Cohort HBV Pediatric Protocol

Table of Contents

Observational Study of Children with Hepatitis B Virus Infection in North America: A Cohort study protocol of the Hepatitis B Research Network (HBRN)

Abstract

Aims

- Primary Aim:
  - To describe participants 6 months to <18 years of age with hepatitis B virus (HBV) infection in a prospective cohort in the United States (US) and Canada and identify predictors of disease activation and progression

- Secondary Aims:
  - To describe clinical, virological, and immunological characteristics of participants with HBV in the US and Canada.
  - To evaluate changes in HBV infection status and HBsAg levels and factors associated with those changes.
  - To verify whether a baseline HBsAg below 1,000 IU/mL and HBV DNA below 1,000 IU/mL is an accurate predictor of people who are, or who will become, inactive carriers, defined as people who are HBsAg positive, HBeAg negative, and have normal ALT and HBV DNA under 1,000 IU/mL on at least two occasions at least 6 months apart.
  - To assess the health related quality of life (HRQOL) of treatment naïve hepatitis B surface antigen (HBsAg) positive children and adolescents
  - To develop a bank of biospecimens (e.g., serum, plasma, DNA, liver tissue) obtained from participants with HBV infection.
  - To identify pediatric participants from 2 years to <18 years of age with chronic HBV infection for potential participation in treatment study to be conducted by the Hepatitis B Research Network (HBRN).

Type of study

- Observational

Cohort study

Patients with HBV infection will be identified by sites participating in the NIH-funded Hepatitis B Research Network (HBRN). Consenting patients who meet entry criteria will undergo a baseline evaluation. Those who are eligible for the treatment study being conducted by the network will be approached about participating. If they are not eligible, unwilling to participate or have completed their participation in the treatment study, they will be followed longitudinally to observe clinical outcomes and changes in their virologic and immunologic status.

It is estimated that approximately 500 patients with HBV infection will be enrolled in the Cohort Study. They will be followed indefinitely until the conclusion of the study.

- **Inclusion criteria**
  - Written informed consent/assent as appropriate
  - At least 6 months to <18 years of age
  - HBsAg-positive

- **Exclusion criteria**

- History of hepatic decompensation based on clinical or laboratory criteria
- Hepatocellular carcinoma (HCC)
- History of solid organ transplant or bone marrow transplant
- Chronic Immunosuppression therapy
- Current Hepatitis B antiviral treatment (except pregnant females)
- Known coinfection with HIV (patients with HDV or HCV coinfection are not excluded)
- Medical or social condition which, in the opinion of the principal investigator, would make the patient unsuitable for the study or interfere with or prevent regular follow up.
- Unable or unwilling to return for regular follow-up

Briefly, the visit schedule will be at baseline, weeks 24 and 48 in the first year of enrollment and every year in subsequent years. Variable follow-up visits may be required if certain events are encountered. For participants experiencing an ALT flare, as defined in section 3.2.1 and Appendix A, follow-up will be every 2-4 weeks until resolved. For HBeAg or HBsAg loss, participants will be asked to return in 12 and 24 weeks, and will then resume follow-up every 48 weeks.

The following outcomes will be noted among participants:

- ALT Flare
- HBsAg or HBeAg loss
- Development of cirrhosis
- Hepatic decompensation
- HCC
- Liver transplantation
- Death
- Reaching age 18 years

1. Background and rationale

1.1. Historical background and goals

Chronic hepatitis B virus (HBV) infection is a leading cause of morbidity and mortality from end stage liver disease and hepatocellular carcinoma (HCC) in the US and worldwide. Of the estimated 1.4 million Americans with HBV infection, up to 25% are expected to die of these liver-related causes. Although significant progress has been made recently in HBV therapy, including approval of six new therapeutic agents for treatment of adults and two therapeutic agents in children, the current knowledge in the management of HBV infection is limited because treatment trials have utilized one to two years of therapy at most, whereas most patients require treatment of much longer duration for optimal long term outcome.

In 2008, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), through the U01 mechanism, established a Research Network, the goal of which is to facilitate and perform clinical, scientific, epidemiological and therapeutic research in HBV infection related chronic liver disease.

1.2. Hepatitis B Research Network

The Hepatitis B Research Network (HBRN) is a cooperative network of Principal Investigators and co-investigators from thirteen sites one Data Coordinating Center (DCC) and one Immunology Center. Clinical centers are responsible for proposing protocols, participating in their overall development, recruiting patients, conducting the research, and disseminating research findings. The clinical centers participate in a cooperative and interactive manner with one another and with the DCC, the Immunology Center and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in all aspects of the HBRN. The DCC supports protocol development; provides sample size calculations, statistical expertise, forms, and data analysis; supports manuscript preparation; and provides overall study coordination and quality assurance, including coordinating the activities of the Steering Committee and other standing committees. The HBRN also collaborates with biosample repositories. A Steering Committee composed of the principal investigators of each clinical center in the Network, the principal investigator of the DCC, the principal investigator of the Immunology Center, and the NIDDK Project Scientist is the main governing body of the HBRN. The Steering Committee has primary responsibility for the general organization of the HBRN, finalizing common clinical protocols and facilitating the development of a standardized nomenclature, diagnostic criteria, histological definitions, and the necessary components to the common database on patients. The Steering Committee is responsible for the conduct and monitoring of studies and reporting of study results.

1.3. Scientific background

1.3.1. HBV introduction

Hepatitis B virus (HBV) infection is a worldwide health problem, which can cause acute liver failure, acute hepatitis, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). It is most prevalent in Asia, Africa, Southern Europe, and Latin America, where the hepatitis B surface antigen (HBsAg) positive rate in the general population ranges from 2 to 20%. Approximately 2 billion people in the world have been infected by HBV, and more than 350 million of them are chronic (HBsAg) carriers.

In endemic areas, HBV infection occurs mainly during infancy and early childhood. Mother-to-infant transmission accounts for approximately half of the transmission route of chronic HBV infections. In contrast to infection in adults, HBV infection during early childhood results in a much higher rate of persistent infection. Ninety percent of infants infected as a neonate and 25-50 percent of children

between the ages of 1 and 5 years that are acutely infected with HBV, will progress to chronic infection, whereas less than 5 % of symptomatic and only 5-10 % of asymptomatic infected adults and teens will develop chronic hepatitis B. A long term follow-up study revealed that the overall prognosis for chronic hepatitis B in horizontally infected Caucasian children was favorable, yet 2% progressed to HCC and 6% had potentially aggressive hepatitis B envelope antigen (HBeAg)-negative hepatitis. (1) Among adults who acquired chronic hepatitis B infection as an infant or child, approximately 15-25 % overall die a premature liver-related death.(2-5) In the United States (US), the total number of persons with chronic hepatitis B is thought to be almost 2 million (6) with many infected adults having acquired their infection during infancy or childhood. Due to the successful implementation of HBV vaccination in the US, the incidence of acute hepatitis B infection in children less than 15 years of age declined by 98 % between 1990 and 2006. (7-9) Currently, the majority of new cases of HBV in children in the US are those who were not fully vaccinated. In many cases these are homeless children, international adoptees, children born outside the US even if the child supposedly received hepatitis B vaccine in their birth country, or those who were born to HBsAg-positive mothers and did not receive immunoprophylaxis or the birth dose of vaccine in a timely fashion.

Most children with chronic HBV infection are *immune tolerant*, with high viral replication (positive HBeAg and high HBV DNA levels) and normal levels of alanine transaminase (ALT).(10)(11) (12)With persistent HBV infection, patients may enter the *immune active* phase with increased ALT levels, and decrease in the HBV DNA levels. Liver injury may accumulate in the life time especially during this phase (13). Active liver inflammation is reflected in the elevation of ALT levels and in active inflammation and/or fibrosis occurring in the liver tissue. During this phase, spontaneous HBeAg / anti-HBe seroconversion may develop after various degrees or duration of active liver injury. With complete HBeAg clearance and seroconversion anti-HBe formation, patients enter the *inactive HBsAg carrier phase.* During this phase viral DNA is low, (< 2,000 IU/ml (10,000 copies/ml) or undetectable), ALT normalizes, liver histology is without inflammation, hepatic fibrosis may regress, and the risk of cirrhosis and HCC declines (14). Unfortunately, approximately 20-30% of patients will subsequently enter the *reactivation phase* (15). In this phase, viral DNA levels increase again while HBeAg remains undetectable. ALT may be either normal or elevated. This is also termed e antigen negative chronic hepatitis B, and is usually due to infection with a mutant virus; some persons may move directly into this phase without going into an inactive HBsAg carrier phase. In this *reactivation phase* the viral variant is more virulent and hence accelerated liver damage is more likely over time.

Review of recent literature to determine the normal ALT reference ranges in children showed the following: the European Hepatitis C virus (HCV) Network studied (16) 1293 HCV-uninfected children born to HCV positive mothers. In this group normal ALT for ≤ 18 months old children were 60 IU/L for males and 55 IU/L for females, whereas for > 18 month old children, 40 IU/L for males and 35 IU/l for females were described. In another study, Schwimmer et al analyzed NHANES data in 982 subjects 12 – 17 years of age without any risk factor for liver disease. The NHANES data showed that the ALT levels in >95% was 25.8 IU/L for males and 22.1 IU/L for females (17). Based on the above two publications, the ALT levels to be use in the HBRN consortium for children >18 months of age - <18 years of age will be 40 IU/L as the upper limit of normal for males and 35 IU/L as the upper limit of normal for females. For children 6 months - 18 months of age, the ALT levels will be 60 IU/L as the upper limit of normal for males and 55 IU/L as the upper limit of normal for females.

Severe liver damage including bridging hepatic fibrosis, liver cirrhosis, and liver cancer, may occur during childhood, especially during the *immune active* and *reactivation phases* (18). Additionally, we

are now realizing that a strict linkage of ALT with progression of liver disease is not always apparent, especially in children (19).

Understanding the status of children infected with HBV in the US with respect to their demographics, family history, route of HBV infection, phase of infection, and related laboratory profiles and potential co-morbidities will provide better insights into this infection and its impact on US children. This information may help guide studies of treatment and prevention among HBV-infected children and their families.

1.3.2. Hepatitis B and pregnancy

Although women infected with hepatitis B generally tolerate pregnancy (20), there are some women who experience ALT flares either during pregnancy or after delivery. Tan et al (21) observed that more HBsAg positive pregnant women had elevated ALT levels than controls, regardless of their HBeAg status. Nguyen et al (22) reported 3 cases of significant liver dysfunction during pregnancy with one case requiring liver transplantation when the patient failed a course of salvage antiviral therapy. Another study observed a post-partum flare in 38% of those who were not treated with lamivudine during pregnancy and among those treated, 62% flared after withdrawal of lamivudine (23). Post-partum flares have been associated with HBeAg seroconversion rates of 12.5 to 17% (24). However, detailed data describing the natural history of hepatitis B in pregnancy are lacking.

Since it is anticipated that at most there would only be a few pregnant subjects less than 18 years of age, there will be no pregnancy specific data collection in the pediatric cohort study. Participants who become pregnant will be referred to the adult hepatologist for clinical care but will be followed in the longitudinal pediatric cohort study until they reach age 18 years.

2. Aims

- Primary Aim:
  - To describe participants 6 months to <18 years of age with hepatitis B virus (HBV) infection in a prospective cohort in the United States (US) and Canada and identify predictors of disease activation and progression

- Secondary Aims:
  - To describe clinical, virological, and immunological characteristics of patients with HBV in the US and Canada.
  - To evaluate changes in HBV infection status and HBsAg levels and factors associated with those changes.
  - To verify whether a baseline HBsAg below 1,000 IU/mL and HBV DNA below 1,000 IU/mL is an accurate predictor of people who are, or who will become, inactive carriers, defined as people who are HBsAg positive, HBeAg negative, and have normal ALT and HBV DNA under 1,000 IU/mL on at least two occasions at least 6 months apart.
  - To assess the health related quality of life (HRQOL) of treatment naïve hepatitis B surface antigen (HBsAg) positive children and adolescents
  - To develop a bank of biospecimens (e.g., serum, plasma, DNA, liver tissue) obtained from participants with HBV infection.
  - To identify and screen pediatric patients from 2 years to <18 years of age with chronic HBV infection for potential participation in other studies within the Hepatitis B Research Network (HBRN).

The HBRN will evaluate a cohort of participants with HBV infection, categorize them into various phases of HBeAg-positive and HBeAg-negative HBV infection and stages of HBV disease, describe the characteristics of these phases, determine the factors associated with transition from one phase to another, and determine the rate of various clinical outcomes.

Specifically, this study will

1. Describe the occurrence of the transitions between various phases of HBeAg-positive and HBeAg-negative HBV infection,
2. Identify immunologic and virologic changes associated with those transitions and
3. Identify predictors of those transitions.

Given that the majority of participants are expected to have normal ALT values, the study will focus on transition into phases with active liver disease. In order to accomplish these goals, projects with the following objectives will be pursued.

2.1.1. Descriptive epidemiology

Data collected at baseline will provide a snapshot of HBV patients seen at the HBRN centers. Descriptive patient characteristics, such as race, ethnicity, country of origin, co-morbidity, and disease and treatment status will be examined.

2.1.2. Categorize participants into different phases of HBV infection

Patients in the immune tolerant phase are believed to have mild histology and HBeAg- negative inactive carriers tend to have no or minimal hepatic inflammation; whereas HBeAg-positive and HBeAg-negative hepatitis B have active inflammation and variable fibrosis. Hepatitis B infection is a dynamic process and it is unclear how patients evolve from “quiescence” to an active phase of the disease, and vice versa.

2.1.3. Relationship of HBV genotype to clinical, biochemical and histological characteristics

The relation between genotype and patient characteristics including age, HBV DNA, ALT, and stage of liver disease for each phase of HBV infection will be analyzed. In addition the relationship between genotype and clinical outcome, including cirrhosis, hepatic decompensation and HCC will be studied.

2.1.4. Health related quality of life (HRQOL) Questionnaires

Health-related quality of life will be measured by the Child Health Questionnaire (CHQ). The CHQ is a validated generic HRQOL instrument that measures health in the physical, psychological and social domains in children (25). The CHQ will be completed by caregivers (eg. biological or adoptive parent or legal guardian henceforth referred to as caregiver) of children less than 10 years of age. Children who are at least 10 years of age will complete the CHQ. Because the CHQ has not been translated and validated in many of the languages spoken by many HBV-infected immigrant populations (African and Asian populations), the CHQ will only be given to participants/caregivers who can understand written English. HRQOL results from the treatment naïve children from this study will be compared to normative data (25) (26).

2.3. Support of Other Studies of the HBRN

In addition to being used for studies as described above, the HBRN will establish and maintain data, biospecimen bank(s) and other resources for ancillary studies of the pathogenesis, diagnosis, natural history and treatment of HBV infection.

3. Screening and enrollment

HBRN sites will keep a screening log of HBsAg-positive patients who are seen at a recruitment site; this screening log will keep only minimal information and will be used primarily to screen patients for eligibility for the Cohort Study. Thus, the presence of hepatic decompensation, HCC, recent antiviral treatment, a history of liver transplantation or HIV infection will be recorded. The other main purpose of the screening log is to ensure that patients are not screened or enrolled more than once. Only de-identified elements of screening log will be forwarded to the DCC for inclusion in the HBRN database.

Patients who appear to meet the eligibility criteria will be approached to participate in the cohort study. Thus all consenting patients with hepatitis B will be enrolled except those who have already reached significant outcomes (decompensation, HCC, liver transplantation), are known to be HIV infected or are currently on antiviral therapy (except pregnant women).

3.1. Phases of HBV Infection ("phenotype")

A patient with HBV infection transitions through a number of phases of HBV infection. One of the aims of the HBRN is to define these phases with objective criteria. Therefore at the outset of the study it is helpful to have some conceptual definitions of these phases. These phases or phenotypes include:

- Acute hepatitis
- Immune tolerant phase
- Immune active phase
    - HBeAg positive chronic hepatitis B
    - HBeAg negative chronic hepatitis B
- Inactive carrier phase
- Indeterminate, i.e., participants who do not fall into one of these categories

Based on historical data and information obtained with their initial study evaluation, each participant will be assigned into one of these five categories (Appendix A).

3.2. Outcome Variables

Once the initial evaluation is completed and a determination made of the phase of hepatitis B for each patient, they will be observed over time for various outcomes. The following outcomes will be noted among patients participating in the cohort study:

Primary outcome:

- HBeAg loss

Secondary outcome

- Hepatitis exacerbation marked by ALT Flare
- HBeAg reversion
- HBeAg Seroconversion
- HBsAg loss
- Cirrhosis
- Hepatic decompensation
- HCC
- Liver transplantation
- Death
- Reaching age 18 years

A Pediatric Outcomes Subcommittee will review the pertinent data and assign a date of occurrence. With regard to ALT flares and deaths, the subcommittee will focus on assigning probable cause(s).

3.2.1. Hepatitis exacerbation marked by ALT Flare

A flare is defined as serum ALT greater than or equal to 10 times the upper limit of normal which corresponds to 1) 550 U/L in females and 600 U/L in males 6 months – 18 months of age, and 2) 350 U/L in females and 400 U/L in males >18 months - < 18 years of age (12). Once a flare is detected, participants will be followed more closely until its resolution (see section 6.2.1.2).

3.2.2. Antigen loss: e and s

HBeAg or HBsAg loss is considered a significant event in the natural history of chronic HBV infection and are usually associated with biochemical and histologic improvement. Loss of these viral markers may also be associated with appearance of corresponding antibodies in serum (anti-HBe or anti-HBs).

HBsAg loss appears to represent a “cure” of HBV infection and is associated with reduction, but not necessarily elimination of the risk of future complications, such as HCC which may still occur particularly in those who lose HBsAg at an older age (e.g. after 50 years) or after the development of cirrhosis.

When HBeAg or HBsAg loss occurs, participants will be followed more closely initially and then return to the regular follow-up schedule (see section 6.2.1.4).

3.2.3. Cirrhosis

The diagnosis of cirrhosis will be made by (1) liver histology, when available or (2) clinical criteria (Appendix A).

Once cirrhosis is diagnosed, patient follow-up should include HCC surveillance.

3.2.4. Hepatic Decompensation

It is likely that the development of cirrhosis and subsequent hepatic decompensation will be preceded and foreseen by the progression of fibrosis. Development of hepatic decompensation will be defined by any of the following events:

- Ascites or hepatic hydrothorax
- Variceal bleeding or portal hypertensive bleeding
- Hepatic encephalopathy
- Child-Turcotte-Pugh (CTP) score of 7 or above

It is anticipated that there will be a small number of patients that will develop decompensation during the follow-up.

3.2.5. Hepatocellular carcinoma (HCC)

HCC may be detected by routine surveillance or may become clinically apparent. The diagnosis of HCC will be made using the AASLD criteria (see Appendix A).

3.2.6. Death

Death may occur related to liver disease (typically hepatic decompensation or HCC) or may occur unrelated to hepatitis B or liver disease. Date and cause of death will be recorded.

4. Selection and enrollment of participants

4.1. Sources of participants

Patients will be recruited from all the centers of the Pediatric Subcommittee of HBRN. Patients who have been seen and are being followed in existing GI and liver clinics as well as those who are newly referred for consultation and seen for the first time at a HBRN center will be recruited.

Some HBRN investigators may engage in outreach activities where HBV patients seen in primary care setting outside the HBRN centers may be accessed. While such activities are allowed and encouraged, those patients need to be invited to a HBRN center for enrollment in the HBRN.

4.2. Inclusion criteria

- Written informed consent/assent as appropriate
- At least 6 months to <18 years of age
- HBsAg-positive

o **Exclusion criteria**

- History of hepatic decompensation based on clinical or laboratory criteria (see 3.2.4 above)
- Hepatocellular carcinoma (HCC)
- History of solid organ transplant or bone marrow transplant
- Chronic Immunosuppression therapy

- Current Hepatitis B antiviral treatment (except pregnant females)
- Known coinfection with HIV (patients with HDV or HCV coinfection are not excluded).
- Medical or social condition which, in the opinion of the investigator, would make the patient unsuitable for the study or interfere with or prevent regular follow-up
- Unable or unwilling to return for regular follow-up

4.3. Participant enrollment procedures

Clinical center investigators and coordinators must be certified by the DCC to begin screening participants. Prior to implementing this protocol, the protocol and consent forms must have Institutional Review Board (IRB) or Research Ethics Board (REB) for Human Research approval.

Once a potential study subject has been identified, details of the study, including possible risks and benefits, will be carefully discussed with him/her and he/she will be asked to sign a consent/assent form. Treatment for HBV will not be offered as part of this Cohort Study.

5. Schedule of visits and procedures

5.1. Screening, consent/assent, and follow-up overview

While many of the subjects likely will be current patients of the HBRN investigators, patients may be referred from physicians outside the Network and some patients may refer themselves. Existing patients who are familiar to the site investigator may be consented and screened at a visit that is part of the ongoing clinical care of the patient.

Patients who are new to the center will undergo initial clinical evaluation to verify HBsAg status and other inclusion/exclusion criteria.

5.2 Initial screening (Screening Log)

HBRN sites will keep a screening log of HBsAg-positive patients seen at their site, beginning with the initiation of the Cohort Study. The following elements will be captured for all HBsAg positive patients:

1. Date screened
2. Year of birth
3. Gender
4. Currently pregnant
5. Race
6. Presence of exclusion criteria: decompensation, HCC, liver transplantation, known HIV positivity or therapy for HIV, current therapy for HBV infection.
7. Date of enrollment in the Cohort Study or, if not enrolled, reason the patient was not enrolled (exclusion criteria, refused, language barrier, unable to comply with follow up or other reason).

Patient identifier and contact information associated with the log entry will be kept securely at the site as it is necessary to prevent repeated screening of the same subject. De-identified data will be entered into the study database so that a summary of those screened may be generated to monitor enrollment and to determine if participants enrolled are representative of patients seen at the site.

5.3. Baseline Evaluation

After informed consent is obtained, the Baseline Evaluation will be done and may, in some instances, be completed over two visits. The second visit must be completed within 12 weeks of the first baseline visit.

The Baseline Evaluation will consist of:

- Participant demographic questionnaire to be completed by the caregiver.
- Health behavior questionnaire to be completed by the adolescent participant (12 years to <18 years).
- Health related quality of life (HRQOL) form to be completed on HBV treatment naïve children only.
    - CHQP for children 5 to < 10 years (by caregiver)
    - CHQ for children ≥10 years (by patient)
- Questionnaire to assess Tanner Stage to be completed by patient between the ages of 8 to <18 years.
- Questionnaire to be completed by the coordinator.
- Questionnaire to be completed by a Physician Investigator.
- Brief physical examination.
- Blood will be drawn for laboratory tests related to hepatitis B and liver disease.
- Blood will be drawn (with consent) to prepare and obtain serum or plasma for storage.*
  * These may be collected at the 24 week follow-up visit if not obtained at baseline.

5.3.1. Participant completed forms

The questionnaire to be completed by the caregiver collects data and information on basic demographics. The questionnaire to be completed by the adolescent participant collects data and information on health behaviors (e.g. smoking and consumption of coffee, tea and alcohol). To assess stage of puberty, participants who are 8 to <18 years of age will complete a self-assessment of Tanner Stage. In addition, for participants who are HBV treatment naïve, the caregiver or subject (based on age) will complete the CHQ form. If the participant or caregiver requires assistance with the completion of any of the forms, this will be recorded.

5.3.2. Coordinator interview and forms

A coordinator will collect the following information:

1. Country of origin of the patient and parents
2. Educational level
3. Family history of hepatitis B liver disease and liver cancer
4. Past medical history
5. Current and past history of antiviral therapy for HBV
6. Medication history
7. Vaccination status of family members
8. Special section for adoptees

5.3.3. Physician Investigator completed form

A physician investigator will record his or her opinion of the following items, based on the initial evaluation data:

- What is the most likely source of the HBV infection?
- What is the most likely time of onset of the HBV infection?
- Is the participant symptomatic of hepatitis B?
- What phenotype best describes the participant?

5.3.4. Physical examination

Vital signs (blood pressure and pulse) and anthropometric measurements (height, weight and waist circumference) will be done and recorded by the coordinator. In addition, a physical examination will be done by a physician or other qualified practitioner focusing on physical signs associated with liver disease.

5.3.5. Laboratory Data: Blood tests related to viral hepatitis and liver disease

When the following laboratory tests are done as a part of routine care, the results will be obtained from the participant's records. Data available 3 months or less prior to the date of consent may be used.

1. Hemoglobin, hematocrit, WBC and platelet count
2. Liver panel (AST, ALT, alkaline phosphatase, GGT, bilirubin [total, direct, indirect], total protein, albumin)
3. Creatinine
4. Alpha fetoprotein
5. INR

In addition, the following serologic/virologic data may be obtained from patient records.

1. HBV DNA level
2. HBeAg
3. anti-HBe
4. IgM anti-HBc (if acute hepatitis B is suspected)
5. anti-HDV
6. anti-HCV
7. anti-HBs
8. anti-HIV
9. anti-HAV
10. Autoimmune markers

The most recent serologic/virologic results will be recorded along with the month and year when the sample was drawn. If recent results are not available from the participant's records, it is recommended that blood be drawn for these tests. For other serologies (anti-HDV, anti-HCV, anti-HIV, and autoimmune markers), there will be no time limits.

5.3.6. Plasma and serum for banking

For participants who consent, blood will be drawn at the baseline evaluation and plasma and serum samples will be banked providing age-dependent blood volume limits are not exceeded (see Appendix D.2 and D.3). Standardized methods for plasma and serum processing will be followed. Plasma and serum samples will be stored locally and ultimately, these samples will be shipped to a central repository for storage to be used for future studies to determine phase of hepatitis B and possibly to predict disease outcomes and response to antiviral therapy.

5.4. Liver histology

Information about liver biopsies obtained as part of standard of care will be collected. In patients who have had a liver biopsy, unstained slides of the biopsy specimen will be requested from the institution where it was done, and they will be shipped to the central pathology site for staining and ultimately central review.

Biopsy slides will be sent for central pathology reading. In situations in which adequate tissue is obtained (greater than 1.5 cm), when a biopsy is done during the study, a portion of the residual tissue will be banked for future studies.

6. Follow-up Evaluation

6.1. Routine follow-up visits

Participants will be followed at predetermined intervals to coincide with accepted standard care visits for managing patients with hepatitis B.

- If the baseline evaluation is completed in one visit, or in two visits that occur within the 12 week window, then time 0 will be the day of the first (or only) baseline visit.
- Routine follow-up will be at weeks 24 and 48 during the first year. The purpose of the first two visits is to determine the stability of the status of the participant.
- Additional follow-up visits may be performed under special circumstances, such as during hepatitis flares (see below).
- Beyond week 48, follow-up evaluations will be conducted at 48 week intervals.

6.1.1. Routine scheduled follow-up data items

Follow-up visits are planned to coincide with accepted standard of care visits for managing patients with chronic hepatitis B. Participants experiencing a disease flare or developing evidence of additional complications of HBV infection will be seen more frequently for clinical monitoring as well. Follow up visits will consist of the following;

- Physical examination
- Blood tests (see schedule)
- Interim history
- Health behaviors by participants 12 years to <18 years (every 48 weeks)
- Brief symptom assessment
- Questionnaire to assess Tanner Stage to be completed by participants 8 to <18 years of age
- Quality of Life (QOL) form: CHQP and CHQ (every 48 weeks) in baseline HBV treatment naïve children only

- Medications
- Assessment for possible interim occurrence of any events related to disease progression (cirrhosis, hepatic decompensation, HCC or liver transplantation)
- Interim antiviral treatment
- Liver biopsy data (if one was performed for clinical indication)
- For participants who consent serum/plasma samples will be collected at each follow-up visit.
- For participants who consent blood will be drawn for DNA extraction, banking, and genetic testing at the week 24 visit. If not obtained at week 24 it may be collected at another visit

6.2. Unscheduled follow-ups

Potential reasons for unscheduled visits may include abnormal liver tests including ALT flares, hepatic decompensation, suspicion for hepatocellular carcinoma, change in HBeAg or HBsAg status, and development of significant non-hepatic condition that affects the management or prognosis of their chronic hepatitis B.

Data items captured at the unscheduled visits will be a subset of those items captured at the routine scheduled follow-up visits, unless specified otherwise in the sections below. For participants who provided consent, blood specimens for storage may be collected at the time of unscheduled visits (e.g. liver biopsy, ALT flare, at time of diagnosis of hepatic decompensation). These specimens will be processed according to the same procedure as routine follow-up samples.

6.2.1. Participants experiencing an ALT flare or HBeAg or HBsAg loss

When the routine protocol follow-up schedule is interrupted by a flare or HBeAg/HBsAg loss a data collection mechanism will be activated as below. Once the event is resolved, the participant will return to the routine protocol follow-up schedule and return for the protocol evaluation that is closest to, but after, the event resolution date. If the event resolution date occurs within 4 weeks of the next scheduled protocol visit, the protocol evaluation visit may be performed in conjunction with the event visit.

6.2.1.1. Evaluating participants experiencing an ALT flare

Flares are defined in section 3.2.1.
The following data will be collected:

- Alcohol use since the last visit for adolescent patients.
- Prescription and non-prescription medications
- Change in comorbidity and metabolic risk profiles (weight, diagnosis of diabetes)
- Other risk factors for acute viral hepatitis or other liver disease such as HDV
- Precipitating event information to determine whether the flare is HBV-related or another event.
- Clinical decisions (e.g., start on antiviral treatment, list for transplantation) made in response to the flare will be recorded.

6.2.1.2. Follow-up plan for participants experiencing an ALT Flare

- Once an ALT flare is diagnosed, the standard of care guideline will be follow-up intervals every 4 weeks. If ALT is greater than 1000 U/L or total bilirubin is greater than 2.5 mg/dl, in the absence of Gilbert's syndrome, follow-up intervals will be every 2 weeks.
- An ALT flare is considered to be resolved when the ALT is 1) <550 U/L in females and <600 U/L in males 6 months – 18 months of age, or 2) <350 U/L in females and <400 U/L in males >18 months - < 18 years of age (12). When the ALT flare is considered to be resolved, the follow-up will revert to the original follow-up schedule.

6.2.1.3. Evaluating participants achieving HBeAg or HBsAg Loss

There are no specific data to be collected in participants who experience HBeAg/HBsAg loss. The regular follow-up data collection adequately captures information of interest.

6.2.1.4. Follow-up in participants achieving HBeAg or HBsAg Loss

The participant will be seen 12 weeks after the visit during which loss occurred, and again 24 weeks after the visit during which loss occurred, and then revert to their original follow-up schedule. The regular follow-up data collection adequately captures information of interest.

6.2.2. Participants who are pregnant

Participants who are pregnant at enrollment or who become pregnant after enrollment will follow the regular follow-up schedule. No specific data collection regarding pregnancy will be collected.

6.3. Follow-up in participants newly diagnosed with cirrhosis

There are no specific data to be collected in patients who are newly diagnosed with cirrhosis. The regular follow-up data collection adequately captures information of interest. The diagnosis of cirrhosis does not change the follow-up interval.

6.4. Follow-up in participants experiencing clinical events

Hepatic decompensation and HCC are expected to be rare. Clinically indicated visits outside of the routine follow-up visits will be captured as an unscheduled visit. As discussed below, detailed information at the time of the diagnosis of these complications will be obtained. There will also be data collection when liver transplantations or death become known for those who had not reached a prior endpoint.

6.4.1. Participants experiencing hepatic decompensation

- Systematic follow-up more than once every 48 weeks is not mandated for participants experiencing hepatic decompensation.
- Information about the event(s) that defined hepatic decompensation including hepatic encephalopathy, ascites, hepatic hydrothorax and variceal or portal hypertensive bleeding will be obtained.
- Blood specimens will be collected at the time when hepatic decompensation is first diagnosed. These specimens will be processed according to the same procedure as routine follow-up samples.

6.5. Participants who have liver biopsy

Information will be collected regarding the technique and possible complications of the procedure.

6.6. Follow-up in participants undergoing liver transplantation

We will record the date of transplantation and whether an incidental HCC was found. If an HCC was found, the HCC form will be completed. Patients undergoing liver transplantation will no longer be followed.

6.7. Follow-up in participants reaching 18 years of age

Patients who reach 18 years of age and are within an adult HBRN clinical center will be offered participation in the adult cohort study and re-consented for the adult protocol.

6.8. Participants who die

Date of death and cause of death will be collected.

7. Informed Consent/assent

Appropriate consent and assent for the cohort study will be obtained from all the participants prior to their enrollment.

8. Statistical and design considerations

8.1. Power analysis

This is an observational study investigating the occurrence of important events such as HBeAg loss or HBeAg seroconversion in children. The endpoints that will be of primary or secondary interest are listed in section 3.2. The power calculation for this study is based on the assumption that 500 children will be followed on average for approximately 2.4 years (1200 person-years). Here we give an example of the statistical powers that will be afforded for different hypotheses of interest based on the primary outcome of HBeAg loss.

Scenario 1. Age effect:
Null hypothesis: There is no age effect, meaning the annual rate of HBeAg loss is same across all age groups
Alternative hypothesis: The rates in various age groups are different from each other.

Assumptions:

1. The annual rates of HBeAg loss are as follows:
    a. 0.5% in less-than-5-year-olds
    b. 3% in 5-15-year-olds
    c. 10% in >15-year-olds
2. The age distribution is uniform, meaning there will be equal number of children in each age group.

The study will have over 99% power to detect such an effect using a chi-square test at 5% level of significance.

Scenario 2. Ethnicity effect (Asian vs. non-Asian):

Null hypothesis: There is no ethnicity effect, meaning the annual rate of HBeAg loss is same across Asians and non-Asians
Alternative hypothesis: The annual rates of HBeAg loss are different across ethnic groups (we assumed 5.3% in Asians and 10% in non-Asians)

Assumption: 75% of the children will be Asian and 25% will be non-Asian.

The study will have 81% power to detect such an effect using a two-sided Fisher's exact test at 5% level of significance.

8.2. Statistical analysis plan

The primary aim of this study is to describe participants 6 months to < 18 years of age with hepatitis B virus (HBV) infection and to identify predictors of disease activation and progression. The participants will be characterized by demographics, comorbid conditions, disease, and treatment history. Distribution of participants across different subgroups such as race, ethnicity and age-group will be

reported using group-specific proportions and their 95% exact or approximate confidence intervals as appropriate. Continuous participant characteristics such as age, HBV DNA levels and ALT will be summarized using mean and standard deviations (for normally distributed variables) or median and inter-quartile range (for non-normal variables). Distribution of ALT levels will also be investigated using the proposed cut-offs of a flare, which is defined as serum ALT greater than or equal to 10 times the upper limit of normal which corresponds to 1) 550 U/L in females and 600 U/L in males 6 months – < 18 months of age, and 2) 350 U/L in females and 400 U/L in males >18 months - < 18 years of age. Participant characteristics across subgroups will be graphically presented using histograms for categorical variables and box plots, histogram, and density plots for continuous variables.

Participants in different phases of HBV infection will be characterized by the distribution of demographic characteristics such as age, gender, race, ethnicity; medical history and sources of infection; and virological characteristics such as genotype. For continuous variables such as age, ALT and HBV DNA levels, group-specific summary statistics such as mean or median will be provided and uncertainty around such measures would be measured by standard deviations or inter-quartile range, as appropriate. Graphical procedures such as box plots and density plots will be used to visually examine the distribution across phases. Comparison of such variables across different phases will be done by analysis of variance or its nonparametric equivalent Kruskal-Wallis test. For categorical variables such as race and genotype, contingency tables will be used for investigating distribution of such characteristics across participants in different phases of HBV infection. Chi-square or its exact equivalent will be used for formal test of association.

Estimating event (e.g. HBeAg loss, disease progression, and others listed in section 3.2) probabilities and identifying demographic, clinical, and histological factors associated with these events are of prime interest. Event probabilities, their variances, and confidence intervals will be estimated using proportions. Both rates per person-month and cumulative probabilities of event-free time, and their associated confidence intervals will be calculated. These statistics will be calculated overall and for important subgroups (e.g., genotypes, ethnicity, age-groups, people with high vs. low HBV DNA levels). Hypothesis tests will also be used to determine whether probabilities, rates, or cumulative probabilities differ between (or among) subgroups using chi-square tests for association, differences in Poisson parameters or log rank statistics, and the proportional hazards model, respectively. For all statistical tests, $p<0.05$ (two-sided) will indicate statistical significance.

Predictors of clinical outcomes (e.g., HBeAg loss, HBeAg seroconversion, HBV-DNA loss,) will be assessed using regression models: logistic regression for event probabilities (e.g., proportion with HBeAg loss, HBeAg seroconversion, HBV-DNA loss death), Poisson regression for rates per person-month (HBeAg seroconversion incidence rates), and proportional hazards models for time to event data (e.g. time to HBeAg loss, HBeAg seroconversion, HBV-DNA loss, death,).

Distribution of variables over time will be analyzed using linear (for continuous variables such as HBV DNA), generalized linear (for binary or count, such as number of adverse events) models, as appropriate. These models will be used to assess the association of baseline and time varying participant characteristics with the longitudinal outcome data.

HRQOL scores across demographic categories will be compared using Wilcoxon test (Kruskal-Wallis test) or t-test (Analysis of Variance) as appropriate; Multivariable linear regression will be used to identify independent factors associated with HRQOL in the statistical models; analyses will include variables previously known to be associated with quality of life (e.g., age, sex). To compare the

HRQOL scores of treatment naïve HBV patients to those of the treatment naïve HCV patients, we will use two-sample procedures such as t-test or Wilcoxon test, as appropriate.

Statistical analysis plan for quantitative HBsAg and HBV DNA cut-offs as predictors of inactive carrier.

One of the goals of this study is to assess the accuracy of "baseline HBsAg below 1,000 IU/mL and HBV DNA below 1,000 IU/mL" as a predictor of inactive carriers, the definition of which is outlined in the secondary aims of this study, At baseline, each participant in the cohort study will be designated as "Inactive" or "Active" based on the following test. If the participant's baseline HBsAg level is below 1,000 IU/mL and HBV DNA level is below 1,000 IU/mL, the participant will be labeled as "Inactive"; Otherwise, the participant will be labeled as "Active". Participants will then be followed up routinely according to the cohort schedule to determine if they become a true inactive carrier.

The analysis will be centered around a 2X2 table, where each patient will belong to one of the four cells at the end of the study.

Table: Test result vs. true hepatitis B activity status. TA = True Active, FA = False Active, TI = True Inactive, FI = False Inactive, DA = Designated Active, OA = Observed Active, DI = Designated Active, OI = Observed Inactive

	Observed status		
Designated status at baseline	**Inactive**	**Active**	**Total**
Inactive	TI	FI	DI
Active	FA	TA	DA
Total	OI	OA	n

We will estimate the positive predictive value (PPV) and negative predictive value (NPV) of the test defined respectively as the proportion of participants who become inactive among those who were designated inactive at baseline, and the proportion who remain active among those who were labeled active at baseline. These are estimated as follows:

PPV = TI/DI

NPV = TA/DA.

We will also estimate retrospective sensitivity (SE) and specificity (SP) as follows:

SE = TI/OI

SP = TA/OA.

In addition, the false positive rate (FPR) and false negative rate (FNR) will be calculated as follows:

FPR = FI/OA

FNR = FA/OI

Standard errors of these quantities will be calculated using exact and approximate (asymptotic) methods for proportions. Exact and Wald 95% confidence intervals will be constructed for each of the above proportions.

We will also calculate Youden's index defined by J = SE – FPR = SE+SP-1, and the corresponding confidence interval. The Youden's index in this setting reflects the likelihood of being labeled as "inactive' among participants who will eventually become inactive versus those who will not.

Exploratory analysis will be performed with HBsAg level <2,000 IU/mL and HBV DNA <2,000 IU/mL as cutoffs to predict inactive carrier as defined in the cohort protocol. Exploratory analysis will also be performed using alternative definitions of inactive carriers as used in EASL and AASLD Practice guidelines.

8.3. Missing data

In general, missing outcome data will be handled as follows in statistical analyses. The primary analyses will be conducted under the assumption that the probability of missing (dropout) depends on observed outcome or covariates (missing at random, MAR), which is a relatively less stronger assumption than missing completely at random (MCAR) where the likelihood of missingness is assumed to be independent of observed or unobserved data. To account for the missing data statistical methods described in section 8.2 will be weighted using inverse-probability-weighting (IPW) method. IPW method provides consistent estimates in the presence of MAR data. In the event the probability of missing depends on unobserved outcome and/or covariates (missing not at random, MNAR), we will use selection models such as MNAR Dale model and Diggle-Kenward model. These models often require strong assumptions on the dropout mechanism which are primarily unverifiable based on the observed data. We will conduct sensitivity analyses to investigate the sensitivity of our conclusions to possible violation of such assumptions by fitting models under MCAR, MAR and MNAR assumptions and compare the model fits using log-likelihoods.

9. Data management

Data will be submitted to the Data Coordinating Center (DCC) via a distributed web-based data entry system. Clinical center coordinators and other project personnel will be trained and certified to collect and enter data using established systems. Clinical centers will have the option to use any front end device (ex. TabletPC, laptop, desktop) that provides access to the Internet.

DCC personnel will closely monitor clinical center adherence to study protocol and data collection practices for complete and accurate research data. Monitoring will be performed via established data management procedures with on-site monitoring visits conducted at designated intervals, or as needed, to facilitate the smooth conduct of the study. At the time of the on-site visit, DCC personnel will have access to all study and patient documents and to clinical center personnel. All patient and study documents will be kept confidential. Identifiers such as patient name and address will not be included on any data sent to the DCC.

DCC personnel meet weekly to discuss study status, recruitment, compliance, review data issues, clinical center participation, and other issues that arise during the course of the study.

10. Study Organization – Sites

This study will be conducted at approximately 7 clinical centers within the United States and Canada. A Data Coordinating Center (DCC) based at the University of Pittsburgh will coordinate operations, develop and implement data and other systems, maintain the database and perform data analyses. This study will use a biospecimen and a genetic repository. A central virology lab, pathology lab, and one or more central testing labs will be utilized to perform research tests and to store specimens identified in the protocol.

11. Human subjects issues

11.1. Overview

The study protocol, consent forms, and data collection forms will be submitted to each clinical center's IRB and to the DCC's IRB. Additionally, each clinical center will submit to their IRB any

recruitment materials to be used at their site. A site may not initiate any patient contact about the HBRN study until the site has IRB approval for the studies and the DCC has certified the site for initiation of patient activities. All study personnel will have completed training in the Protection of Human Subjects per NIH guidelines. Given the epidemiology of HBV infection, subjects included in this study will include a large proportion of racial/ethnic minorities (Asian, American Indian/Alaska Native, Native Hawaiian or other Pacific Islander, Black or African American, Hispanics or Latino) as well as non-Hispanic white subjects. We anticipate that the patients recruited from diverse sources, including community and tertiary referral populations, will capture the entire spectrum of HBV infection.

11.2. Standard of care
All subjects enrolled in the HBRN studies will receive standard of care for HBV infection and identified associated medical problems as defined by the HBRN. This will include provision of health care, laboratory testing, HCC surveillance, counseling and educational materials at enrollment and on an ongoing basis during follow-up.

11.3. Enrollment in other HBRN studies
The HBRN is planning to conduct a clinical trial in immunotolerant children and adolescents aimed at improving effectiveness of therapy and exploring the effects of treatment in this category of patients. All subjects to be enrolled in these treatment studies will have been enrolled in the longitudinal study and have completed at least the baseline visit. Those found to be potentially eligible for enrollment in a treatment study will be offered participation and caregivers will be asked to sign a separate consent form. Once they are enrolled in the immunotolerant trial, they will be deemed to be in inactive status in the longitudinal study unless they withdraw their consent. Subsequently when participation in the immunotolerant trial is completed (either by virtue of study completion or early withdrawal), subjects will be asked to resume follow-up according to the cohort protocol.

11.4. Institutional Review Board (IRB) approval
A site may not initiate patient activities in the HBRN Network until the site has IRB approval. Consent forms must have IRB approval. Sites must provide the DCC with copies of the initial IRB approval notice and subsequent renewals, as well as copies of the IRB approved consent forms.

11.5. Consent/Assent forms
Prototype consents and assents will be prepared for the study. Individual sites may add material but may not delete material thought to be necessary for informed consent. Sites may reformat and reword information to conform to their local requirements. A copy of the approved site-specific consent form must be submitted to the DCC for review and archive. A signed consent form will be obtained from the caregiver. The subject's assent (for enrollees who are 12 to <18 years of age or as per site assent age requirements) must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the caregiver, and this fact will be documented in the subject's record.

11.6. Subject confidentiality
Clinical sites are responsible for the confidentiality of the data associated with participants in the HBRN studies in the same manner they are responsible for the confidentiality of any patient information within their spheres of responsibility. All forms used for the study data will be only identified by coded identifiers to maintain subject confidentiality. All records will be kept in locked file cabinets at the clinical centers with access limited to HBRN study staff. All study staff will identify

patients by the patient identifier number generated at the clinical center. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB or Data and Safety Monitoring Board (DSMB). Clinical information may be reviewed during site visits by the DCC and the NIDDK Project Officer. Participants grant permission to share research data with these entities in the consent document. Federal regulations govern the protection of patient's rights relative to data confidentiality and use of research data.

Consent procedures and forms, and the communication, transmission and stoppage of patient data will comply with individual site IRB and NIH requirements for compliance with The Health Insurance Portability and Accountability Act (HIPAA). The DCC will require that clinical centers provide documentation from the site IRBs with the appropriate authorization or consent form.

11.7. Data and Safety Monitoring

Data and safety will be monitored by the NIDDK in conjunction with an NIDDK-appointed Data and Safety Monitoring Board (DSMB). This board serves in a consultative capacity to inform the NIDDK decisions regarding conduct of the study. The description of DSMB activities is included in the DSMB Charter.

The DSMB charter will be developed by the NIDDK. It will monitor all aspects of the study (e.g., recruitment, protocol deviations, breeches of confidentiality, data quality, attrition, descriptive characteristics), and recommend protocol modifications, including early study termination. Reports will be prepared by the DCC. Tables showing study progress will be presented by clinical center and overall. These will include recruitment, protocol deviations, attrition, breeches of confidentiality, and data quality. The DCC will maintain a cumulative summary of breeches of confidentiality to be forwarded to the DSMB for their meetings via conference call or in person. Based on the data presented, the DSMB will recommend continuation or termination of the study. A summary of the DSMB findings will be forwarded to all investigators for submission to their respective IRBs.

11.8. Participant withdrawal from Study

If a participant chooses to withdraw from a HBRN study, all data collected up to the point of withdrawal will remain in the study database, but no further data may be collected. The participant must submit a written request to withdraw to the clinical center personnel. This is consistent with HIPAA guidelines and regulations. A participant may also withdraw consent for use of data or stored specimens – in this case, any specimens collected from this subject will be destroyed and data deleted.

References

1. Bortolotti F, Guido M, Bartolacci S *et al.* Chronic hepatitis B in children after e antigen seroclearance: final report of a 29-year longitudinal study. Hepatology 2006;43:556-62.

2. Beasley RP, Hwang LY, Lin CC *et al.* Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. Lancet 1981;2:1129-33.

3. Evans AA, Chen G, Ross EA *et al.* Eight-year follow-up of the 90,000-person Haimen City cohort: I. Hepatocellular carcinoma mortality, risk factors, and gender differences. Cancer Epidemiol Biomarkers Prev 2002;11:369-76.

4. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008;48:335-52.

5. Fattovich G, Olivari N, Pasino M *et al.* Long-term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years. Gut 2008;57:84-90.

6. Cohen C, Evans AA, London WT *et al.* Underestimation of chronic hepatitis B virus infection in the United States of America. J Viral Hepat 2008;15:12-3.

7. Chen CJ, Iloeje UH, Yang HI. Long-term outcomes in hepatitis B: the REVEAL-HBV study. Clin Liver Dis 2007;11:797,816, viii.

8. Chen CJ, Yang HI, Su J *et al.* Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.

9. Fung J, Lai CL, But D *et al.* Prevalence of fibrosis and cirrhosis in chronic hepatitis B: implications for treatment and management. Am J Gastroenterol 2008;103:1421-6.

10. Hsu HY, Chang MH, Hsieh KH *et al.* Cellular immune response to HBcAg in mother-to-infant transmission of hepatitis B virus. Hepatology 1992;15:770-6.

11. Lee PI, Chang MH, Lee CY *et al.* Changes of serum hepatitis B virus DNA and aminotransferase levels during the course of chronic hepatitis B virus infection in children. Hepatology 1990;12:657-60.

12. Schwimmer JB, Dunn W, Norman GJ *et al.* SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. Gastroenterology 2010;138:1357,64, 1364.e1-2.

13. Chang MH, Hsu HY, Hsu HC *et al.* The significance of spontaneous hepatitis B e antigen seroconversion in childhood: with special emphasis on the clearance of hepatitis B e antigen before 3 years of age. Hepatology 1995;22:1387-92.

14. Ni YH, Chang MH, Chen PJ *et al.* Viremia profiles in children with chronic hepatitis B virus infection and spontaneous e antigen seroconversion. Gastroenterology 2007;132:2340-5.

15. Weinbaum CM, Williams I, Mast EE *et al.* Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. MMWR Recomm Rep 2008;57:1-20.

16. England K, Thorne C, Pembrey L *et al.* Age- and sex-related reference ranges of alanine aminotransferase levels in children: European paediatric HCV network. J Pediatr Gastroenterol Nutr 2009;49:71-7.

17. Schwimmer JB, Dunn W, Norman GJ *et al.* SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. Gastroenterology 2010;138:1357,64, 1364.e1-2.

18. Chang MH, Chen PJ, Chen JY *et al.* Hepatitis B virus integration in hepatitis B virus-related hepatocellular carcinoma in childhood. Hepatology 1991;13:316-20.

19. Livingston SE, Simonetti JP, McMahon BJ *et al.* Hepatitis B virus genotypes in Alaska Native people with hepatocellular carcinoma: preponderance of genotype F. J Infect Dis 2007;195:5-11.

20. Gambarin-Gelwan M. Hepatitis B in pregnancy. Clin Liver Dis 2007;11:945,63, x.

21. Tan HH, Lui HF, Chow WC. Chronic hepatitis B virus (HBV) infection in pregnancy. Hepatol Int 2008;2:370-5.

22. Nguyen G, Garcia RT, Nguyen N *et al.* Clinical course of hepatitis B virus infection during pregnancy. Aliment Pharmacol Ther 2009;29:755-64.

23. ter Borg MJ, Leemans WF, de Man RA *et al.* Exacerbation of chronic hepatitis B infection after delivery. J Viral Hepat 2008;15:37-41.

24. Jonas MM. Hepatitis B and pregnancy: an underestimated issue. Liver Int 2009;29 Suppl 1:133-9.

25. Landgraf JM, Abetz L, Ware JE. The Child Health Questionnaire User's Manual.
, 1st ed., edn. The Health Institute, New England: Boston, MA:, 1996.

26. Rodrigue JR, Balistreri W, Haber B *et al.* Impact of hepatitis C virus infection on children and their caregivers: quality of life, cognitive, and emotional outcomes. J Pediatr Gastroenterol Nutr 2009;48:341-7.

Appendix A: Definitions

A.1. Acute hepatitis B

Presence of HBsAg and IgM anti-HBc with serum ALT values greater than 300 U/L and absence of known history of HBsAg positivity. Probable acute hepatitis B is when all above criteria are met except serum ALT is less than or equal to 300 U/L or if there is any suspicion of chronic disease.

A.2. Phases or phenotypes of chronic hepatitis B

Immune tolerant: Presence of HBsAg and HBeAg and normal ALT levels on two occasions or more at least 6 months apart. HBV DNA levels of greater than 1,000,000 IU/mL.

HBeAg-positive chronic hepatitis: Definite presence of HBsAg and HBeAg and abnormal serum ALT levels (at least twice the ULN) on two occasions or more at least 6 months apart. HBV DNA levels of greater than 10,000 IU/mL. **Probable:** Presence of HBsAg and HBeAg and HBV DNA greater than 10,000 IU/mL, but ALT levels between 1-2 times the ULN.

HBeAg-negative chronic hepatitis: Definite presence of HBsAg without HBeAg but with abnormal serum ALT levels (at least twice the ULN) on two occasions or more at least 6 months apart. HBV DNA levels of greater than or equal to 1,000 IU/mL. **Probable:** Presence of HBsAg without HBeAg and HBV DNA greater than or equal to 1,000 IU/mL, but ALT levels between 1-2 times the ULN.

Inactive carrier: Definite presence of HBsAg without HBeAg and normal ALT levels and HBV DNA levels of less than 1,000 IU/mL on two occasions or more at least 6 months apart. **Probable:** Presence of HBsAg without HBeAg and HBV DNA between 1,000-10,000 IU/mL, but ALT levels normal.

Indeterminate: Does not fit into any of the above categories.

Table 1: Criteria for determining Phases or Phenotypes of chronic hepatitis B (CHB)

	HBeAg	ALT	HBV DNA (IU/mL)
Immune tolerant	Positive	Normal	>1,000,000
HBeAg+ CHB	Positive	Elevated*	>10,000
HBeAg- CHB	Negative	Elevated*	≥1,000
Inactive carrier	Negative	Normal	<1,000**

* ≥ 2 times ULN = definite, 1-2 times ULN = probable (See A.3 below for definitions of normal ALT)
** HBV DNA 1,000-10,000 IU/mL with normal ALT and no HBeAg-probable

Notes:

- The phase will be assigned based on HBeAg, ALT and serum HBV DNA level, regardless of the presence or absence of anti-HBe in serum
- Probable: 1) if all other criteria for chronic hepatitis B are met and serum ALT is elevated 1-2x ULN, participant will be assigned to “probable” chronic hepatitis B (HBeAg positive or negative) or 2) if all other criteria for immune tolerant are met and serum HBV DNA is between 100,000 and 1,000,000 IU/mL, participant will be assigned to “probable” immune tolerant or 3) if all other criteria for inactive carrier are met and serum HBV DNA is between 1,000 and 10,000 IU/mL, participant will be assigned to “probable” inactive carrier

- Indeterminate: The phase or phenotype will be "indeterminate" if the participant does not clearly fall into any one of the above definite or probable categories, or two or more sets of results at baseline are contradictory.

A.3. Normal ALT

The following normal ALT levels will be used regardless of the laboratory at which the test is done.

Age	Male	Female
6 months - ≤ 18 months	≤ 60 U/L	≤ 55 U/L
>18 months - <18 years	≤ 40 U/L	≤ 35 U/L

A.4. ALT flare

Serum ALT greater than or equal to 10 times the upper limit of normal which corresponds to 550 U/L in females and 600 U/L in males 6 months - ≤18 months and 350 U/L in females and 400 U/L in males >18 months - <18 years.

A.5. Loss/Seroconversion/Seroreversion

A.5.1. HBeAg

In a participant who is previously HBeAg-positive and anti-HBe-negative, disappearance of HBeAg from the serum is HBeAg *loss*.

If this is accompanied by emergence of anti-HBe, it is HBeAg *seroconversion*.

Among HBeAg-negative participants, reappearance of HBeAg is considered HBeAg *seroreversion*.

A.5.2. HBsAg

By definition, a participant with HBV infection is HBsAg-positive. Anyone who is HBsAg-positive regardless of the presence or absence of anti-HBs is considered to be HBV-infected. A subsequent disappearance of HBsAg from the serum is HBsAg *loss*.

If this is accompanied by emergence of anti-HBs, it is referred to as HBsAg *seroconversion*.

A.6. Clinical events

Clinical events will be adjudicated by a Pediatric Outcomes Subcommittee consisting of at least three clinical investigators and representatives from the DCC and the NIDDK. The subcommittee will develop criteria for judging outcomes and adjudicate them as definite, probable or unlikely. Cases to be adjudicated include etiology of ALT flares, cirrhosis, hepatic decompensation, HCC, and cause of death.

A.6.1. Cirrhosis

In the absence of histological diagnosis, cirrhosis is defined as:

- Any one of the following
    - Presence of ascites or hepatic hydrothorax
    - Variceal or portal hypertensive bleeding
    - Hepatic encephalopathy
    - Child-Turcotte-Pugh (CTP) score of 7 or above

or in the absence of hepatic decompensation

- Any two of the following (in the absence of another explanation)
    - Splenomegaly
    - Nodular liver
    - Platelet count below 120,000/mm^3

A.6.2. Hepatic decompensation

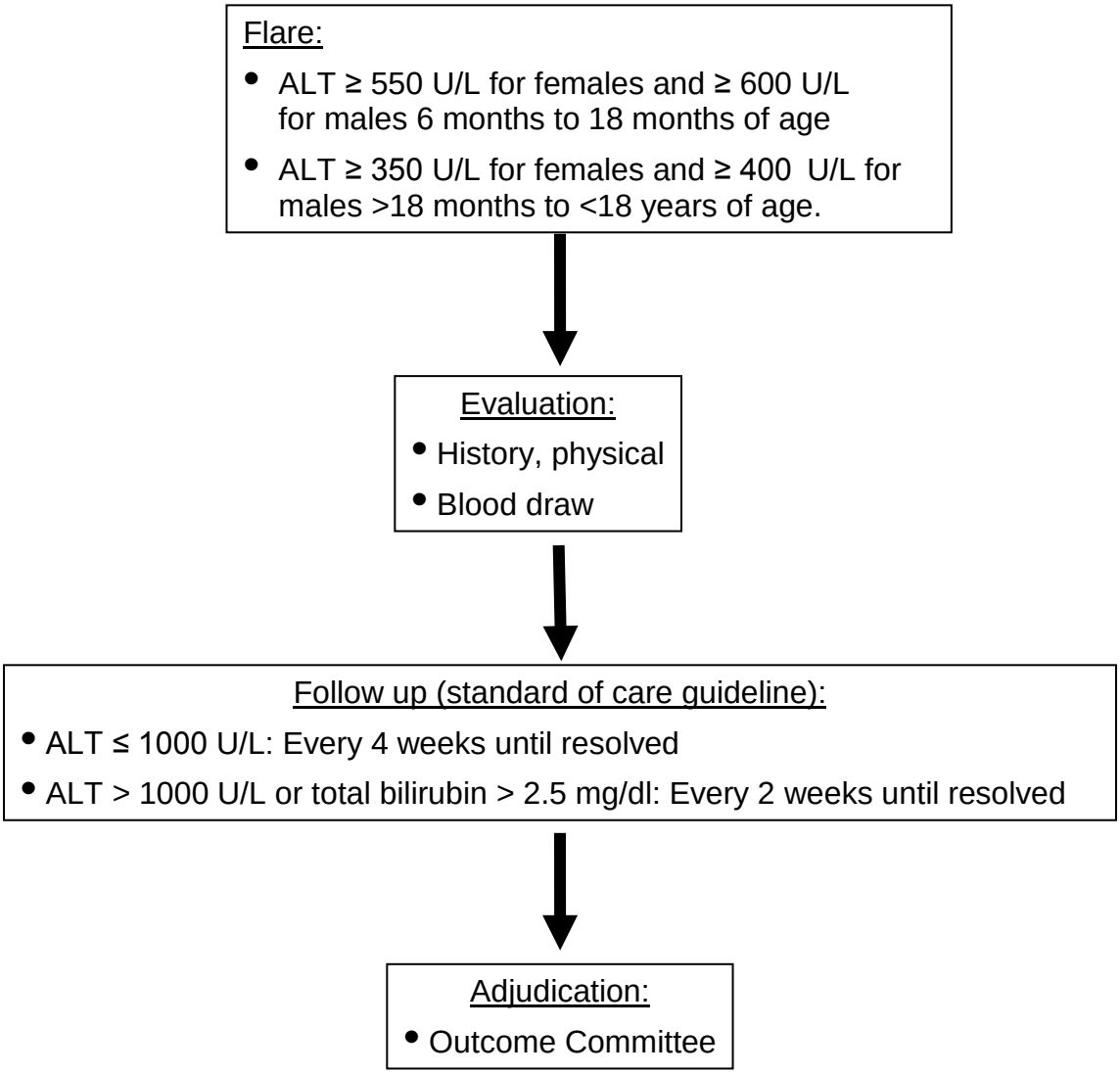
Development of hepatic decompensation will be defined by any of the following events:

- Ascites or hepatic hydrothorax
- Variceal or portal hypertensive bleeding
- Hepatic encephalopathy
- Child-Turcotte-Pugh (CTP) score of 7 or above

A.6.3. Hepatocellular carcinoma (HCC)

The diagnosis of HCC will follow the current AASLD guidelines.

Appendix B: Evaluation and Follow-up for participants experiencing an ALT flare



Appendix C: Participating Pediatric centers

1. Johns Hopkins University/University of Minnesota are multi-PI consortium together with the University of Washington and University of Texas Southwestern
2. University of California, San Francisco
3. University of Toronto
4. Saint Louis University

Appendix D

D.1: Data Collection Schedule

Item Cohort Calendar (weeks)	Baseline	Scheduled 24	Scheduled 48	Beyond week 48 Repeat every 48 weeks
Informed consent	X			
Demographics (year of birth, sex, race)	X			
Country of origin, education	X			
Family Hx, risk factors	X			
Health behaviors	X		X	X
CHQP or CHQ	X		X	X
Tanner stage forms	X		X	X
Symptoms	X	X	X	X
Medical history	X	X	X	X
Medication history (HBV Tx)	X	X	X	X
Medication history	X	X	X	X
Height, Weight, Waist circumference	X	X	X	X
Blood pressure	X	X	X	X
Brief physical exam	X	X	X	X
CBC	X		X	X
ALT	X	X	X	X
Hepatic panel (AST, bilirubin, albumin)	X	X	X	X
GGT	X		X	X
Creatinine	X		X	X
INR	X		X	X
Qualitative HBsAg	X		X	X
IgM anti-HBc	Xb			
Qualitative HBeAg	X	Xf	Xf	Xf
Anti-HBs	X		X	X
Anti-HBe	X	Xf	Xf	Xf
Quant HBV DNA	X	X	X	X
HBV genotype and subtype	Xa			
Anti-HCV	Xa			
Anti-HDV	Xa			
Anti-HIV	Xa			
Anti-HAV	Xa			
Autoimmune markers	Xa			
Abdominal Imaging	Xc			
Liver biopsy	Xc			
Plasma and serum banking (central repository)	Xd	Xd	Xd	Xd
DNA banking (genetics)		Xe		

X: Record available data

a: Results at any time

b: In new participants with uncertain diagnosis of hepatitis B

c: Results within 2 years if available and repeat when clinically indicated

d: Can be obtained based on the blood volume restriction detailed in Appendix D.2

e: Can be obtained at any visit if not at week 24

f: May not be repeated in HBeAg- participants unless a flare

Additional samples may be collected at the time of a special visit (e.g. liver biopsy, ALT flare).

D.2: Research Whole Blood Draw Schedule

The amount of blood obtained for research purposes will be dependent upon the weight of the patient and the amount of blood needed for clinical purposes. Center specific guidelines will be followed in regard to the maximum amount of whole blood taken during a single draw. Whole blood volumes in the following table will be used as a guide.

Item	**Weight (kg)**								
	Baseline			**Week 24**			**Week 48 and every 48 weeks thereafter**		
	<20	**20-40**	**>40**	**<20**	**20-40**	**>40**	**<20**	**20-40**	**>40**
Volume for central labs (mL)	**10**	**10**	**25**	**7.5**	**10**	**17.5**	**10**	**7.5**	**17.5**
Genetics^				**5**	**5**	**5**			
Storage (central repository)	**4**	**8**	**8**				**4**	**8**	**8**
Total (mL)	**14**	**18**	**33**	**12.5**	**15**	**22.5**	**14**	**15.5**	**25.5**

* Can be obtained at any visit if not obtained at baseline or week 24, if blood volume permits.
** Can be obtained at any visit if not obtained at baseline, if blood volume permits.
^ Can be obtained at any visit if not obtained at week 24, if blood volume permits.