Childhood Liver Disease Research Network (ChiLDReN)

FibroScan™ in Pediatric Cholestatic Liver Disease (FORCE) Study Protocol

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1 Introduction and Overview of ChiLDReN Studies

ChiLDReN is a National Institute of Diabetes, and Digestive and Kidney Diseases (NIDDK) sponsored consortium of leading clinical centers that provide care for children with various forms of cholestatic liver disease. The network has been in existence since 2002 and has developed advanced infrastructure for the conduct of clinical investigations in 8 rare pediatric liver diseases. The primary disorders under study by ChiLDReN are Alagille syndrome, alpha-1 antitrypsin deficiency, bile acid synthesis defects, biliary atresia, cystic fibrosis liver disease, idiopathic neonatal hepatitis, mitochondrial hepatopathies and progressive familial intrahepatic cholestasis. These cholestatic disorders collectively comprise a large proportion of infants and young children with chronic liver disease who develop significant morbidity and mortality.

2 FORCE Study Overview

Noninvasive monitoring of liver fibrosis is an unmet and critical need within the clinical management of children with chronic liver disease. While liver biopsy is often used in the initial diagnostic evaluation of children with liver disease, subsequent surveillance liver biopsy is rarely performed in children because of its inherent invasiveness and risks. Therefore, our understanding of the natural history of fibrosis progression in children is limited. The patchy nature of fibrosis in many important pediatric liver diseases [e.g. biliary atresia (BA) and cystic fibrosis liver disease (CFLD)] limits the utility of sequential liver biopsy even if it were to be employed in clinical practice in pediatrics. Thus, non-invasive means of assessing liver fibrosis throughout the liver would be highly desirable and clinically useful in pediatric hepatology. ChiLDReN is poised and uniquely qualified to conduct a comprehensive longitudinal assessment of the utility of FibroScan™-specific elastography, liver stiffness measurement (LSM) as a measure of hepatic fibrosis in children with serious chronic cholestatic liver disease.

3 Background, Study Rationale

Fibrosis and severity of disease in pediatric liver disease is clinically important

The progression and resolution of liver fibrosis is an intricate process involving both parenchymal and non-parenchymal liver cells in addition to infiltrating immune cells. Repetitive or chronic hepatocyte turnover is a critical step in activating inflammatory and pro-fibrogenic pathways [1]. The fibrogenic response, characterized by scar formation due to increased production and deposition of extracellular matrix proteins, has mechanical and physical consequences [2] , including increased stiffness of the liver. Progressive hepatic fibrosis occurs in most children with BA and in a subset of children with alpha-1 antitrypsin deficiency (A1AT) and Alagille syndrome (ALGS). This progressive fibrosis ultimately results in cirrhosis, portal hypertension and well-known, significant clinical complications (including ascites, variceal hemorrhage, spontaneous bacterial peritonitis and end-stage liver disease) [3, 4]. In BA, fibrosis is prominent early in the course of disease and would be an ideal target for newly developed anti-fibrotic pharmacotherapies.

At what, if any, point fibrosis becomes irreversible is not fully understood. However, increasing evidence has shown that early stages of cirrhosis may be reversible independent of underlying etiology [5-8]. The early detection of fibrosis and significant liver disease is challenging as clinically available serum chemistries to measure liver inflammation or damage can often be normal even in advanced cirrhosis [9]. Understanding the degree of injury and the stage and rate of progression of fibrosis in children with chronic liver disease is vital in the complex decision-making of initiating novel medical or surgical therapies and the timing of liver transplant evaluation.

The current gold standard for detecting liver fibrosis is invasive

Liver biopsy is generally accepted as the most sensitive and relied upon procedure to assess degree of liver fibrosis (F0-F4 histological staging) [10]. However, liver biopsy has risks including pain, limited physical activity for up to 2 weeks, hemorrhage and occasional hypotension, infection, inconvenience, and cost of an overnight admission or prolonged observation period, child and parental anxiety, need for sedation or general anesthesia, and in rare cases, mortality [11, 12]. While regarded as both a necessary and relatively low-risk procedure, significant complications have been reported in children [13-15]. It is limited by sampling error (small tissue core size, limited area of liver sampled) and inter-observer variability in interpretation of biopsy histology. Lastly, standard scoring systems have a maximal reading of F4 or F6 (cirrhosis), without the ability to routinely quantify fibrosis beyond cirrhosis. It has been presumed, without proof, that liver biopsy would be a gold standard for any trial of an anti-fibrotic agent. This presumption may be flawed based upon the patchy nature of fibrosis and may be impractical due to issues related to sequential liver biopsy in children, especially those with advanced disease, thrombocytopenia and/or coagulopathy.

FibroScan™ is a non-invasive modality to detect and quantify global liver fibrosis

Traditional gray scale ultrasound imaging (sonography) uses high-frequency sound waves to view soft tissues and organs and is an excellent imaging modality for the pediatric population, limiting the use of radiation. However, conventional ultrasound is limited by operator experience and technique, interpretation bias, poor correlation with liver histological fibrosis, and by the inability to differentiate between liver steatosis and fibrosis, a particularly important distinction given the childhood obesity epidemic [16, 17]. Further, a non-invasive imaging modality that can differentiate between mild and moderate fibrosis (i.e. F1/F2 vs F2/F3) has not yet been validated in pediatric liver disease.

FibroScan™ (*Echosens*), a complementary non-invasive ultrasound tool, measures the inherent elasticity of the liver, which may be altered by pathologic processes such as inflammation, tumor, hepatic congestion, and importantly fibrosis [18, 19]. Elasticity of tissue in the context of elastography is the ratio of tension (stress) needed to produce a relative change in length (strain), and quantifies how much pressure must be placed on tissue in order to cause elastic deformation [20, 21]. FibroScan™ has the ability to assess small changes in pliability of liver tissue across the entire liver, thus providing a more global assessment of liver fibrosis than a single core of liver tissue. A noninvasive method to capture this information is desperately needed to advance the care of children with significant liver disease and to provide for measurements during clinical trials. Moreover, global assessment of fibrosis might serve as both a predictor/descriptor of disease course but also as a critical biomarker for clinical research. FibroScan™ and obtained liver stiffness measurement (LSM) have great potential to fill this void. There are limited large-scale cross-sectional data on the correlation of FibroScan™ LSM in children with clinical status and no data on changes in FibroScan™ LSM over time in pediatric liver diseases. One of the major limitations is the availability of correlative liver biopsies.

FibroScan™ data are available for 116 children from France, although liver biopsy histology for comparison was available for only 33 children [22]. Despite this limitation there was strong correlation of FibroScan™ LSM and fibrosis stage (AUROC 0.88 [0.68-0.95]). A recent cross-sectional study of 31 Chinese children with BA comparing LSM with Metavir fibrosis stage demonstrated a similar AUROC of 0.866 for ≥ F4, further suggesting a cut-off value of 15.15 kPa for ≥ F4, with a sensitivity, specificity, positive predictive value and negative predictive value of 0.857, 0.917, 0.750, and 0.957, respectively [23]. In addition, FibroScan™ LSM in children with BA following hepatoportoenterostomy appears to correlate with important clinical features of portal hypertension (PHT), including splenomegaly and/or the presence of esophageal

varices (EV) or gastric varices (GV) [24, 25]. In this study from Thailand, 73 patients with BA (male:female=32:41; age 9.11 ± 5.64 years) were compared with 50 normal controls (male:female = 19:31; age 11.00 ± 3.31 years). The LSM of patients with BA was significantly higher than controls (27.37 ± 22.48 and 4.69 ± 1.03 kPa; $p < 0.001$). Patients with EV or GV also had significantly higher LSM than those without (37.7 $\pm$ 21.6 [n = 39] vs 11.0 $\pm$ 8.7 [n = 34], $p<0.001$). Similar findings were observed for children with splenomegaly relative to those without (38.9 $\pm$ 22.0 [n = 39] vs 9.9 $\pm$ 6.0 [n = 34], $p <0.001$), [25]. As for EV/GV diagnosis, the AUC's were 0.89 (95% CI 0.80 to 0.98) for transient elastography (TE) and 0.87 (95% CI 0.78 to 0.96) for Aspartate Aminotransferase (AST) to Platelet Ratio Index (APRI), respectively. The sensitivity (and specificity) of TE (cut-off value of 12.7 kPa) and APRI (cut-off value of 1.92) in predicting EV/GV were 84% (77%) and 84% (83%), respectively, whereas the sensitivity (and specificity) of splenomegaly in predicting EV/GV were 92% (85%). Small studies in children with CF liver disease have also shown FibroScan™'s efficacy as a tool to detect and quantify severity of CFLD [26] as well as its correlation with development of EV [24].

FibroScan™ technology can also now provide simultaneous determination of liver steatosis measured by controlled attenuation parameter (CAP) and LSM, in children of any age [27]. FibroScan™ among obese children predicted clinically significant fibrosis (≥ 2) well in a cohort of children with nonalcoholic fatty liver disease, although none of the children had cirrhosis [28].

Notably, none of the aforementioned studies describe the longitudinal capture of LSM in children using FibroScan™, while studying its correlation with varying stages of PHT (i.e. Early PHT: splenomegaly OR thrombocytopenia, Late PHT: splenomegaly, thrombocytopenia, AND variceal bleed OR ascites). In addition, there are no published studies specifically investigating ALGS or A1AT. As such there is an urgent need to begin to characterize elastrography in children with these disorders.

Non-invasive liver biomarkers may complement FibroScan™ in detecting, monitoring progression or regression of fibrosis, or in predicting clinical outcome

The rapid progression of liver disease in some children indicates a need to identify early markers of liver fibrosis to help facilitate early intervention. Empirically identified markers identified by genomic, proteomic, and metabolomic technologies, as well as targeted serum marker analysis, offer new strategies with which to diagnose and predict outcomes in pediatric liver diseases [29-32]. Preliminary studies in children with fibrotic liver diseases have identified specific markers reflecting matrix re-modeling, hepatic stellate cell activation, collagen turnover, and chemoattractant expression in this age group [30-33]. These include Tissue Inhibitor of Metalloproteinase-1 (TIMP-1), Lysyl hydroxylase (LH), Prolyl hydroxylase (PH), hyaluronic acid (HA), and interleukin-8 (IL-8), among others. FibroScan™ LSM has also correlated well with APRI in 100 children with BA, particularly when using size specific probes in children with varying thoracic circumferences [34].

The FORCE protocol includes the collection of serum and plasma at the time of FibroScan™ LSM. This rich resource will position the network to conduct future investigations to correlate LSM with known and to-be-identified serum biomarkers of fibrosis. The ability of these biomarkers to predict change in LSM over time will also be assessed. In addition, unbiased investigations using techniques like SomaLogic (Boulder, CO) may identify new biomarkers that correlate with and explain the pathophysiology of liver stiffness (e.g. fibrosis) in pediatric cholestatic liver disease.

ChiLDReN's unique position

Biliary atresia is the leading indication for liver transplantation in children, accounting for approximately 30% -40% of all pediatric liver transplants. Longitudinal studies that have enrolled large numbers of patients with BA and the aforementioned liver diseases (A1AT and ALGS) are well established and on-going in ChiLDReN, and have successfully coupled prospectively collected clinical data with laboratory data and a rich biorepository of specimens. These databases are subsumed within the following protocols registered on ClinicalTrials.gov; PROBE = NCT 00061828, BASIC = NCT00345553 and LOGIC = NCT00571272. As of November 30, 2015, 569 participants with BA have been enrolled in PROBE, 563 pre-transplant participants with BA have been enrolled in BASIC, and 377 participants with alpha-1- antitrypsin deficiency (A1AT) and 314 participants with Alagille Syndrome (ALGS) have been enrolled in LOGIC. Baseline cross-sectional analyses of features and clinical associations of portal hypertension in BA and A1AT deficiency have been published by ChiLDReN [3,4]. The protocols for PROBE, BASIC, and LOGIC incorporate the collection of biosamples at fixed intervals using standard operating procedures, including liver tissue (when clinically obtained or at the time of transplant), plasma/serum, genomic DNA and urine. A rich bank of clinical information exists and will be continually collected and will be available for correlation with FibroScan™ LSM.

This study will pioneer evaluating and possibly validating the role of non-invasive and radiation-free FibroScan™ technology to detect and quantify liver fibrosis in children with chronic liver disease, while also investigating the prognosticating ability of LSM, serum and simple biomarkers of fibrosis. The potential to offer non-invasive alternatives for assessing liver fibrosis in children may provide much needed insight into the natural history of pediatric liver disease. Evaluation/correlation of these diagnostic tools in a multi-center setting have far-reaching implications.

The underlying hypothesis of this proposal is that FibroScan™-based elastographic LSM in children with BA can differentiate those with clinical features of portal hypertension from those without. In addition, it is presumed that fibrosis is progressive in BA and FibroScan™ can identify that progression over a two-year time period in children with BA. Exploratory analyses of the same concepts will be conducted in ALGS and A1AT, where there is a marked paucity of high quality data.

4 Study Objectives

4.1 Primary Objective

- Aim 1 - To prospectively assess whether FibroScan™ LSM are associated with the clinical and laboratory features of portal hypertension in children with BA.

  Hypothesis 1: *FibroScan™ LSM results can differentiate children with clinical features of portal hypertension from those without.*

4.2 Secondary Objectives

- Aim 2 - To prospectively measure changes in LSM over time by FibroScan™ in children with BA.

  Hypothesis 2: *FibroScan™-based LSM in children with BA will reliably reveal increases over 2 years of follow-up.*

- Aim 3 - To confirm the feasibility of obtaining valid FibroScan™ LSM in children with cholestatic liver diseases.

  Hypothesis 3: *Valid results of FibroScan™-based LSM can be obtained in more than 80% of children with BA, A1AT, and ALGS.*

- Aim 4 - To prospectively assess whether FibroScan™ LSM are associated with conventional laboratory determinants of liver disease.

  Hypothesis 4: *FibroScan™-based LSM in children with BA, A1AT, and ALGS will correlate with conventional laboratory determinants of liver disease including but not limited to, PELD score, total bilirubin, albumin and INR that are components of the PELD score, platelet count, and the APRI.*

4.3 Exploratory Objectives

- Aim 5 - To prospectively assess whether FibroScan™ LSM are associated with the clinical and laboratory features of portal hypertension in children with *A1AT and ALGS.*

  Hypothesis 5: *FibroScan™ LSM results can differentiate children with clinical features of portal hypertension from those without.*

- Aim 6 - To collect a set of biosamples that correspond to the prospectively assessed FibroScan™ LSM and clinical data.

  Hypothesis 6 - *FibroScan™-based LSM will correlate with serum biomarkers of fibrosis.*

- Aim 7 - To prospectively explore changes in LSM over time by FibroScan™ in children with A1AT and ALGS.

  Hypothesis 7: *FibroScan™-based assessment of LSM in children with A1AT and ALGS will reliably reveal increases over 2 years of follow- up.*

5 Methods

5.1 Study Schema

The research protocol will encompass the prospective longitudinal analysis of FibroScan™ LSM in children with BA, A1AT, or ALGS. FibroScan™ Liver Stiffness Measurement's (LSM) are non-invasive and painless. Eligible participants must be actively followed at participating ChiLDReN sites and be concurrently enrolled in PROBE, BASIC, or LOGIC. There will be three assessments: one at baseline and two subsequent annual follow-up visits. At each visit, comprehensive clinical data and biosamples will be collected according to the PROBE, BASIC, or LOGIC protocols. Clinical data in these longitudinal studies include but are not limited to interval clinical events, history of clinical symptoms of liver disease, physical examination findings, and routine laboratory and imaging studies. Liver biopsy samples, when clinically indicated, are also captured by these longitudinal databases. Specialized clinical information specifically relevant to FORCE and FibroScan LSM will also be recorded. These parameters will generally not overlap with the parent protocols, with the exception of spleen size assessment on

physical examination, which will be specifically assessed for FORCE. Information relevant to the performance of FibroScan LSM will be collected (e.g. thoracic diameter, duration fasting prior to the study, ability of participant to cooperate with the LSM). In addition there will be collection of serum/plasma samples, available for future study of serum biomarkers as described above.

5.2 Study Population, Participant Selection and Recruitment

All children with an established diagnosis of BA (excluding those with known situs inversus or polysplenia/asplenia), who are actively followed at participating ChiLDReN sites and enrolled in PROBE or BASIC will be eligible for the trial. In addition, all children with A1AT or ALGS actively followed at one of these sites and enrolled in LOGIC will also be eligible. No lower limit age restrictions are incorporated in this protocol to afford an accurate assessment of feasibility in children of all ages. The number of evaluable participants to be entered in the study at all clinical sites will be 450 (250 BA, 100 A1AT, 100 ALGS). An evaluable participant is a participant with a Baseline visit with at least 10 consecutive valid measurements and an IQR/median <30%. We will recruit to replace those participants who do not complete the initial LSM of the study.

5.2.1 Inclusion criteria:

- Age 21 years or less at the time of enrollment
- Participants enrolled in a ChiLDReN based prospective observational cohort study (PROBE, BASIC, or LOGIC)
- Willingness and ability to participate in the study for up to 24 months
- One of the following three diagnoses
    - Biliary atresia per ChiLDReN criteria (see Appendix A) or,
    - Alpha-1 antitrypsin deficiency (PiZZ or SZ) per ChiLDReN criteria (see Appendix A) or,
    - Alagille Syndrome per ChiLDReN criteria (see Appendix A)

5.2.2 Exclusion criteria:

- BA with known situs inversus or polysplenia/asplenia
- Presence of clinically significant ascites detected on physical examination
- Open wound near expected FibroScan probe application site
- Use of implantable active medical device such as a pacemaker or defibrillator
- Known pregnancy
- Prior liver transplant
- Unable to give informed consent or assent

5.2.3 Participant Selection

Enrollment into this study will potentially be offered to all current and future participants enrolled into PROBE, BASIC, and LOGIC at participating ChiLDReN sites who have a diagnosis of BA, A1AT, or ALGS, who have their native liver and who meet the inclusion and exclusion criteria for this study. Once the target enrollment number for each disease (BA 250, A1AT 100, ALGS 100) is reached, new enrollment will cease. Each site's investigators and coordinators will work with the ChiLDReN Data Coordinating Center (DCC) to identify potential participants for this study who are already enrolled in ChiLDReN longitudinal studies and who meet all of the inclusion and exclusion criteria for this study. Potential participants will be contacted by mail or by phone prior to their scheduled follow-up visit to minimize visits to the study center involved in participation in FORCE.

5.2.4 Duration of enrollment

Initial enrollment in FORCE will occur over the first two years of this study, with continued participation for the 2-year follow-up duration of this study. Liver transplantation and/or presence of clinically significant ascites at the time of the 1-year or 2-year follow-up visit will be a study terminating event.

5.2.5 Number of Participants

During the 4-year duration of this study, the plan is to enroll up to 600 participants to ensure a total of 450 evaluable participants; 250 with biliary atresia, 100 with alpha-1 antitrypsin deficiency, and 100 with Alagille syndrome. We anticipate that most of those enrolled will be existing participants in PROBE, BASIC, or LOGIC, however new participants in these studies will also be eligible for enrollment in this study as long as they meet enrollment criteria. The following are estimates of the number of participants with each disorder that will be enrolled into the study as existing PROBE, BASIC, or LOGIC participants and as new PROBE, BASIC, and LOGIC participants to be enrolled each year:

- Biliary Atresia – 282 existing PROBE and BASIC participants and 30 new PROBE and BASIC enrollees per year
- α1-AT deficiency – 87 existing LOGIC participants and 6 new LOGIC enrollees per year
- Alagille syndrome – 84 existing LOGIC participants and 8 new LOGIC enrollees per year.

5.2.6 Participant Discontinuation/Withdrawal

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant non-adherence
- Lost to follow-up
- Inability to obtain a valid baseline scan
- Development of significant clinical ascites detectable on physical exam
- Receipt of a liver transplant
- Appropriate FibroScan probe unavailable
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Investigator believes that participation is not in the best interest of the participant

The reason for participant discontinuation or withdrawal from the study will be recorded in the study database. Participants who sign the informed consent form, and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced.

5.3 Schedule of Visits

Baseline	Informed consent FibroScan measurement FORCE clinical data and serum/plasma collection Clinical data collection & biosamples per PROBE, BASIC, or LOGIC
12 month f/u	FibroScan measurement FORCE clinical data and serum/plasma collection Clinical data collection & biosamples per PROBE, BASIC, or LOGIC
24 month f/u	FibroScan measurement FORCE clinical data and serum/plasma collection Clinical data collection & biosamples per PROBE, BASIC or LOGIC

5.4 Data Collected

Clinical data including interval events, medical history, physical examination data and laboratory values, along with biosamples will be collected at each of the 3 visits per the PROBE, BASIC, or LOGIC study protocols.

5.4.1 FibroScan™ measurements

Operators will be trained and certified at each site to perform FibroScan™ measurements to ensure consistent and standardized acquisition of complete data. Training will take place at each site by a designated trainer from Echosens.

Fasting will not be specified for these procedures. No sedation will be administered for these FibroScan™ assessments. The exam time is estimated to be 10 to 20 minutes.

The thoracic perimeter of the patient will be measured and recorded. The thoracic perimeter value along with the skin to capsule distance (determined by the FibroScan device) will determine probe selection and exam type. The device records and displays the validity of each measurement based on standardized criteria determined by the FibroScan device. Ten valid measurements are obtained. Repeated measurements are performed until 10 valid values are obtained.

After the end of the examination the gel is removed from the participant's chest wall with a soft tissue. Gel is also removed from the probe with a soft towel and it is then disinfected with a solution containing quaternary ammonia. The report is printed and a non-identifying study ID label is applied. The report will be placed in the research binder and not in the clinical chart.

FibroScan™ is based on vibration controlled transient elastography at 50Hz. FibroScan measures 2 parameters:

1. "Liver stiffness" quantifies liver fibrosis and is measured in kPa.
2. "Controlled Attenuation Parameter (CAP)" quantifies liver steatosis and is measured in dB/m.

In addition, quality control data are collected:

- Invalid measurements and success rate
- Number and list of valid measurements
- Inter quartile range (IQR) (kPa or dB/m) of all valid measurements within the examination (reflects the dispersion of stiffness or CAP measurements)
- IQR/med. (%) indicates the IQR/median ratio and should remain as low as possible to ensure reliable results (goal < 30%). An exam with an IQR/median of ≥ 30% will be considered an invalid exam. Participants with an invalid exam at Baseline will be withdrawn from the study. Participants with an IQR/median of ≥ 30% at the Year 1 exam will remain in the study.

5.4.2 Biosample Collection

Blood will be drawn on the same day as the FibroScan™ assessment for routine clinical evaluation and for biobanking for future assessment of serum/plasma biomarkers of fibrosis. The relevant laboratory parameters for FORCE (total bilirubin, ALT, AST, alkaline phosphatase, gGTP, albumin, CBC with platelet count and PT/INR) are considered to be standard of care for children with chronic liver disease and will be obtained as part of routine clinical practice.

5.5 Sample Size and Power Calculations

The sample size for this study is based on logistical considerations – the number of participants in PROBE, BASIC, and LOGIC who meet FORCE eligibility criteria and are willing to enroll in the study at the participating ChiLDReN sites in the two-year recruitment period (we based these considerations on an estimate of at least 9 sites). Thus, this section describes the magnitude of effects possible to be detected with adequate power, or the possible power to detect meaningful group effects, for Aims 1 (primary) and 2 (key secondary).

Aim 1: To prospectively assess whether FibroScan™ LSM are associated with the clinical and laboratory features of portal hypertension in children with biliary atresia.

> Hypothesis 1: *FibroScan™ results can differentiate children with clinical features of portal hypertension from those without.*

The definitions of PHT and no PHT that we will use for the primary analysis are termed "definite" vs "absent" based on the research definition adopted by the Network. Clinically evident PHT (CEPHT) is defined as "definite" (dCEPHT) when there is either (1) a history of a complication of PHT (esophageal or gastric variceal (EV) bleed or ascites [as defined by prescription of diuretics]) or (2) clinical findings consistent with PHT (both splenomegaly [spleen palpable > 2 cm below the costal margin] and thrombocytopenia [platelet count < 150,000/ml]). CEPHT is denoted as "possible" (pCEPHT) if only one of the two clinical findings is present in the absence of a complication; while CEPHT is "absent" if none of the criteria is met. (Note that secondary analyses will consider the comparison of "definite" + "possible" CEPHT vs. "absent" CEPHT and comparisons of each category, i.e., "definite" vs "possible" vs "absent" CEPHT).

We estimate the proportion of participants with PHT, based on the literature, preliminary data from the ChiLDReN database, and clinical experience (for estimates for normal liver), in Table

1. We apply these estimates to the overall expected number of eligible participants to define the sample sizes for the dCEPHT and absent CEPHT groups, assuming that 80% of eligible participants will consent to enroll in the Echosens study.

Table 1. Estimates of PHT prevalence in biliary atresia

Disease Group	dCEPHT	pCEPHT	No CEPHT (cirrhosis without features of CEPHT, ~normal liver)
BA [3]	49%	17%	34% (34%, 0%)

For the power calculation, we conservatively assume the true difference in population mean FibroScan™ LSM between definite and no clinically evident portal hypertension is 12 kPa. This estimate is based on an integration of data from studies of children with BA [25] and of adults with cirrhosis. Chongrisawat studied 73 BA patients (mean age 9 years [SD=5.6]) and 50 normal controls (mean age 11 years [SD=3.3]. The mean difference in liver stiffness scores between the BA participants with and without splenomegaly is 29 kPa (95% CI: 21.6, 36.4) [see Table 2]. In adults with cirrhosis, mean liver stiffness score is about 12 kPa, while for adults with normal livers their mean score is in the range of 4-6 kPa.

Table 2. Mean (SD) liver stiffness FibroScan™ scores in BA patients and normal controls*

Sample	N	Mean $\pm$ SD, kPa
BA with splenomegaly	39	38.9 $\pm$ 22.0
BA without splenomegaly	34	9.9 $\pm$ 6.0
BA	73	27.4 $\pm$ 22.5
Normal controls	50	4.7 $\pm$ 1.0

*[25]

We assume the standard deviation (SD) is 22.0 kPa and 6.0 kPa for participants with and without clinically evident portal hypertension, respectively. Using a 2-sided Type I error rate of 5% under these assumptions, there will be more 99% power to detect a 12 kPa difference between participants with and without clinically evident portal hypertension in the BA disease group. Alternatively, there is at least 80% power to detect mean group differences of 6.2 kPa in BA participants [see Table 3]. Note that these assumptions are conservative because they do not account for new enrollees into the PROBE and BASIC studies.

Table 3. Power and mean group differences, assuming Type 1 error = 5% (2-sided), SD = 22 kPa (dCEPHT group) and 6 kPa (absent CEPHT group)

Disease Group (Study[ies])	Total Sample Size[1]	Projected Sample Size[2]	Projected Sample Size in dCEPHT, Absent CEPHT Groups[2]	Power to detect 12 kPa Group Difference[3]	Group Difference for $\geq$ 80% Power[3]
BA (PROBE, BASIC)	282	192	112, 79	>99%	6.2

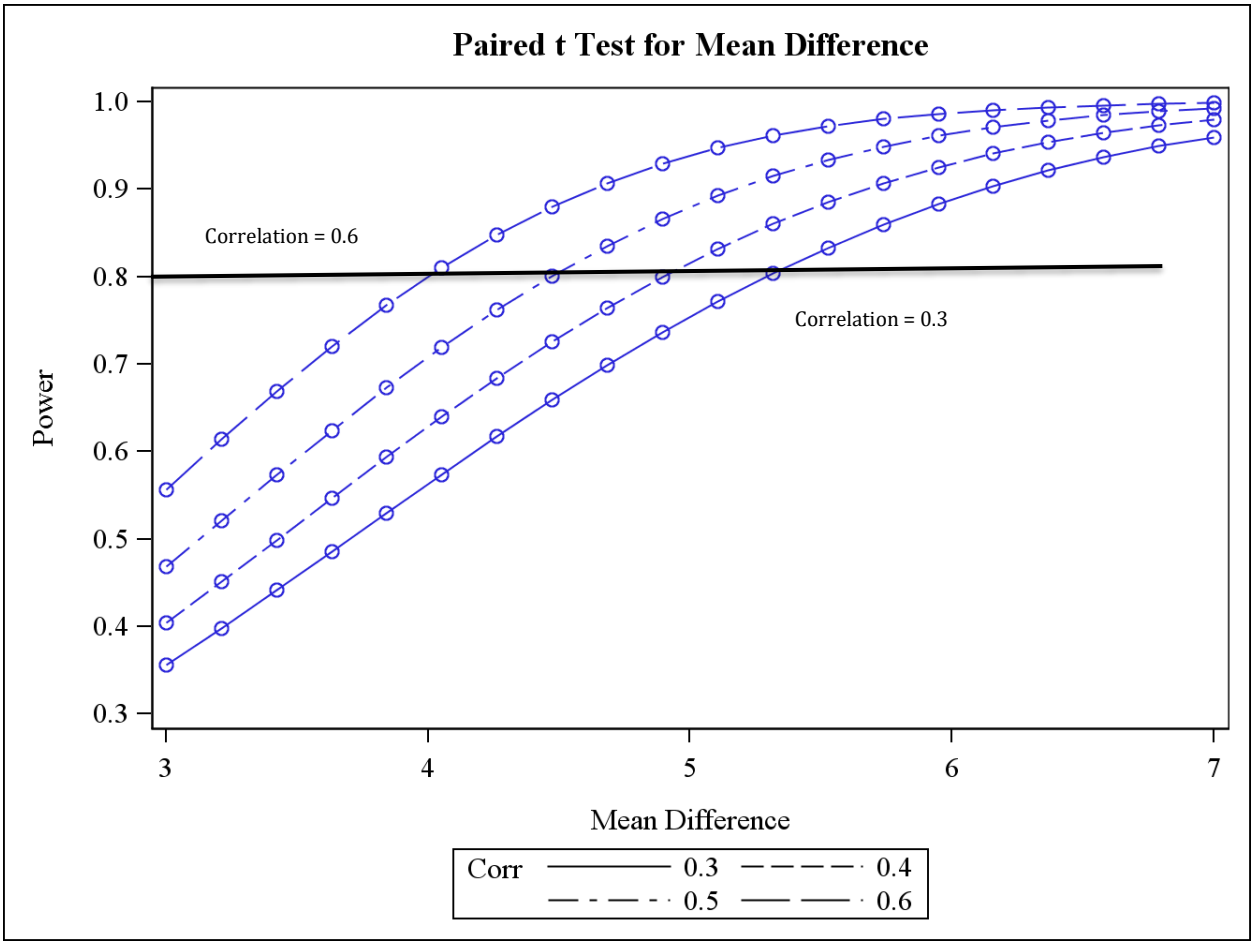
[1] Based on 12/31/2015 database that does not include projected enrollment in studies
[2] Assumes 80% of eligible participants will consent to enroll in FORCE study, as well as proportions of participants with dCEPHT and absent CEPHT from Table 2
[3] Based on two-sample t test for mean difference with unequal variance [SAS 9.3]

Aim 2: To prospectively measure changes in liver stiffness over time by FibroScan™ in children with BA.

Hypothesis 2: *FibroScan™-based assessment of liver stiffness in children with BA will reliably reveal increases over 2 years of follow-up.*

From the projected 226 participants with BA who will enroll in this study, we assume that up to 15% will drop-out by 2 years (liver transplant, death, or lost to follow-up), resulting in 192 participants for whom we can estimate the change from baseline to 2 years. For this within-patricipant assessment, the degree of correlation between the two FibroScan™ values affects the power --- greater correlation results in greater power under equivalent assumptions. We assume that the range of correlation ranges between 0.3 and 0.6. Conservatively, we estimate that the SD of the change from baseline is 22 kPa. With these assumptions, we have sufficient power (≥80%) to detect mean changes from baseline to 2 years of 5.3 kPa for correlation = 0.3 to 4.0 kPa for correlation = 0.6 (based on paired t test, SAS 9.3); see Figure 1. If the correlation is greater or if the SD is less, then a smaller mean change can be detected with sufficient power. Our approach for assessing power and effect sizes is conservative; utilizing all measurements via more complicated statistical models can provide greater power or equivalently smaller mean changes in FibroScan™ LSM.

Figure 1. Power for Various Changes from Baseline to 2 Years in FibroScan Values for Correlations Ranging from 0.3 to 0.6, N=192



5.6 Statistical Analysis

Descriptive statistics, both tabular and graphical, will be used to characterize the distribution of FibroScan™ values at enrollment into the FORCE study and at 1 and 2 years of follow-up, by disease group (BA, A1AT, ALGS). In addition, changes from enrollment to year 1 and to year 2 will be summarized. A detailed Statistical Analysis Plan will be prepared prior to the completion of the study.

5.6.1 Primary Aim

The primary aim of the study seeks to compare the distribution of FibroScan™ LSM at enrollment between participants with and without portal hypertension. A linear model will be fit to FibroScan™ values at enrollment to assess the impact of portal hypertension on LSM, controlling for important covariates such as age, gender, and race. The coefficient for the clinically evident portal hypertension effect will provide the difference in FibroScan™values associated with clinically evident portal hypertension. If the distributional assumptions of the models are not met, transformations (e.g., log of the FibroScan™ values) or rank-based (non-parametric) methods may be employed.

Secondary analyses will be performed to compare “definite” + “possible” CEPHT vs. “absent” CEPHT and compare each category, i.e., “definite” vs “possible” vs “absent” CEPHT. The same approach (linear models) will be used to test for differences in FibroScan™ measurements among the CEPHT groups, with an omnibus test used for the comparison of the three CEPHT groups.

5.6.2 Secondary Aims

The key secondary aim (Aim 2) compares the one- and two-year FibroScan™ values to those at enrollment in participants with BA. A linear mixed effects model will be used to assess whether mean LSM changes significantly over time, with FibroScan™ measurements as the dependent variable, fixed effect of time (enrollment [year 0], Year 1, Year 2) and subject-specific random effects to account for both heterogeneity among subjects, and correlation among measurements taken on the same subject as independent variables. To test whether there is a significant change in LSM over time, we will test the coefficient for the time is significantly different from zero. We will also explore the impact of other factors on the changes in FibroScan™ by incorporating other independent variables (e.g., age at hepatoportoenterostomy, baseline total bilirubin, number of interval episodes of cholangitis) and their interaction with time into the linear model. To test whether there is a significant difference in the way FibroScan™ values change over time between two groups (e.g., younger vs older at time of hepatoportoenterostomy) or with a factor (e.g., baseline total bilirubin), we will test whether the interaction term is significantly different from zero. The main analytic approach will use restricted maximum likelihood methods and an unstructured variance-covariance structure. Extensions of the linear mixed model (e.g., inclusion of polynomial time factors) or other semiparametric (e.g., piecewise linear) methods will be used if LSM does not change linearly.

For Aim 3, we will estimate the proportion of participants in whom a valid FibroScan™ LSM can be obtained, by disease group.

Per Goldschmidt 2013 [35] we will define two measures of the feasibility of FibroScans™ in our populations: “technically possible” and “acceptable quality”. The proportion of participants with a “technically possible” FibroScan™ is defined as the number of subjects with at least 10 FibroScan™ measurements obtained divided by the number assessed. The proportion of participants with FibroScans™ of “acceptable quality” is defined as the number of participants

with FibroScan™ LSM with the ratio of the interquartile range and median of the 10 measurements <30%, of which at least 6 are completed, divided by the number assessed. The proportions and their 95% confidence intervals will be provided using the Wald method; however, the Wilson-Score methods will be used if the sample sizes are small or the proportion is small for a disease group. We will perform separate analyses for participants <2 years of age and for those >2 years of age.

In Aim 4, we will correlate FibroScan™ LSM values at enrollment with PELD score, total bilirubin, albumin, INR, platelet count, and APRI by disease group. The analysis will be limited to participants for whom a PELD can be calculated (i.e., those for whom the individual components of the PELD score are available). Note that PELD will be calculated for all pediatric participants including those greater than 12 years of age. PELD is calculated as

$$\text{PELD} = 4.80 \times [\ln \text{serum bilirubin (mg/dL)}] + 18.57 \times [\ln \text{INR}] - 6.87 \times [\ln \text{albumin (g/dL)}] + 4.36(\text{<1 year old}) + 6.67(\text{growth failure})$$ [www.unos.org]

APRI is calculated as

$$\text{APRI} = \frac{\text{AST/upper limit of normal AST} \times 100 \text{ [U/L]}}{\text{Platelet Count } (10^9/\text{L}) \text{ [U/L]}}$$

Descriptively, we will use scatterplots to graphically explore the relationships between FibroScan™ LSM and the clinical features of these liver diseases, with Pearson correlation coefficients (or Spearman, if the distributions are skewed). Linear mixed effects models (akin to that described for Aim 2) will be used to assess the relationship between FibroScan™ LSM and each parameter over time (i.e., time-varying covariate), controlling for other important covariates (such as gender, age, race). We will explore the nonlinearity assumption of these models using more flexible methods, such as LOESS (locally weighted scatterplot smoothing, a nonparametric approach).

5.6.3 Exploratory Aims

In Aim 5, we will use methods comparable to Aim 1 (for BA participants) to assess whether FibroScan™ LSM can differentiate children with and without clinical features of portal hypertension. Separate analyses will be conducted for participants with A1AT and with ALGS.

In Aim 6, we will develop a biorepository of serum/plasma samples for future correlative analyses with FibroScan™ LSM.

In Aim 7, we will use methods comparable to Aim 2 (for BA participants) to summarize the changes in FibroScan™ LSM in participants with A1AT and with ALGS (separately).

6 Human Subjects

6.1 Protection of Human Subjects

6.1.1 Institutional Review Board

This study and analysis will be performed under Institutional Review Board (IRB) oversight. Prior to the initiation of the study, an IRB approval for study of human subjects will be obtained separately from the IRB of each of the participating FORCE clinical study centers and the DCC. Revisions to the study protocol and changes in the study design will also be submitted to the

individual IRBs for approval prior to implementation. A clinical center may not initiate any participant contact about the FORCE study until the site has received an activation letter from the DCC.

Participants will be enrolled in the FORCE protocol with full and written informed consent, which will include the gathering of protected health information (PHI). Each participating center will be responsible for obtaining such human subjects research authorization and will create an informed consent document detailing the procedures described above in the language required by their respective organizations. All key personnel at the participating centers will have successfully completed IRB-required training and certification for human subjects research. Additionally, participants will satisfy HIPAA researchers' privacy requirements.

6.1.2 Patient Confidentiality

Special procedures for ensuring patient confidentiality will be implemented. Data transmission and the distributed data systems will have multiple layers of security as discussed in Section 8, Study Management. Each study participant will be assigned an identification number. Only this number will be used to identify participants in any individual tabulation. The PHI that is collected will represent the minimum necessary to successfully execute the study.

PHI entered into the database at the site level will only be visible to study personnel accessed through a triple password regimen. The PHI is encrypted at the site level. Site personnel will have the decryption key and it will not be available to the DCC. It is expected that only group data will be published. If individual participant data are to be published, no identifying information will be included. The study files will be maintained in a secure location. Access to computerized data will be restricted to study personnel. Password authorization will be enforced. Previous use of this security system and a secured server indicates that this technique is very successful in assuring the protection of confidential information.

Authorized representatives of the Sponsor, the NIDDK, National Institutes of Health (NIH), participating FORCE clinical study centers, DCC monitoring staff, as well as the IRBs at each site will have access to medical records and records from participants in this study. Such access is necessary to ensure the accuracy of the findings.

6.1.3 Recruitment and Informed Consent/Assent

Each site will contact the parents/guardians or the potential participant (if 18 years or older) to provide information about this study and offer enrollment. Initial contact with the family/participant may be conducted by one or more of the following: phone contact by a study coordinator or investigator/co-investigator, direct mailing to the family, or by an investigator/co-investigator or study coordinator during an outpatient clinical or research visit, or during admission to the hospital. If the family/participant is interested in possibly enrolling in this study, the parents/guardian/participant will then meet with the study investigator(s) and study coordinator. The investigator and coordinator will discuss the objectives of the study and the study design, possible benefits, and potential risks with the family, and review the IRB approved consent form. Printed information about the study and the consent form will be provided to the family. The IRB-approved consent form will include the purpose of the trial, the responsible parties and investigators, potential benefits, risks of participation, the right to refuse to be in the study, the right to withdraw from the study under no penalty, contact numbers and information about the responsibility for injury and payment for medical care. If the participant, parent or guardian consents to enroll into the study, written informed consent will be obtained from the parents or guardians and written assent will be obtained from the participant in accordance with local IRB regulations. For participants 18 years of age and older, consent will be obtained directly from the participant. Affected siblings with A1AT or ALGS and evidence of liver disease

who are enrolled in LOGIC are eligible to participate in this study. The study team will endeavor to arrange the baseline visit for this study so that it coincides with the visit schedule of the other ChiLDReN longitudinal study for which the participant is already enrolled.

If desired by the parent/legal guardians, the results of the FibroScan™ LSM will be provided. These results will not be placed in the official clinical record. Guidance will be given to the family as to current understanding of the measurement results and that the clinical implications of the findings are not clear (see appendix document – lay explanation of FibroScan™ measurement).

6.1.4 Risks to the Patient and Adequacy of Protection Against Risk

Participants enrolled in the FORCE Study will experience more than the normal amount of testing that is customary for patients with pediatric liver disease. Venipuncture carries risks of pain and bruising at the puncture site as well as syncope. Individuals may experience minor discomfort or soreness over the area where the ultrasound probe contacts the abdomen. There is also a small risk of allergic reaction to the water-based gel used to improve conduction between the probe and the participant's skin during the procedure. All scans will be performed by a FibroScan™certified operator. There is a potential risk of breach of confidentiality that is inherent in all research protocols, and steps to minimize this risk are described above. Steps to minimize risk and address any discomfort are addressed below.

Risks of FibroScan™: There are no known direct risks from the FibroScan™ medical device, which uses ultrasound waves. Participants may experience minor pain or soreness during the scan. There is a small risk of allergic reaction to the water-based gel used during the procedure. There is no radiation exposure. All scans will be performed by FibroScan™-certified operators.

If the participant experiences excessive discomfort from a study procedure, the procedure will be stopped.

6.1.5 Unauthorized Data Release

The data sets will be stored on a secure server with restricted access (requires a unique username and password) at the DCC and every precaution will be taken to keep the information private. However, there is always the possibility of unauthorized release of data about participants. Such disclosure would be extremely unlikely to involve a threat to life, health, or safety. It is conceivable that such disclosure could have psychological, social, or legal effects on the patient. Using the standard security procedures (described above under patient confidentiality) can effectively minimize the risk of unauthorized disclosure of data. All study personnel who have access to patient data will be educated regarding the need to protect confidentiality and the procedures to be followed to ensure such protection. All staff will also be required to sign a standard medical record confidentiality agreement. The computer system on which data are maintained uses standard password protection procedures to limit access to authorized users. After the study is completed, the database will be stored on the NIDDK Data Repository. The database in the Repository will be de-identified to obviate further privacy and security considerations.

6.1.6 Adverse Event Monitoring and Reporting

6.1.6.1 Definition of an Adverse Event

An adverse event (AE) is any untoward medical occurrence or unfavorable and unintended sign in a research participant that occurs during or as a result of a research procedure. For this study, each center will review the list of study procedures and identify the specific procedures

that are not standard-of-care at their institution and these will be considered research procedures. Complications that are a result of research procedures will be reported and tracked as adverse events.

The research procedures (ultrasound scanning and phlebotomy) present minimal risk; we anticipate few adverse events. All adverse events must be recorded. The onset and end dates, severity and relationship to study procedure(s) will be recorded for each adverse event. All adverse events will be reported by FORCE investigators to the DCC. Any action or outcome (e.g., hospitalization, additional therapy, etc.) will also be recorded for each adverse event. Participants will be questioned and/or examined by the investigator or his/her designee for evidence of adverse events.

Serious Adverse Event. The term serious is based on patient outcomes associated with events that could threaten a patient's life or functioning. An event should be considered serious if it results in any of the following:

- Death,
- Life-threatening (patient was at risk of death as a result of the event, does not include hypothetical risk of death if the event had been more severe),
- Inpatient hospitalization or prolongation of existing hospitalization,
- Persistent or significant disability or incapacity,
- Congenital anomaly or birth defect,
- Medical or surgical interventions required to prevent one of the outcomes listed above.

The phrase "related to study" implies causality or attribution to the study procedures. For purposes of defining a serious adverse event (SAE), if a causal relationship cannot be ruled out, then an AE should be considered 'related to the study procedure(s)'. As noted above, it is very unlikely that any adverse events will be attributable to this study.

6.1.6.2 Reporting Responsibility

All adverse events must be recorded. The onset and end dates, severity and relationship to study procedure(s) will be recorded for each adverse event. Any action or outcome (e.g., hospitalization, additional therapy, etc.) will also be recorded for each adverse event.
All Adverse Events (AEs) and Serious Adverse Events (SAEs) must be reported by the investigator to the DCC. The DCC will review reports of all related SAEs and other relevant information immediately, and may request additional information from sites for analysis of these events. Sites will report SAEs according to the time frames outlined below. All events that are serious and related (possibly or probably) must be reported to the DCC within 24 hours of the investigator being informed of the event. Follow-up information about a previously reported serious and related adverse event may be reported to the DCC within 7 working days of the investigator receiving the information; however, important follow-up information must be submitted within 24 hours. All deaths connected to a study procedure must be reported to the DCC within 24 hours of the investigator being informed of the event. The NIH will appoint an independent Data and Safety Monitoring Board (DSMB) that will provide study oversight. SAEs will also be reviewed by the study's DSMB during its regular meetings.

6.2 Benefits to the Participant

There are no direct benefits to the participants for participation in the study.

6.3 Inclusion of Women

This study includes women up to and including age 21 at the time of enrollment.

6.4 Inclusion of Minorities

Racial and ethnic minorities will be recruited into the study. We anticipate that the representation of racial and ethnic minorities will correspond to the fraction of minorities in the population presenting to the participating clinics' pediatric liver disease patients.

6.5 Inclusion of Children

Since this is a pediatric study, children under the age of 18 will be enrolled into this study.

6.6 Data Safety and Monitoring Plan

Accepted principles of data and safety monitoring will be observed throughout the conduct of the FORCE study. The DSMB will review the study protocol prior to enrollment and will review all subsequent protocol revisions. The DSMB will also evaluate the occurrence of adverse events related to study participation as well as study accrual updates.

FORCE Principal Investigators will be responsible for monitoring the enrollment of participants, submission of data to the DCC, and monitoring and reporting of adverse events related to study participation. The DCC will be responsible for monitoring for effective conduct of the protocol and accurate and timely data submission.

IRBs will be provided feedback on a regular basis.

A minimum of two people will be trained and certified in the FibroScan™ exam at each clinical center by Echosens™, the maker of the FibroScan™ device. At the time of the initial training, the Echosens representative will assemble and calibrate the FibroScan™ medical device.

Training of study coordinators and study monitoring activities will be conducted by the DCC to ensure patient confidentiality and privacy and to maximize the reliability, accuracy, and timeliness of study data.

The FORCE clinical sites, the DCC, and relevant research center staff will conduct regular meetings to review recruitment/enrollment progress, data collection activities, and participant retention. The DCC will produce regular reports regarding enrollment, data quality, and timeliness and share the reports with NIDDK, the Steering Committee, and the participating clinical center. Data will be routinely exported from the system, examined for accuracy and completeness, and backed up to secure storage devices. Upon completion of data collection, final processing and cleaning of data will be conducted. A technical report detailing specific project methodology, response rates, and other details will be produced.

7 Study Organization

7.1 Clinical Centers

The participating FORCE clinical study centers, made up of the ChiLDReN clinical centers, will have primary responsibility for developing the study protocol, maintaining high rates of follow-up and data collection, obtaining data of high quality, and interpreting, presenting, and publishing findings from the study.

7.2 Data Coordinating Center

The DCC contributes biostatistical expertise and shares in scientific leadership of the research group. The DCC has developed a communication infrastructure that includes meetings, teleconferences, email and bulletins, interactive Web-based encounters, and written correspondence. The DCC assists in protocol development and preparation of scientific publications. The DCC has the major responsibility of creating a database and data collection systems for the participating FORCE clinical study centers, ongoing evaluation of data quality, performance monitoring of the FORCE clinical study centers, and statistical analyses of the data. The DCC has also created a comprehensive Manual of Operations (MOO) that will govern the conduct of the study. The manual details the protocols, protocol clarifications and amendments, summary of the regulatory requirements for the study, instructions for enrollment, data collection, data management, visit schedules, and detailed instructions on the use of the electronic data submission. The DCC is responsible for clinical monitoring of the study.

University of Michigan
Arbor Research Collaborative for Health
Ann Arbor, MI
Principal Investigators: John C. Magee, MD, FACS and Robert M. Merion, MD, FACS

7.3 Steering Committee

The primary governing body of the ChiLDReN study is the Steering Committee, consisting of each of the Principal Investigators of the ChiLDReN clinical study centers, the Principal Investigators of the DCC, and the NIDDK Project Scientist. The Steering Committee develops policies for the study pertaining to access to patient data, performance standards, and publications and presentations. It approves study protocols and meets to discuss the progress of the study and to consider problems arising during its conduct. The Steering Committee may establish subcommittees to further develop specific components of the study protocol. Small working groups may be established to prepare manuscripts and presentations.

8 Study Management

8.1 Data Collection, Data Collection Forms, Data Entry

The DCC will utilize the Web-based ChiLDReNLink as the data management nucleus for the FORCE study. ChiLDReNLink is a database platform developed by Arbor Research Collaborative for Health (Arbor Research). The DCC will utilize ChiLDReNLink to create electronic case report forms to capture all relevant study data for all investigational/research protocols that are developed and implemented during the course of FORCE. The ChiLDReNLink system allows real-time monitoring of study data for protocol adherence, quality assurance, adverse event reporting, discrepancy reporting, and other trends.

8.2 Data Management

Study data will be entered into the electronic data entry system by study coordinators at each study site. These data will be encrypted and transferred to the DCC and stored on a secure server at Arbor Research. Access to the server and data entry system is limited and requires a unique username and password combination. The servers are backed up daily and physically stored in a locked facility.

All analysis of the data sets will utilize de-identified (coded) data sets.

8.3 Quality Control and Database Management

The first steps in ensuring protocol compliance are good protocol design and careful orientation of study personnel. Following final agreement on protocols, and prior to study initiation at any of the FORCE clinical study centers, the DCC and Echosens will organize a Training and Certification session for FORCE study coordinators/data entry personnel.

The electronic data entry system will have built-in data checks as part of study quality assurance. Protocol compliance will be assessed by monitoring the submission of data at required intervals. Data inconsistencies and discrepancy reports will be reviewed by the Clinical Monitors so that necessary queries can be generated and sent to the FORCE clinical study centers for verification and resolution.

Periodic requests may be generated for the submission of random source documents to assess the quality of data acquisition and data entry at each site. Additionally, the Clinical Monitor or Clinical Study Process Manager will visit each site at least once a year to review source documents, monitor regulatory compliance, and assess protocol adherence.

In addition to source document verification, the Clinical Monitor and Clinical Study Process Manager will produce reports from the database to look for inconsistencies in submitted data, particularly for repeated measures data elements, even if data do not fall outside of built-in validation routines.

Studies of intra-subject and inter-subject data variability by FORCE clinical study center as well as intra-center and inter-center data variability will be used to further ascertain random or systematic data quality issues.

8.4 Data Security/ Data Transfer

For this study, personnel at each study center will collect and enter data into the web-based data entry system. The following data security contingencies are in place:

- Compliance with Industry Standards Regarding Data Security (HIPAA and 21 CFR Part 11)
- Audit trails are maintained for all activity and all changes to any data element
- All servers, web servers, firewalls, etc. are configured and maintained according to industry best practice guidelines for backup, security, continuity of operations, and protection of PHI
- All data are available only to authorized users from each site after secure login with encryption, with all site activity audited at the user level
- All transmissions between the internet and the database are encrypted using a 128-bit encryption algorithm
- There is a comprehensive security plan in place

Detailed instructions on the use of the database platform, data element definitions, and a code list will be provided in a MOO. Each study site will be provided a copy of the MOO and the entire manual will be available on the study website, and in the Help area of the database user interface.

8.5 Resource Sharing Plan

During the study, data and biosamples will be shared with internal and external investigators according to the guidelines agreed upon by the Steering Committee. Upon study completion, study data and materials will be transferred to the NIDDK Data Repository. Minutes of the meetings of the Steering Committee, Project Executive Committee, subcommittees, and the DSMB will be kept on file at the DCC.

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10 Appendices

Appendix A: ChiLDReN diagnostic criteria for Biliary Atresia, Alpha-1 antitrypsin deficiency and Alagille Syndrome

Appendix B: Lay Explanation of FibroScan™ Liver Stiffness Measurement