NOS in the consensus classification also show nuclear beta-catenin staining despite morphologic overlap with features of hepatocellular carcinomas. At present, there is no immunostain to differentiate hepatocellular carcinoma from hepatoblastoma with confidence, though in general most pediatric hepatocellular carcinomas do not show the same intense nuclear staining as hepatoblastomas. Beta-catenin staining is usually associated with strong glutamine synthetase and cyclin D1 staining in hepatoblastomas. Possible genetic markers (trisomies for chromosomes 2, 20, and 8; abnormalities of chromosome 1p) are being investigated and may help differentiate these 2 entities, but only approximately 35%-40% of hepatoblastomas carry the abnormalities.[1]

Immunohistochemistry with glypican-3, beta-catenin, and glutamine synthetase (GS) aids in distinguishing hepatoblastoma from normal liver. Normal fetal liver is negative for glypican and shows only pericentral hepatocyte staining while staining diffusely in the tumor cells. Nuclear beta-catenin is only seen in tumor. Immunohistochemistry may be useful for identifying the small cell component of hepatoblastoma, as well. The small cells usually stain strongly and uniformly with beta-catenin in a nuclear pattern and are negative for glypican-3. This is in contrast to embryonal and fetal cells, which are cytoplasmic glypican-3 positive in most instances and show variable nuclear beta-catenin. The SCU component may also stain for vimentin and cytokeratin.

Evaluation of the SCU component with an INI1 stain is critical, particularly if the SCU component forms a significant portion of the biopsy. Any loss of INI1 in the SCU component may warrant reclassification on review as a malignant rhabdoid tumor with a different Children's Oncology Group treatment protocol. While this loss of INI1 is unusual in the usual SCU components that form small foci in between other epithelial components, it is prudent to do the stain and report the findings. Interestingly, stain for INI1 may be stronger in the nuclei of SCU component than surrounding cells; the significance of this is still to be determined.

It is also important to realize that fetal pattern hepatoblastoma may resemble the fetal hepatocytes trapped in benign liver tumors, such as mesenchymal hamartoma (MH) and infantile hemangioma (IH), and this needs to be recognized in a biopsy. Use of immunohistochemistry may be helpful in some instances but usually needs more than 1 stain for confirmation. The fetal liver trapped in an MH or IH may show fine glypican-3 staining but will usually lack beta-catenin nuclear staining. Also, the lesional cells of IH will stain with CD31 and Glut1, while MH may show epithelial lined cysts or myxoid matrix with a prominent biliary component. The biliary elements in hepatoblastoma (Cholangioblastic pattern) usually show nuclear beta-catenin staining.

References:

1. Lopez-Terrada D, Alaggio R, de Davila MT, et al. Towards an international pediatric liver tumor consensus classification: proceedings of the Los Angeles COG liver tumors symposium. *Mod Pathol.* 2014;27(3):472-491.