# Protocol for the Examination of Biopsy Specimens From Patients With Hepatoblastoma

**Version:** 4.1.0.0
**Protocol Posting Date:** December 2022
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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**Accreditation Requirements**
The use of this case summary is recommended for clinical care purposes but is not required for accreditation purposes. The core and conditional data elements are routinely reported. Non-core data elements are indicated with a plus sign (+) to allow for reporting information that may be of clinical value.

**Summary of Changes**
**v 4.1.0.0**
- WHO 5th edition updates
- Expert Consultation updated from Conditional to Optional




Replaced by version 5.0.0.0 on September 20, 2023, Obsolete as of June 2024 (8 months after newest release date)

**Reporting Template**

**Protocol Posting Date: December 2022**
**Select a single response unless otherwise indicated.**

**CASE SUMMARY: (HEPATOBLASTOMA: Biopsy)**

**EXPERT CONSULTATION**

**+Expert Consultation (Note A)**
___ Pending - Completion of this CAP Cancer Protocol is awaiting expert consultation
___ Completed - This CAP Cancer Protocol or some elements have been performed following expert consultation
___ Not applicable

**SPECIMEN**

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

**TUMOR**

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

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

**Histologic Type (Note C) (select all that apply)**
*Ancillary studies (immunohistochemistry, molecular) may be performed to clarify histologic type.*
___ Hepatoblastoma, epithelial type, fetal pattern (mitotically inactive / well differentiated)
___ Hepatoblastoma, epithelial type, fetal pattern (mitotically active / crowded)
___ Hepatoblastoma, epithelial type, embryonal pattern
___ Hepatoblastoma, epithelial type, pleomorphic pattern (poorly differentiated)
___ Hepatoblastoma, epithelial type, macrotrabecular pattern
___ Hepatoblastoma, epithelial type, small cell undifferentiated pattern
___ Hepatoblastoma, epithelial and mesenchymal type, without teratoid features
___ Hepatoblastoma, epithelial and mesenchymal type, with teratoid features





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___ Hepatoblastoma, other (specify, i.e., blastemal, cholangioblastic, squamoid or glandular patterns): _____
___ Hepatocellular neoplasm, not otherwise specified
**+Histologic Type Comment: _____**

**ADDITIONAL FINDINGS**

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

**SPECIAL STUDIES (Note E)**

**Serum Alpha-Fetoprotein (AFP) Level at Diagnosis (Note E)**
*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

**Beta-catenin IHC**
___ Not performed
___ Pending
___ Negative
___ Positive (specify pattern): _____
___ Cannot be determined (explain): _____

**+Glypican-3 IHC**
___ Not performed
___ Pending
___ Negative
___ Positive
**+Pattern of Glypican-3 IHC Staining: _____**
___ Cannot be determined (explain): _____

**INI-1 IHC**
___ Not performed
___ Pending
___ Expression retained
___ Expression lost
___ Cannot be determined (explain): _____





Replaced by version 5.0.0.0 on September 20, 2023, Obsolete as of June 2024 (8 months after newest release date)

**+Other Ancillary Studies (specify): _____**

**COMMENTS**

**Comment(s): _____**

## Explanatory Notes

### A. Expert Consultation

Expert consultation is not required. This question has been added to annotate, if so desired, that the case has been sent out for consultation and thus items of the CAP protocol could not be completed pending expert consultation. Completion of the CAP protocol will then be performed following consultation.

### B. Procedures

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.

The current recommendation for hepatoblastoma diagnosis is a tumor biopsy, except for rare cases for which upfront resection may be performed. This is the recommendation made by the international consensus classification[1] and will be followed in future Children's Oncology Group (COG) studies in alignment with other international protocols. Hepatoblastomas are usually solitary lesions that occupy one of the lobes of the liver but 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. Any tumor that is not radiologically PRETEXT I or II 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 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 for diagnosis as long as multiple (5-10) needle cores are obtained.[2] 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, which are treated differently. Regardless of the procedure type, every attempt should be made 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), following

institutional practices and when feasible. 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.[1].

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.
2. 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.

**C. Histologic Type and Associated Immunohistochemistry**

Not only are hepatoblastomas rare, but their diversity significantly limits the experience of any single center or pathologist.[1] 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**

*Benign and tumor-like conditions*
- Hepatocellular adenoma (adenomatosis)
- Focal nodular hyperplasia
- Macroregenerative nodule

*Premalignant lesions*
- Dysplastic nodule

*Malignant*
- Hepatoblastoma
  - Epithelial
    - Fetal with low mitotic activity (well-differentiated fetal pattern)
    - Fetal, mitotically active (crowded fetal)
    - Embryonal
    - Pleomorphic (poorly differentiated)
    - Small-cell undifferentiated
    - Epithelial mixed (any/all above)
    - Cholangioblastic
    - Epithelial macrotrabecular pattern
  - Mixed epithelial and mesenchymal
    - Without teratoid features
    - With teratoid features
- Malignant rhabdoid tumor of the liver (INI-1 expression lost)
- Hepatocellular carcinoma (HCC)
  - Classic HCC
  - Fibrolamellar HCC
- Hepatocellular neoplasm, not otherwise specified (HCN-NOS)

Modified from Lopez-Terrada et al.[2]

Detailed descriptions of the various epithelial patterns and subtypes of hepatoblastoma can be found in recent reviews.[3,4] More concise descriptions are provided below to aid accurate classification.

*Epithelial patterns: Fetal with low mitotic activity (well-differentiated/mitotically inactive fetal)*
The designation of "pure fetal hepatoblastoma" is restricted to primary resection specimens where the entire (100%) tumor consists of well-differentiated/mitotically inactive fetal pattern hepatoblastoma. By definition, a diagnosis of "pure fetal hepatoblastoma" cannot be made on a biopsy specimen, although the biopsy may demonstrate varying proportions of this epithelial pattern. "Pure fetal hepatoblastoma" is the least common amongst the histologic subgroups of HB but its recognition is important as it may obviate the need for chemotherapy. The current Children's Oncology Group (COG) study is treating stage I "pure fetal hepatoblastoma" as very low risk tumors treated with surgery alone.[2,5,6,7]

Well-differentiated/mitotically inactive fetal pattern is 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 at low-power. Nuclei are usually inconspicuous and, by definition, the mitotic rate is low (2 or fewer mitoses per 10 high-power fields). Rare interspersed extramedullary hematopoiesis (EMH) may be seen.

Immunohistochemistry may aid in differentiating this pattern from uninvolved background liver, which may show overlapping histologic features particularly in very young patients. The well-differentiated fetal (WDF) areas typically show a 1-2+ fine stippled pericanalicular (cytoplasmic) staining pattern with glypican-3 (GPC3) and variable nuclear staining for beta-catenin. Glutamine synthetase (GS) is usually diffusely positive in tumor cells whereas background liver shows a pericentral zonal distribution. SALL4 is negative in WDF.

*Epithelial patterns: Fetal with mitoses (crowded/mitotically active fetal)*
This is the most common pattern seen in biopsy specimens and resections. By definition, >2 mitoses per 10 high-power fields are seen. Cells are of similar size as those seen in WDF pattern but show more granular cytoplasm and larger nuclei. EMH is frequently seen. Beta-catenin shows more frequent nuclear staining compared to WDF but is not diffuse, with variable cytoplasmic staining. GPC3 typically shows a course diffuse cytoplasmic staining pattern that is 2-3+. GS shows diffuse strong staining and SALL4 is negative.

*Epithelial patterns: Embryonal*
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. A transition from crowded fetal to embryonal pattern can be seen and may be subtle or abrupt. Rosettes and tubular structures may be seen in this pattern. Mitoses are frequent. Nuclear staining for beta-catenin is more diffuse than fetal patterns. GPC3 is typically strongly positive (3+ staining), with the exception of some primitive embryonal components that may be negative for GPC3. GS usually shows variable staining. SALL4 is frequently strongly nuclear positive.

*Epithelial patterns: Pleomorphic*
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 (previously also called "anaplastic fetal") epithelial components are seen in post-chemotherapy

resection specimens, but this pattern can also be present in diagnostic biopsy specimens. Tumor cells are usually positive for GPC3 and beta-catenin (nuclear).

*Epithelial patterns: Macrotrabecular*
Unlike the epithelial patterns noted above (i.e., fetal, embryonal, pleomorphic), the macrotrabecular pattern is an architectural pattern, with arrangement of cells in trabeculae 5 cells thick and greater. The original descriptions of 20-cell-thick plates were problematic, and most cases represented hepatocellular carcinoma (HCC), not HB. Particularly in biopsy specimens, if tumor cells demonstrate pleomorphic cytomorphology with macrotrabecular arrangement, then consideration should be given to hepatocellular neoplasm (HCN), NOS (HCN-NOS) or HCC.

*Other epithelial patterns*
Squamoid and glandular tumor components may be seen in HB. Biliary-like profiles at the edges of tumor nodules, designated cholangioblastic, can also be seen and is distinct from ductular reaction seen at the junction with background liver.[8] The biliary-like profiles of cholangioblastic pattern show nuclear beta-catenin staining (versus membranous beta-catenin staining only in ductular reaction) and are typically positive for CK19 and pankeratin, with less frequent CK7 expression.

*Primitive cell patterns: Small cell undifferentiated (SCU) and blastemal*
The SCU pattern has been the most controversial pattern in HB. Earlier studies included a category of “pure small cell undifferentiated HB” with poor prognosis which are now known to represent malignant rhabdoid tumor with SMARCB1 alterations and loss of INI-1 expression. If this category is excluded, small foci of SCU in otherwise conventional HB no longer appears to be significant and the last COG trial showed no prognostic value to this histologic pattern.[9] Nests of SCU pattern, characterized by small blue cells with scant mitoses and cytoplasm, are often identified within areas of embryonal pattern HB.

More frequently, nests of cells with similar morphology to SCU are seen in areas of CF and at the periphery of nodules of HB and are designated blastemal. It is possible that the two patterns (SCU and blastemal) are related and represent primitive cells in HB capable of multidirectional differentiation. The full significance of these patterns is still to be determined but should be recognized as primitive components of HB that are not seen in either HCN-NOS or HCC. SCU and blastemal cells show nuclear expression of beta-catenin and co-expression of cytokeratins (pankeratin, CK19, CK7) and vimentin.

*Mixed epithelial-mesenchymal HB*
In the consensus classification, mesenchymal HB is noted as part of a mixed epithelial-mesenchymal HB with or without teratoid elements. It is unusual to find a pure mesenchymal HB, except in rare cases post-chemotherapy where epithelial elements have responded to therapy and only the mesenchymal elements remain, mainly osteoid and bone. Other mesenchymal elements that can be seen include cartilage (mature or immature), muscle or rhabdomyoblastic areas, and spindle cell mesenchyme. Of note, nests/aggregates of blastemal HB can be seen in the vicinity of mesenchymal components, most often osteoid. Nuclear beta-catenin may be seen in any of the mesenchymal components. GPC3 and SALL4 are usually negative but may highlight epithelial components in between.

Presence of neural elements such as primitive neuroepithelium, melanin, glial or ganglion cells may all represent features of teratoid differentiation in HB.[10] Still other unusual patterns of teratoid HB include glandular elements admixed with primitive neuroepithelium, with cytoplasmic supranuclear and subnuclear

vacuolation in the glandular epithelium resembling yolk sac tumor.[11] These glands are different from the occasional intestinal-type glands that may be seen in epithelial HB and seem to occur in the vicinity of immature neuroepithelium. These glands show nuclear staining for beta-catenin and are also positive for GPC3 and SALL4, also similar to yolk sac tumor. The neuroepithelial elements show variable nuclear beta-catenin and are negative for GPC3 and may show variable staining for SALL4. They usually show multilayering when arranged in rosette form, helping to differentiate them from embryonal rosettes, although this distinction may sometimes be difficult.

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.
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**D. 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 1986-2002[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; of note, a component of yolk sac tumor may be present in teratoid hepatoblastoma. 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<br>(0-1 y) | Infantile hemangioma<br>Mesenchymal hamartoma<br>Teratoma | Hepatoblastoma, especially small cell undifferentiated<br>Rhabdoid tumor<br>Yolk sac tumor<br>Langerhans cell histiocytosis<br>Megakaryoblastic leukemia<br>Disseminated neuroblastoma |
| Early childhood<br>(1-3 y) | Infantile hemangioma<br>Mesenchymal hamartoma | Hepatoblastoma<br>Rhabdomyosarcoma<br>Inflammatory myofibroblastic (pseudo) tumor |
| Later childhood<br>(3-10 y) | Perivascular epithelioid cell tumors (PE-Comas), including angiomyolipoma in liver and clear cell tumor of ligamentum teres / falciform ligament | Hepatocellular carcinoma<br>Embryonal (undifferentiated) sarcoma<br>Angiosarcoma<br>Cholangiocarcinoma<br>Endocrine (gastrin) carcinoma |
| Adolescence<br>(10-16 y) | Hepatocellular adenoma<br>Focal nodular hyperplasia<br>Biliary cystadenoma | Fibrolamellar hepatocellular carcinoma<br>Hodgkin lymphoma<br>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 2.2% in the incidence of hepatoblastoma from 2004-2015.[6] This increase may be in part explained by surviving premature infants. Hepatoblastomas in Japan accounted for 58% of all malignancies in children who weighed less than 1000 g 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 1500 g 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.

| 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 a1-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

Hepatoblastomas are genomically stable embryonal neoplasms generally carrying a very low rate of somatic mutations.[7,8,9,10] Karyotyping of hepatoblastomas initially demonstrated few recurrent chromosomal abnormalities including trisomies of chromosomes 20, 2 and 8, and abnormalities involving gains of chromosome 1q, sometimes associated with t(1;4)(q12;q34) or other unbalanced translocations.[11] However, aberrant activation of the Wnt/β-catenin pathway appears to be the main hepatoblastoma driver, with close to 90% harboring CTNNB1 mutation.[7,12] NFE2L2 has been reported to represent the second most commonly mutated gene in small series of hepatoblastomas (5% to 10%) and associated with poor prognosis. The presence of TERT promoter mutations is characteristic of the hepatocellular neoplasm, not otherwise specified (HCN-NOS) provisional subtype. Several recent hepatoblastoma genomic profiling studies have reported variants and copy number alterations in additional genes[7,9,10] involving pathways potentially implicated in hepatoblastoma development and clinical behavior, including Notch, Sonic Hedgehog, PI3K/AKT, EGFR and Hippo pathway (YAP), among others.[7,8,13,14]

Several hepatoblastoma genomic profiling studies have attempted to better understand the biological factors associated with hepatoblastoma prognosis, response to therapy, and define biological groups to develop a more precise risk stratification. Transcriptomic profiling initially demonstrated two distinct genotype-phenotype hepatoblastoma subtypes, one with a more mature phenotype corresponding to fetal histology, and a second one recapitulating early fetal life liver, and with embryonal histology.[15] Later genomic studies demonstrated additional molecular risk-associated subtypes, with high-risk tumors being characterized by high NFE2L2 activity, high LIN28B, HMGA2, SALL4, and AFP expression, as well as low let-7 expression and HNF1A activity.[7] Recently, HB epigenomic profiling demonstrated genome-wide dysregulation of RNA editing in HB and identified additional epigenomic clusters, including an aggressive subgroup identified by characteristic methylation features, strong 14q32 locus expression, as well as CTNNB1 and NFE2L2 mutations and a progenitor-like phenotype.[16] Unfortunately, none of these transcriptomic or epigenomic prognostic-associated clusters have yet been clinically validated in large prospective studies and are currently not being used for risk stratification. Systematic banking of hepatoblastoma tumor material remains of great importance to further investigate the clinical relevance of these molecular abnormalities and biological groups, so they could be incorporated in more precise risk stratification algorithms.

| 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 |

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**E. Tumor Markers**

Alpha-fetoprotein (AFP) is a circulating tumor marker elevated in all cases of HB. Historically, it was thought that tumors with an AFP level less than 100 ng/mL carried a poor prognosis, particularly given the perceived link between low AFP with small cell undifferentiated (SCU) histologic pattern. This concern has since been refuted in a publication from a recently concluded Children's Oncology Group trial demonstrating that the presence of SCU pattern is not associated with a poor prognosis.[1] There is consensus opinion from HB experts that low AFP (<100 ng/mL) values can be seen in association with small tumors, incidentally diagnosed on imaging obtained for an unrelated reason or during surveillance for a known cancer predisposition syndrome. Tumors associated with a normal AFP, previously perceived to be HB, are now, in hindsight, known to be malignant rhabdoid tumors or tumors of a different histology altogether.

Clinically, AFP is a useful diagnostic biomarker to monitor response to therapy and to evaluate for disease progression. There are two important factors to keep in mind when interpreting the clinical utility of AFP. First, there are tumors other than HB that secrete AFP, including pediatric hepatocellular carcinomas, germ cell tumors, and rare pancreatic tumors. Second, AFP is markedly elevated in the perinatal period and in the subsequent months of life which can impact the diagnostic relevance of this lab value. The Children's Hepatic tumors International Collaboration (CHIC) risk-stratification tool derived from the retrospective analysis of 1200 patients with HB treated on clinical trials conducted within four consortia demonstrates that gradations of AFP at diagnosis <100, 100-1000, or >1000 might be relevant for prognosis.[2] While work in the germ cell tumor literature links the kinetics of AFP decline during therapy with long-term outcome, there is limited data in hepatoblastoma linking log-fold decline of AFP to outcome and more work is being done to clarify this relationship.[3,4,5]

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**F. 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.

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