NASH CRN

Nonalcoholic Steatohepatitis

Clinical Research Network

NAFLD Database

Standard Operating Procedures

Part I: Clinical Center Operations

16 July 2008

NAFLD Database SOP Part I: Clinical Center Operations

Contents

NAFLD Database SOP Part I: Clinical Center Operations

1. Design overview

1.1. Design synopsis

Objectives

- To investigate the etiology, natural history, diagnosis, treatment, and prevention of nonalcoholic fatty liver disease (NAFLD) as defined by steatosis, steatohepatitis, and/or fibrosis
- To develop a specimen bank comprising liver tissue, serum, plasma, and DNA obtained from NAFLD or cryptogenic cirrhosis patients
- To provide a resource for clinical trials and ancillary studies of the pathogenesis, natural history, and treatment of NAFLD or cryptogenic cirrhosis

Type of study

- Observational

Population

- Patients at least age 2 years with definite or suspected NAFLD or cryptogenic cirrhosis

Inclusion criteria

- Histologic diagnosis of NAFLD (past or present) or histologic diagnosis of cryptogenic cirrhosis or suspected NAFLD on the basis of imaging studies suggestive of NAFLD or clinical evidence of cryptogenic cirrhosis
- Absence of regular or excessive use of alcohol within 2 years prior to initial screening
- At least age 2 years at time of initial screening
- Consent

Exclusion criteria

- Clinical or histologic evidence of alcoholic liver disease
- Evidence of other chronic liver disease
- History of total parenteral nutrition, biliopancreatic diversion, or bariatric surgery
- Short bowel syndrome
- Suspected or confirmed hepatocellular carcinoma
- Known HIV positive
- Other condition that is likely to interfere with study followup

Recruitment

- To sample size goal
- 36 month period

Duration of followup

- 48-192 weeks

1.1. Design synopsis

Outcome measures

- Liver histology scores (derived from historical liver biopsy at entry, standard of care biopsy done during screening or followup, or liver biopsy done for NASH CRN trials)
- ALT, AST levels
- Lipid profile
- Imaging studies of NAFLD
- Glucose, insulin levels
- Body mass index
- Health related quality of life
- Alcohol consumption
- Nutritional intake
- Physical activity
- Medication for NAFLD

Visit schedule

- Screening/entry into NAFLD Database: enrollment must occur within 120 days of initiation of screening
- Followup visits at 24 weeks, 48 weeks, 96 weeks, 144 weeks, and 192 weeks

Sample size

- Total of 1500 patients (~200/clinic)

1.2. Data collection schedule

	Screening visits		Followup visits: Weeks from enrollment				
	s1	s2/enr	24	48	96	144	192
Consent and HIPAA authorization	X	.	.	.	.	.	.
Baseline medical history	X	.	.	.	.	.	.
Followup medical history (including interim drinking history)	.	.	X	X	X	X	X
Liver symptoms questionnaire	X	.	.	X	X	X	X
Detailed physical examination including Tanner stage (if < age 18)	X	.	.	X	X	X	X
Focused physical examination	.	.	X	.	.	.	.
Imaging studies of liver*							
CT scan†	.	A	A	A	A	A	A
Ultrasound†	.	A	A	A	A	A	A
MRI†	.	A	A	A	A	A	A
Liver biopsy†	.	A	A	A	A	A	A
Provision of standard of care materials	.	X	.	.	.	.	.
Database eligibility confirmation	.	X	.	.	.	.	.
Alcohol use questionnaires							
AUDIT (age ≥8)	X	.	.	.	.	.	.
Lifetime drinking history (Skinner; age ≥18)	X	.	.	.	.	.	.
Diet questionnaires							
Block Food Questionnaire (adult)	.	X	.	X	X	X	X
Brief Block Food Questionnaire (age 2-17)	.	X	.	X	X	X	X
Activity questionnaires							
Physical Activity Questionnaire (adult)	.	X	.	X	X	X	X
Modifiable Activity Questionnaire (age 8-17)	.	X	.	X	X	X	X
Quality of life questionnaires							
SF-36 (adult)	.	X	.	X	X	X	X
Pediatric Quality of Life							
Child's form (age 5-7, or age 8-12)	.	X	.	X	X	X	X
Adolescent form (age 13-17)	.	X	.	X	X	X	X
Parent's form (age less than 18)‡	.	X	.	X	X	X	X
Hematology	.	X	.	X	X	X	X
Liver panel	.	X	.	X	X	X	X
Clinical chemistry	.	X	.	X	X	X	X
HbAlc§	.	X	.	X	X	X	X
Lipid profile (fasting)	.	X	.	X	X	X	X
Glucose and insulin levels (fasting)	.	X	.	X	X	X	X
Etiologic tests	X	.	.	.	.	.	.
Specimens for banking¶	.	X	.	X	X	X	X

* At least one imaging study for suspected NAFLD or suspected cryptogenic cirrhosis.
† A = as available
‡ Parents complete the Parent form of the Pediatric Quality of Life questionnaire until the child is age 18 (separate parent versions are used for child or teen patients as appropriate)
§ In established or newly diagnosed diabetes.
¶ Specimens for banking and ancillary studies include: liver tissue, serum, plasma, and DNA.

1.3. Whole blood draw schedule: mL of blood to be drawn at screening and followup visits

Procedure*	s1	s2	24	48	96	144	192	Total
Fasting glucose and insulin	.	5	.	5	5	5	5	**25**
Fasting lipid	.	5	.	5	5	5	5	**25**
CBC	.	5	.	5	5	5	5	**25**
Clinical chemistry	.	5	.	5	5	5	5	**25**
Hepatic panel	.	5	.	5	5	5	5	**25**
HbA1c	.	5	.	5	5	5	5	**25**
Plasma: TGF-β	.	5*	.	5	5	5	5	**25**
Serum: fibrosis	.	10*	.	10	10	10	10	**50**
Serum: banking	.	30*	.	30	30	30	30	**150**
Genetics	.	20*	.	.	.	.	.	**20**
Other screening	40	.	.	.	.	.	.	**40**
Total	**40**	**95**	**0**	**75**	**75**	**75**	**75**	**435**

All NAFLD Database study visits except for the first screening visit are fasting visits and need to be scheduled for early morning. Fasting is defined as nothing by mouth except water in the 12 hours prior to blood draw.

*For children younger than 8 years of age, blood draw will be as much as possible per institutional guidelines.

1.4. Study population composition

- 1500 patients age 2 or older with:
  - suspected NAFLD (capped at 20%)
  - definite NAFLD (65%)
  - suspected or definite cryptogenic cirrhosis (15%); no more than half of these may have suspected cryptogenic cirrhosis
- ~200 patients per clinical center
- ~25% pediatric
- 30 - 40% diabetic

2. Eligibility and enrollment

2.1. Inclusion and exclusion criteria

Inclusion criteria

In order to be enrolled in the Database, patients must satisfy all of the following:

- Alcohol use history consistent with NAFLD
- Imaging study suggestive of NAFLD or biopsy materials meeting histologic definition of NAFLD or cryptogenic cirrhosis (histology must be confirmed by local NASH CRN pathologist) or imaging study and clinical findings meeting the criteria for suspected (clinical) cryptogenic cirrhosis
- Ability and willingness of patient or legal guardian/representative to give written, informed consent
- Age at least 2 years at initial screening visit
- Ability and willingness to participate in followup

Exclusion criteria

Patients who satisfy any of the following will not be eligible:

- Clinical evidence of alcoholic liver disease: Regular and excessive use of alcohol within the 2 years prior to interview defined as alcohol intake greater than 14 drinks per week in a man or greater than 7 drinks per week in a woman. Approximately 10 g of alcohol equals one 'drink' unit. One unit equals 1 ounce of distilled spirits, one 12-oz beer, or one 4-oz glass of wine.
- Total parenteral nutrition within 3 months of interview
- Short bowel syndrome
- History of gastric or jejunoileal bypass preceding the diagnosis of NAFLD. Bariatric surgery performed concomitant with or following the diagnosis of NAFLD does not exclude enrollment of patients.
- History of biliopancreatic diversion
- Evidence of advanced liver disease defined as a Child-Pugh-Turcotte score of equal to or greater than 10
- Evidence of chronic hepatitis B as marked by the presence of HBsAg in serum (patients with isolated antibody to hepatitis B core antigen, anti-HBc, are not excluded)
- Evidence of chronic hepatitis C as marked by the presence of anti-HCV or HCV RNA in serum
- Low alpha-1-antitrypsin level and ZZ phenotype (both determined at the discretion of the investigator)

- Wilson's disease
- Known glycogen storage disease
- Known dysbetalipoproteinemia
- Known phenotypic hemochromatosis (HII greater than 1.9 or removal of more than 4g of iron by phlebotomy in an individual age 18 or older)
- Prominent bile duct injury (florid duct lesions or periductal sclerosis) or bile duct paucity
- Chronic cholestasis
- Vascular lesions (vasculitis, cardiac sclerosis, acute or chronic Budd-Chiari, hepatoportal sclerosis, peliosis)
- Iron overload greater than 3+
- Zones of confluent necrosis, infarction, massive or sub-massive, pan-acinar necrosis
- Multiple epithelioid granulomas
- Congenital hepatic fibrosis, polycystic liver disease
- Other metabolic/congenital liver disease
- Evidence of systemic infectious disease
- Known HIV positive
- Disseminated or advanced extrahepatic malignancy
- Concomitant severe underlying systemic illness that in the opinion of the investigator would interfere with completion of followup
- Active drug use or dependence that, in the opinion of the study investigator, would interfere with adherence to study requirements
- Inability to provide informed consent

2.2. Calculation of Child-Pugh-Turcotte score

Child-Turcotte-Pugh score for severity of liver disease will be calculated as follows:

	Points
1. Serum albumin (g/dL; recorded on the LR form)	
greater than 3.5	1
2.8 – 3.5	2
less than 2.8	3
2. Serum total bilirubin (mg/dL; recorded on the LR form)	
less than 2.0	1
2.0 – 3.0	2
greater than 3.0	3
3. Prothrombin time (INR; recorded on the LR form)	
less than 1.7	1
1.7 – 2.3	2
greater than 2.3	3
4. Ascites: use all available information from all sources and best medical judgement	
None	1
Mild, easily managed	2
Severe, refractory	3
5. Encephalopathy: use all available information from all sources and best medical judgement	
None	1
Mild, easily managed	2
Severe, refractory	3

2.3. ICD-9-CM codes for medical conditions pertaining to eligibility and surveyed on medical history forms

The NAFLD Database eligibility determination may require data abstraction from patient charts and administrative records, in addition to administering interviews and ordering study specific tests. In addition, the patient's status relative to a long list of medical conditions is queried on the Baseline Medical History (BG) form and on the Followup Medical History (HI) form. The International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) codes for these conditions are specified below as an aid to data abstraction.

Viral hepatitis (070)

Malignant neoplasm of liver and intrahepatic bile ducts (155)

Malignant neoplasm of gallbladder and extrahepatic bile ducts (156)

Acquired hypothyroidism (244)

Diabetes mellitus (250)

Ovarian dysfunction (256)
- Polycystic ovaries (256.4)

Disorders of carbohydrate transport and metabolism (271)
- Glycogen storage disease (271.0)

Disorders of lipoid mechanism (272)
- Pure hypercholesterolemia (272.0)
- Pure hyperglyceridemia (272.1)
- Mixed hyperlipidemia (272.2)
- Other and unspecified hyperlipidemia (272.4)
- Lipodystrophy (272.6)

Gout (274)

Disorders of mineral metabolism (275)
- Disorders of iron metabolism (275.0)
  - Hemochromatosis, iron overload

2.3. ICD-9-CM codes for medical conditions pertaining to eligibility and surveyed on medical history forms

Disorders of copper metabolism (275.1)
Wilson's disease

Disorders of fluid, electrolyte, and acid-base balance (276)
Acidosis (276.2)
Fluid overload, retention (276.6)
Other metabolic disorders (277)
Cystic fibrosis (277.0)
Amyloid degeneration of liver (277.3)
Disorders of bilirubin excretion, Gilbert's syndrome (277.4)
Alpha-1 antitrypsin deficiency (277.6)
Dysmetabolic syndrome X (277.7)

Obesity (278)
Obesity, unspecified (278.0)
Morbid obesity (278.01)
Localized adiposity (278.1)

Disorders involving the immune mechanism (279)
Autoimmune hepatitis, not elsewhere classified (279.4)

Coagulation defects (286)
Acquired coagulation factor deficiency due to liver disease (286.7)

Schizophrenic disorders (295)

Affective psychoses (296)
Major depressive disorder, bipolar affective disorder

Neurotic disorders (300)
Anxiety states (300.0)
Obsessive-compulsive disorders (300.3)

Personality disorders (301)

Epilepsy (345)

2.3. ICD-9-CM codes for medical conditions pertaining to eligibility and surveyed on medical history forms

Muscular distrophies and other myopathies (359)

Hypertensive disease (401-405)
- Essential hypertension (401)

Ischemic heart disease
- Acute myocardial infarction (410)
- Other acute and subacute forms of ischemic heart disease (411)
- Old myocardial infarction (412)
- Angina pectoris (413)
- Other forms of chronic ischemic heart disease (414)

Ill-defined descriptions and complications of heart disease (429)
- Cardiovascular disease, unspecified (429.2)
  - Arteriosclerotic cardiovascular disease, cardiovascular arteriosclerosis

Cerebrovascular disease
- Transient cerebral ischemia (435)
- Acute, but ill-defined, cerebrovascular disease (436)
  - Apoplexy, cerebral seizure, cerebrovascular accident, stroke
- Other and ill-defined cerebrovascular disease (437)

Atherosclerosis (440)

Disease of capilleries (448)
- Nevus, non-neoplastic (448.1)
  - Spider nevi, **spider angiomata**, or vascular (arterial) spider is a common skin stigma in cirrhosis usually forming on the skin above the level of diaphragm attachment. It consists of a central arteriole, from which many small vessels radiate. The central arteriole is sometimes elevated, and its pulsation can be felt. Similar nevi also develop in the shoulder and upper arm, but they usually lack of the central arteriole.

Portal vein thrombosis, portal obstruction (452)

Budd-Chiari syndrome, hepatic vein thrombosis (453)

Esophageal varices with bleeding (456.0)

2.3. ICD-9-CM codes for medical conditions pertaining to eligibility and surveyed on medical history forms

Acute and subacute necrosis of liver (570)
- Acute hepatic failure
- Acute or subacute hepatitis, not specified as infective
- Necrosis of liver
- Parenchymatous degeneration of liver

Chronic liver disease and cirrhosis (571)
- Chronic hepatitis (571.4)
- Cirrhosis of liver without mention of alcohol (571.5)
  - Cryptogenic, macronodular, micronodular, posthepatic, postnecrotic
  - Portal cirrhosis
- Biliary cirrhosis (571.6)
  - Chronic nonsuppurative destructive cholangitis
  - Cholangitic cirrhosis
  - Cholestatic cirrhosis
- Other chronic nonalcoholic liver disease (571.8)
  - Fatty liver, without mention of alcohol
- Unspecified chronic liver disease without mention of alcohol (571.9)

Liver abscess and sequelae of chronic liver disease (572)
- Abscess of liver (572.0)
- Portal pyemia (572.1)

- Hepatic coma (572.2)
  - Hepatic encephalopathy
  - Hepatocerebral intoxication
  - Portal-systemic encephalopathy
  - **Asterixis**: An abnormal tremor consisting of involuntary jerking movements, especially in the hands, frequently occurring with impending hepatic coma.
- Portal hypertension (572.3)
- Hepatorenal syndrome (572.4)
- Other sequelae of chronic liver disease (572.8)
  - Hepatopulmonary syndrome

Other disorders of liver disease (573)
- Chronic passive congestion of liver (573.0)
- Hepatitis, unspecified (573.3)

2.3. ICD-9-CM codes for medical conditions pertaining to eligibility and surveyed on medical history forms

Toxic (noninfectious) hepatitis

Cholelithiasis (574)

Other disorders of gallbladder (575)

Other disorders of biliary tract (576)

Diseases of pancreas (577)

Intestinal malabsorption (579)
Postoperative blind loop syndrome (579.2)
Short bowel syndrome

Nephritis, nephrotic syndrome, and nephrosis (580-589)

Hydronephrosis (591)

Calculus of kidney and ureter (592)

Other disorders of kidney and ureter (593)

Other conditions in the mother classifiable elsewhere, but complicating pregnancy (648)
Abnormal glucose tolerance in pregnancy, **gestational diabetes** (648.8)

Erythematous conditions (695)
Unspecified erythematous condition, **erythema** (695.9) Redness of the palmar skin caused by dilatation and congestion of the capillaries. **Palmar erythema** represent extensive arteriovenous anastomoses in the thenar and hypothenar eminences and digital pads of the fingers.

Other diseases of skin (701)
Acquired **acanthosis nigricans** (701.2) An eruption of velvety wartlike growths accompanied by hyperpigmentation mainly in the skin of the neck.

Diffuse diseases of connective tissue
Systemic lupus erythematosus (710.0)

2.3. ICD-9-CM codes for medical conditions pertaining to eligibility and surveyed on medical history forms

Rheumatoid arthritis (714.0)

Disorders of muscle, ligament, and fascia (728)
Muscular calcification and ossification (728.1)
Contracture of palmar fascia (728.6)
Other disorders of muscle, ligament, and fascia (728.8)
Interstitial myositis (728.81)

Other disorders of soft tissues (729)
Myalgia and myositis, unspecified (729.1)
Other congenital anomalies of digestive system (751)
Anomalies of gallbladder, bile duct, and liver (751.6)
Congenital cystic disease of liver (751.62)

General symptoms (780)
Sleep disturbances (780.5)
Sleep disturbance, unspecified (780.50)
Insomnia with **sleep apnea** (780.51)
Hypersomnia with **sleep apnea** (780.53)
Other and unspecified **sleep apnea** (780.57)

Symptoms involving skin and other integumentary tissue (782)
Edema (782.3) An accumulation of an excessive amount of watery fluid in cells, tissues, or serous cavities.
Jaundice, unspecified (782.4) Yellowish discoloration of the whites of the eyes, skin, and mucous membranes caused by deposition of bile salts in these tissues. Jaundice is a sensitive indicator of liver dysfunction. As a sign and symptom, jaundice and hyperbilirubinemia are among the frequently used "liver function" tests.

Symptoms concerning nutrition, metabolism, and development (783)
Abnormal weight gain (783.1)
Polyphagia (783.6)

Other symptoms involving abdomen (789)
Abdominal pain (789.0)
Hepatomegaly (789.1)
Splenomegaly (789.2)

2.3. ICD-9-CM codes for medical conditions pertaining to eligibility and surveyed on medical history forms

Ascites (789.5) Lymph fluid that has leaked into the peritoneal cavity, is one of the principal clinical manifestations cirrhosis and portal hypertension.

Nonspecific findings on examination of blood (790)
- Abnormal glucose (790.2)
  - Impaired fasting glucose, elevated fasting glucose (790.21)
  - Impaired glucose tolerance test (oral), elevated glucose tolerance test (790.22)
  - Abnormal non-fasting glucose (790.29)
- Nonspecific elevation of levels of transaminase or lactic acid dehydrogenase (790.4)
- Other nonspecific findings on examination of blood (790.9)
  - Abnormal coagulation profile (790.92)

Nonspecific abnormal findings on radiological and other examination (793)
- Abdominal area, including retroperitoneum (793.6)

Nonspecific abnormal results of function tests (794)
- Liver, abnormal liver scan (794.8)

2.4. Metabolic syndrome (definition)

Metabolic syndrome is defined by ALL of the following:

1. Waist circumference
   102 cm or greater in males
   88 cm or greater in females

2. Hypertriglyceridemia: 150 mg/dL or greater

3. Low high density lipoprotein (HDL) levels
   less than 40 mg/dL in males
   less than 50 mg/dL in females

4. High blood pressure with systolic 130 mm Hg or greater and diastolic 85 mm Hg or greater

5. High fasting glucose: 110 mg/dL or greater

2.5. Guidelines for repeat determinations of eligibility

While certain inclusion and exclusion criteria are more objective and are unlikely to change, others are more subjective and may change over time. Thus, participants who are deemed ineligible at the time of initial screening may be rescreened at a later time as follows:

- Age < 2 years – the participant may be rescreened after his or her 2nd birthday
- Unwilling to participate – the participant may be rescreened after 3 months at the discretion of the investigator
- TPN within 3 months of interview – the participant may be rescreened if he/she has been off TPN for at least 6 months and strong evidence of NAFLD remains on repeat evaluation
- Child-Pugh-Turcotte score of 10 or 11 – the participant may be rescreened after 3 months at the discretion of the investigator

2.6. Co-enrollment in PIVENS or TONIC treatment trial

- When a NAFLD Database patient enrolls in a NASH CRN main treatment trial (PIVENS or TONIC), the visit schedule and requirements of the main treatment trial take precedence over the requirements for the NAFLD Database – Database requirements are suspended for the duration of the participant's time in the treatment trial. The Database Closeout Form (CO) should be completed to suspend the Database visits while the patient is enrolled in the treatment trial. The treatment trial protocol should provide instructions, but if you cannot find the answer to your question, call the Data Coordinating Center

- Data requirements are not suspended while a patient participates in a NASH CRN ancillary study or pilot or feasibility study

2.7. Transferring patient between studies

Procedures for transferring patients between the NAFLD Database and PIVENS are given below. The specific procedure that needs to be followed varies depending on whether the patient completed enrollment in the NAFLD Database. Please note the following definitions:

- Registered in Database/PIVENS = RG form for Database/PIVENS has been completed but patient has not enrolled/been randomized in Database/PIVENS (ie, patient is in screening for the Database/PIVENS or has been closed out of the Database as ineligible)
- Enrolled in Database = Database enrollment task has been run and the patient was found eligible and was enrolled (ie, patient is in followup for the NAFLD Database)
- Randomized in PIVENS = Randomization task was run and the patient was found eligible and was issued a treatment assignment

1. Patient enrolled in NAFLD Database who now wants to screen for PIVENS

- Whenever possible, the clinical center should wait at least 8 weeks after enrollment in the NAFLD Database before registering the patient in PIVENS. The rationales for this are: (1) we want complete, fresh data in PIVENS and a patient is more likely to be willing to complete forms and procedures if there has been a noticeable duration since he/she completed forms for the Database and (2) to encourage patients who are likely PIVENS candidates to enter directly into PIVENS; the clinical center can use physician discretion regarding registering the patient (ie, patient can be registered before the suggested 8-week time limit), but this should be the exception rather than the rule. The ineligibility symbol has been removed from this item on the PIVENS RG form.
- Have the patient sign the PIVENS consent form
- Complete and key the PIVENS RG form but do NOT issue a new patient ID number and code
- Blood for serum and plasma repository
  - Blood must be collected for the serum and plasma repository even if already banked for the Database regardless of the time between enrollment in the Database and registration in PIVENS

- Blood for genetics repository
  - If not already collected, have the patient sign the PIVENS genetic consent and collect a sample and complete the PIVENS BC and CG forms
  - If blood was already collected, do not send another sample unless the yield was unsatisfactory
  - If the yield on the sample drawn when the patient screened for the Database was satisfactory, leave the Database BC and CG forms in the data system and complete the PIVENS BC form answering 'yes' to the question about prior blood draw for the Database; the patient does not need to sign the PIVENS genetic consent nor does the PIVENS CG form need to be completed
  - If the yield on the sample drawn when the patient screened for the Database was unsatisfactory, have the patient sign the PIVENS genetics consent form, draw the replacement sample, and complete the PIVENS BC and CG forms; the Database BC and CG forms should remain in the data system
- Lab results reported on the Database LR and LS forms may be used on the PIVENS LR and LS forms if they were obtained within the time windows specified on the forms
- All interviews and patient questionnaires (drinking history, AUDIT, baseline history, liver symptoms, quality of life, physical activity, and food questionnaire) must be completed anew for PIVENS
- The physical exam (PE) form must be completed anew for PIVENS
- If the biopsy used for PIVENS is the same one that was used for the NAFLD Database (keep in mind that the biopsy for PIVENS must meet date and medication requirements not imposed in the NAFLD Database), the local pathologist must review the slides again and complete the PIVENS HF form. The PIVENS SD form must be completed; transcribe information from the Database SD (or SE or SF) form. For items relating to slide sequence numbers, transcribe the slide numbers for the slides that were previously sent for the Database (ie, only 10 unstained slides need to be sent from a single biopsy). If flash frozen liver tissue was obtained, the PIVENS LT form must be completed; transcribe information from the Database LT form. Where the PIVENS LT form asks for the duplicate LT label to be affixed to the LT form, write in the label information from the Database LT form and write in the margin "see Database LT form". There will be more than 1 form in the data system pointing to the same numbered slides and liver tissue vials (Database SD/LT and PIVENS SD/LT forms), but this is ok since the patient enrolled in the Database.
- If the patient is eventually randomized in PIVENS, have the patient complete PIVENS visits and forms; you do not need to complete the MV form for the missed Database visits, but you do need to complete the Database Closeout (CO) form to suspend the patient's participation in the NAFLD Database. The CO form can be completed prior

to or after randomization in PIVENS, but our advice is to complete it upon randomization in PIVENS. The patient remains enrolled in the NALFD Database while participating in PIVENS, but the patient is not subject to completion of NAFLD Database visits.

- Retain all Database forms completed for the patient in the patient's NASH CRN file
- Retain the patient's Database visit windows schedule since it will be needed once PIVENS is completed

2. Patient registered in NAFLD Database but never enrolled, now wants to register in PIVENS

- The patient should be closed out of the NAFLD Database by completing and keying the Database ED form to document the reason(s) why the patient didn't enroll in the Database. Answer as many of the questions in sections B, C, D, E and F of form ED as you can, coding an item as 'm' if you do not know the answer; if the patient is eligible for the Database but is opting to go directly into PIVENS, answer 'no' to item 25 (no longer consents) and check 'Other reason' in item 26c and write in 'opted to go directly into PIVENS'
- Have the patient sign the PIVENS consent form
- Complete and key the PIVENS RG form but do NOT issue a new patient ID number and code
- Blood for serum and plasma repository
  - If more than 8 weeks have elapsed since the previous blood draw or if serum and plasma were not obtained for the Database, you must do the blood draws for serum and plasma for PIVENS
  - If less than 8 weeks have elapsed since the previous blood draw for serum and plasma and the serum and plasma have been kept frozen or were sent to the repository at Fisher:
    - The blood draw for serum and plasma does not need to be repeated
    - Transcribe information from the Database BP form to the PIVENS BP form; the date in item 4 of the PIVENS BP form should be the current date, but enter the actual date of blood draw (ie, the date in item 9 on the Database BP form) in item 9 of the PIVENS BP form; when you are asked to apply duplicate labels to the PIVENS BP form, write in the label information and in the margin write "see Database BP form"; key the PIVENS BP form. If the Database BP form has been keyed, it can remain in the data system.
- Blood for genetics repository
  - If not already collected, have the patient sign the PIVENS genetic consent and collect a sample and complete the PIVENS BC and CG forms
  - If blood was already collected, do not send another sample unless the yield was unsatisfactory

  - If the yield on the sample drawn when the patient screened for the Database was satisfactory, key the Database BC and CG forms (if not already keyed) and complete the PIVENS BC form answering yes to the question about prior blood draw for the Database; the patient does not need to sign the PIVENS genetic consent nor does the PIVENS CG form need to be completed
  - If the yield on the sample drawn when the patient screened for the Database was unsatisfactory, then have the patient sign the PIVENS genetic consent form, draw the replacement sample, and complete the PIVENS BC and CG forms based (the Database BC and CG forms can remain in the data system)
- Interviews and questionnaires must be completed on the PIVENS forms
  - Data from the Database AD and LD forms may be transcribed to the corresponding PIVENS forms, but the patient should be queried regarding any changes since the previous interviews; the date in item 4 on each PIVENS form should be the date you review the information with the patient
  - The PIVENS BG form should be completed anew – it is different from the Database BG form, and the PIVENS BG form data help establish that the biopsy is a medication free biopsy (medication use is not an issue with Database biopsies)
  - The patient should complete the PIVENS LQ, QF, and PA forms anew
  - The patient should complete the Block Food Questionnaire anew and the BD form should be completed anew
- The physical exam (PE) form must be completed anew
- If the same biopsy is used for PIVENS that was used for the Database (keep in mind that the biopsy for PIVENS must meet date and medication requirements not imposed in the NAFLD Database), the local pathologist must review the slides again and complete the PIVENS HF form. The PIVENS SD form needs to be completed; transcribe information from the Database SD (or SE or SF form). For items relating to slide sequence numbers, transcribe the slide numbers that were previously sent for the Database (ie, only 10 unstained slides need to be sent from a single biopsy). If flash frozen liver tissue was obtained, the PIVENS LT form must be completed; transcribe information from the Database LT form. Where the PIVENS LT form asks for the duplicate LT label to be pasted onto the LT form, write in the label information from the Database LT form and write in the margin ”see Database LT form”. The Database SD/SE/SF and LT forms can remain in the data system.
- Retain all Database forms completed for the patient in the patient’s NASH CRN file

3. Patient registered in PIVENS, but found to be ineligible, now wants to register in the Database

- The patient should be closed out of PIVENS by completing and keying the PIVENS EC form to document the reason(s) the patient was found ineligible
- Have the patient sign the Database consent form
- Complete and key the Database RG form but do NOT issue a new patient ID number and code
- Blood for serum and plasma repository
    - If serum and plasma were not obtained in PIVENS, do the blood draws for serum and plasma in the Database
    - If more than 8 weeks have elapsed since the blood draw for serum and plasma, do the blood draws for serum and plasma for the Database anew
    - If 8 weeks or less have elapsed since the previous blood draw for serum and plasma and the serum and plasma have been kept frozen or were sent to the repository at Fisher:
        - The blood draw for serum and plasma does not need to be repeated
        - Transcribe the data from the PIVENS BP form to the Database BP form; the date in item 4 of the Database BP form should be the current date, but enter the actual date of blood draw (ie, the date from item 9 on the PIVENS BP) in item 9 of the Database BP form; when you are asked to apply duplicate labels to the Database BP form, write in the label information and write "see PIVENS BP form" in the margin. Key the Database BP form; if the PIVENS BP form has already been keyed, it may remain in the data system.
- Blood for genetics repository
    - If blood was not already collected, have the patient sign the Database genetic consent and collect a sample and complete the Database BC and CG forms
    - If blood was already collected, do not send another sample unless the yield was unsatisfactory
    - If the yield on the sample drawn when the patient screened for PIVENS was satisfactory, make sure that the PIVENS BC and CG forms have been keyed. Complete the Database BC form: answer "No" to item 9 on the Database BC form and write "collected when screening for PIVENS" in the specify line. Key the Database BC form. The patient does not need to sign the Database genetics consent and the Database CG form does not need to be completed.
    - If the yield on the sample drawn when the patient screened for PIVENS was unsatisfactory, then have the patient sign the Database genetic consent form, draw the replacement sample, and complete the Database BC and CG forms based (the PIVENS BC and CG forms can remain in the data system)

- Interviews and questionnaires must be completed on the Database forms
  - Data from the PIVENS BG, AD, and LD forms may be transcribed to the corresponding Database forms, but the patient should be queried regarding any changes since the previous interviews (**NOTE**: care should be taken in transcribing the BG form since the BG forms are similar, but are not the same); the date in item 4 on each Database form should be the date that the information is reviewed with the patient
  - Patient should complete the Database LQ, PA, and QF forms anew even if they were completed for PIVENS
  - The Database physical examination (PE) form must be completed anew if more than 8 weeks have elapsed since the PIVENS PE form was completed; if fewer than 8 weeks have elapsed, repeat the measurement data anew (height, weight, circumferences, skin fold etc) and review the Examination findings (section C) for any findings that are likely to have changed; if the Examination findings are unlikely to have changed, transcribe those data to the Database PE form; otherwise, complete the Examination findings anew; date the PE form with the current date (ie, the date when the measurements were redone)
  - If the patient completed the Block Food Questionnaire in PIVENS within the past 8 weeks, he/she does not need to complete it again for the Database. The data from the PIVENS BD form should be transcribed to the Database BD form (revision 2 only – revision 1 is inadequate for this situation). Put the current date in item 4 of the Database BD form but enter the date when the Block food questionnaire was completed in item 8 of the BD form. The date in item 8 should match the date completed on the questionnaire booklet (bubble areas) and on the label on the booklet. Where you are asked to affix a label to the BD form, transcribe the information from the label affixed to the PIVENS BD form and write in the margin ”see PIVENS BD form”. The PIVENS BD form can remain in the data system.
- If the same biopsy is used for the Database that was used for PIVENS, the local pathologist must review the slides again and complete the Database HF/HE form. If slides were previously sent for PIVENS, the Database SD/SE form must be completed referencing the slide numbers for the slides that were sent (ie, only 10 unstained slides need to be sent from a single biopsy). If flash frozen liver tissue was obtained, the Database LT form must be completed. Where the Database LT form asks for the duplicate LT label to be affixed to the LT form, write in the label information from the PIVENS LT form and write in the margin ”see PIVENS LT form”. The PIVENS SD and LT forms can remain in the data system.

- Retain the PIVENS forms in the patient's NASH CRN file

These procedures are complicated and there are bound to be problems when it comes to applying the protocols to real patients. As always, contact the DCC if you have questions or if you run into problems when trying to key forms or enroll/randomize a patient.

2.8. Roll over into NAFLD Database after completion of PIVENS or TONIC

- Patients who complete participation in PIVENS or TONIC should resume participation in the NAFLD Database (if previously enrolled in the Database) or be invited to join the NAFLD Database (if not previously enrolled). A PIVENS/TONIC Closeout (CO) form should be completed at the F120 visit (or at the close of the F120 visit window) for all patients randomized in PIVENS/TONIC.

- If the patient was previously enrolled in the NAFLD Database, the patient resumes participation in the Database by completing the visit that is open on the patient's Database visit time windows guide

- If the patient was not previously enrolled in the NAFLD Database, the patient will receive a new visit schedule upon the keying of the CO form into the database. This new visit schedule will use the PIVENS or TONIC randomization date as the effective enrollment date into NAFLD database.

2.9. Enrollment and eligibility checking

Enrollment steps

- Complete collection of baseline data and key baseline data forms
- Run electronic check on eligibility (ie, run the Enrollment Task and resolve any ineligibility conditions)
- Run the Enrollment Task; if the patient is eligible, this task will officially enroll the patient in the Database and materials needed in followup will be generated (ie, labels, visit time window)

Overriding eligibility criteria

- Requests overriding eligibility criteria must be made in writing to the DCC (direct the request to Aynur Ünalp-Arida); the request must specify the eligibility criteria for which override is requested and the request must be justified; the request must come from the principal investigator of the clinical center
- The DCC may require agreement to the override from other NASH CRN investigators
- Override requests require time to review and the review process will not be shortened

Enrollment date

- The date the clinical center runs the Enrollment Task and enrolls the patient
- The "time zero" for reckoning the time windows specified on the patient's Database visit time window guide is the date of enrollment

3. Certification

3.1. Certification overview

What is certification?

- It is an internal (ie, related to the study) procedure designed to identify the staff responsible for specific data items or data collection procedures or decisions about eligibility
- It is a managerial and quality assurance tool for the study

Who and what does it apply to?

- It applies to:
  - NAFLD Database staff
  - Each clinical center
- Certification for the NAFLD Database is required before any patient visits or data collection may occur; patients may not begin any screening examinations, sign any consent statements, or complete any study forms until the clinical site has been certified for the study
- More than one staff member may be certified for a function and it is recommended that more than one staff member be certified for a function

Why do we require it?

- Primary purpose is to help assure consistent conduct of the study over time, within and across clinics. The conduct of procedures should be similar across patients and in serial testing of the same patient over the duration of followup.
- Study procedures may vary from the usual practice of a participating clinical center, but it is important that methods be carried out in the same manner within and across clinical centers.
- It identifies the staff and sites that carry out study procedures and identifies to staff that they and their site are a part of the NAFLD Database study.
- It provides a mechanism for tracking who collected key data items or made key decisions.
- The certification process may help a clinic prepare for study activities by presenting the training, facility, and equipment needs in an organized fashion and requiring acquisition or completion of these items before study specific activities may begin.

Is separate certification in each NASH CRN study required?

- Certification for NAFLD Database procedures will constitute the initial round of NASH CRN certification activities. As the PIVENS and TONIC studies are about to begin patient activities, certification requirements related to the specific study that is to be started will be issued through notification of each clinical center by a numbered Policy and Procedure Memorandum.

3.2. Clinical center certification

General comments

- Each clinical center participating in the NAFLD Database must be certified for that participation
- Completion of the Clinical Center Certification (CC) form will be required
- IRB approval for the NAFLD Database protocol and consents/assents will be required

Purpose of clinical center certification

- Provide information regarding how the clinical center will conduct different aspects of the protocol, who will staff the study, and how the pediatric and adult components of the NAFLD Database study will interact and efficiently communicate
- Guide a clinical center through the steps of getting ready for the NAFLD Database – provide a checklist of what needs to be in place before patient activities begin

Requirements for certification of a site

- Complete the Clinical Center Certification (CC) form
- Certify at least one person for each function that requires certification (a person may be certified for more than one function)
- Obtain IRB approval of the most current NAFLD Database protocol and consent and assent documents
- Receive written notice of approval (email) from the Data Coordinating Center that the site is certified

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3.3. Personnel certification

Staff functions requiring certification

- Clinical Coordinator
- Study Physician
- Pathologist
- Data Entry Technician

Requirements

- Everyone
  - Read the NAFLD Database protocol
  - Complete the Knowledge Assessment (KA) form; this is a written general knowledge assessment about the Database (open book)
  - Complete the Personnel Certification (PC) form; this form identifies the functions applied for and provides an assurance of data confidentiality and integrity
- Additional requirements for Study Physician
  - Identify whether physician will complete evaluations of pediatric patients, adult patients, or both
  - Study Physician must be an MD preferably a hepathologist
- Additional requirements for Pathologist
  - Be approved by David Kleiner and Elizabeth Brunt
- Additional requirements for Data Entry Technician
  - Complete the Data Entry Certification/Decertification Request (DC) form
  - Complete the data system tutorial

Process

- Send required materials to the DCC
- The DCC will send written notice of approval for certification or pending certification
- Each staff member will be issued a Personnel Identification Number (PIN)

Staff PINs

- Each staff member certified for at least one function will be issued a PIN which will consist of 3 digits – the first digit will identify the clinical center and the next two digits will be a sequential number assigned by the Data Coordinating Center
- The PIN is used when completing forms
- The Data Entry Technician uses his/her PIN when signing on to the NAFLD data system
- Staff can be certified for more than one function but will have only one PIN

4. Human subjects

4.1. Background

Consent to participation in the NAFLD Database must be completed before screening for the Database may begin. The patient must consent to procedures offered to and performed on him/her for screening, as well as to the followup visits which the patient will face in the future.

The consent process is a dynamic process involving explanations, time to think, questions, clarifications, and advice that a patient may seek from relatives, friends or anybody else considered relevant. We wish to inform the prospective participant as much as possible and as accurately as possible about what will be offered to him/her, how it will be done, what are the reasonable risks and benefits, what are the alternatives, and what is expected of the patient. We wish to answer patients' questions in a consistent and complete way.

The NAFLD Database consent and assent process has three major stages:

- The patient is asked to consent or assent to screening and enrollment into the NAFLD Database
- The patient is asked to consent or assent to the collection, storage, and use of blood samples for genetic research
- The patient is asked to sign the HIPAA authorization to disclose protected health information

4.2. Institutional review board process

Seven prototype consent and assent statements have been prepared for the NAFLD Database:

- Consent for screening and enrollment in the Database
- Consent for screening and enrollment in the Database (parent)
- Assent for screening and enrollment in the Database (adolescent)
- Assent for screening and enrollment in the Database (child)
- Consent for the collection, storage, and use of blood samples for current and future genetic research
- Consent for the collection, storage, and use of blood samples for current and future genetic research (parent)
- Assent for the collection, storage, and use of blood samples for current and future genetic research (adolescent)

Clinics are expected to use these materials in their submissions to their institutional review boards (IRBs) for approval to participate in the NAFLD Database. Each clinic must send copies of the consent statements to be used in their clinic, stamped with their IRB's seal, to the Data Coordinating Center prior to initiating patient activities in the NAFLD Database. Data Coordinating Center staff will review and compare the approved local consents and assents to the prototype consents and assents. Specific local additions to and editing of the prototypes may be required at individual institutions, but deletion of material and major rewording of text may need to be explained and justified. Once a consent or assent form has been approved by an institution's IRB, it cannot be changed without the IRB's approval.

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 clinical center may not initiate any patient contact about the NAFLD Database until the site has IRB approval for the Database 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.

HIPAA authorization forms will be prepared by each clinical center according to local clinical center institutional requirements and guidelines.

4.3. Consent administration

NAFLD Database consents and assents

It is assumed that patients referred to a clinical center for screening have heard about the NAFLD Database, but their level of knowledge and expectations may well differ. We wish to standardize the consent administration across clinical centers as much as possible. Administration of the Database consents and assents involves two tasks:

(1) A NAFLD Database staff member must sit down with the patient and if the patient is a child, with the child's parent or guardian, and review the contents of the statement; explain the risks, benefits, and responsibilities of participation; review the alternatives to participation; and answer questions. In the case of a child patient, a NAFLD Database staff member must also sit down with the child and review the assent statement.

(2) A NAFLD Database certified study physician (i.e., a NAFLD Database certified adult hepatologist or a NAFLD Database certified pediatric counterpart) must sign the consent statement, taking overall responsibility for the patient's informed and voluntary consent.

Staff at each clinical center should be designated to carry out these tasks. The rationale for requiring that the consent statement be signed by a study physician is to help assure that the physician signing the consent or assent is one who has a broad role in the study.

Generally, the consent and assent statements should be offered to the patient to read through at least a day before signature is requested. The consent will then be reviewed with the patient by the staff member designated to obtain consent; the consenter may opt to read the statement to the patient, pausing to explain issues as needed. This activity should take place in a quiet, private and relaxed setting in the clinical center.

The patient should sign the consent statement in the presence of the NAFLD Database staff member after all questions have been answered and when the patient has asserted orally that he/she is ready to sign the consent. After the patient has signed and dated the consent, the patient should meet with a NAFLD Database study physician for the physician to sign the consent statement; ordinarily this meeting should take place on the same day that the patient signed the consent statement. The physician should ask the patient to confirm his/her voluntary consent and query the patient about any questions or concerns the patient may have about participation. Both signatures on the consent form must be in a non-erasable ink pen. If the physician cannot meet with the patient on the same day that the patient signs the consent statement, the physician may sign on another day.

Consent for genetic research

The consent for collection and banking of blood for genetic research should be administered in the same way that the NAFLD Database consent is administered, except that it should not be signed until the patient has been determined to be eligible for the NAFLD Database.

4.4. Time considerations for obtaining consent

- The **NAFLD Database Consent and HIPAA authorization** must be obtained at the start of the initial visit (visit s1); documents from the referring physician (if any) should have been reviewed prior to the visit and the patient judged eligible for screening prior to the visit. Signature of this consent is required prior to sending the patient for any NAFLD Database diagnostic tests. A check for signature of this consent statement occurs on the Registration (RG) form.

- The **Database Consent for Collection, Storage, and Use of Blood Samples for Current and Future Genetic Research** must be obtained after eligibility for the NAFLD Database has been established, at visit s2. Signature of this consent is required prior to drawing blood for genetic research for the NAFLD Database; a check for signature of this consent statement occurs on the Blood Collection for DNA (BC) form. Signature of this consent statement is not required for NAFLD Database eligibility (i.e., the patient may choose not to participate in the genetic research component of the NAFLD Database).

- A patient may be given the consent statements to review prior to the initiation of visit s1 to meet patient needs with respect to review time. Whenever a consent is first given to a patient for review, it should be made clear to the patient that the consent should not be signed until requested by a NAFLD Database staff member. The consents may be mailed to the patient prior to visit s1. Whatever timing is used by a clinic, the patient should be allowed enough time to reflect about the proposed NAFLD Database procedures, pose questions, and consult with other individuals that he/she considers relevant to their participation in the NAFLD Database. Patients may request and should be given time to "think it over" at home and come back at a later time.

4.5. Consent handling

- Signed consent and assent statements are important legal documents. These signed statements should be kept in the patient's NAFLD Database clinical center file together with his/her other NAFLD forms and documents. These forms are not part of the individual's institutional medical record, but part of his/her study record in the NAFLD Database. Consent and assent statements will be examined during site visits.

- Consents and assents should be annotated with the patient's study identifiers (ID number and code).

- The NAFLD Database consent statement is an "all or none" form. The patient either accepts it in its entirety and signs it, or does not. The patient must consent to the evaluation procedures, the follow-up evaluations, and the banking of his/her serum, plasma, and liver tissue specimens. If the patient refuses any part, the patient may not enroll in the NAFLD Database.

- The NAFLD Database Consent for Genetic Research has been made a separate consent statement so that the patient can opt out of genetic research and still participate in the NAFLD Database.

4.6. Informing participants of changes to consent statement after enrollment

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As new data become available during the conduct of the NAFLD Database, the consent statements may need to be changed to reflect the current assessment of risks and benefits to participants in the study.

Procedures for dissemination of revisions of consent statements from the DCC

- Changes deemed necessary will be made to the prototype consent statements
- Revisions of the prototype consent statements will be distributed to sites via a numbered Policy and Procedure Memorandum (PPM) with instructions to submit the revised consent to their IRB

Procedures for reviewing changes to consent statements with participants

- Clinical center personnel will develop a chronology of IRB approved changes to the consent statements used at their site
- At each followup visit, staff will use the chronology of consent changes to review with the participant any changes to the consent since the last visit. This review does not require obtaining the participant's signature on a new consent statement, unless the local IRB requires obtaining a signature.
- Review changes to the consent statements with participants at followup visits
- This review process is not intended to be a reaffirmation of consent. The clinical center, if required by their local IRB, may develop procedures for reaffirmation of consent.

4.7. Consenting roll over patients from PIVENS and TONIC

Participants, whether new or rejoining, must sign the most recent version of the IRB approved informed consent for NAFLD Database.

If the patient previously consented to the NAFLD Database

- Review any changes to the NAFLD Database consents or assents & follow institutional specific IRB guidelines

If the patient did not consent to the NAFLD Database previously

- Consent as for a new NAFLD Database patient

4.8. HIPAA considerations

NAFLD Database study staff have access to patient health information and to patient identifiers, such as name, address, and telephone number. Study records are to be kept in a secure place. Only people working on the NAFLD Database should have access to these records. However, these records could be reviewed to make sure that the study is being done as it should. People who may see medical records supporting study records are:

- Officials of your institution
- Your institution's research ethics committee
- Monitors from the NASH CRN Data Coordinating Center at the Johns Hopkins University, or other individuals selected by the NASH CRN Steering Committee to monitor the study
- Members of the Data and Safety Monitoring Board (DSMB) to monitor overall progress of the study
- Government officials from the Office of Human Research Protections or the National Institutes of Health or the Food and Drug Administration

Each clinical center should take steps to protect patient privacy. The assigned patient ID number and code should be used to identify patients on forms and in the data files. Personal information such as name, address, and telephone number should be kept only at the clinical center where a patient completes visits.

People outside the clinical center who will receive NAFLD Database study data include:

- The NASH CRN Data Coordinating Center at the Johns Hopkins University in Baltimore, Maryland (or its successor) to maintain the central study database
- The NASH CRN Data and Safety Monitoring Board to review the NAFLD Database data for performance and safety
- The NIDDK Genetics Repository at Rutgers, the State University of New Jersey in New Brunswick, New Jersey (or its successor) will receive patients' blood to obtain DNA; the blood samples for a particular patient will be identified by the patient's study ID number and code, not by name
- The NIDDK Biosample Repository at Fisher Bioservices in Germantown, Maryland (or its successor) will receive patients' plasma, serum, and liver tissue; the samples for a particular patient will be identified by the patient's study ID number and code, not by name
- The NASH CRN investigators, as well as outside researchers, to analyze and report NAFLD Database study data. Patient identity will not be disclosed in any reports or publications resulting from the study. While the NAFLD Database is ongoing, the use of the NAFLD Database study data must be approved by the NASH CRN Steering Committee and by the research ethics committee at your institution.

4.8. HIPAA considerations

Patient agreement to join the NAFLD Database indicates that the patient also agrees to the use of study data as described above. If a patient does not agree to the described uses of study data, the patient may not participate in the NAFLD Database. The only exception is refusal to provide blood for genetic research; patients may refuse to provide blood for genetic research and still enroll in the NAFLD Database.

5. Study visits

5.1. Overview of visit schedule

Screening (must be completed within 120 days of starting)

- s1: Consent, baseline history, physical exam, blood draw for laboratory measures (Hepatitis B, Hepatitis C, Hepatitis A, iron, HFE gene analysis, ceruloplasmin, alpha-1 antitrypsin, autoantibody studies, immunoglobulin levels, and TSH) if not available from archival records, liver biopsy (if available), questionnaires on alcohol use and liver symptoms

- s2: Blood draw for laboratory measures (hematalogy, chemistries, HbA1c, liver panel, lipids, and glucose and insulin), specimen banking, and genetic research; and liver imaging studies as needed; questionnaires on quality of life, diet, activity level

Enrollment

- en: Enrollment is an event, not a visit; enrollment occurs when the clinical center staff run the enrollment task on the NAFLD Database data system and the patient is found to be eligible

Follow-up

- f024: Followup medical history, focused physical examination, collection of materials from any interim liver biopsy, and findings from any interim liver imaging studies

- f048: Followup medical history, detailed physical examination, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies, questionnaires on liver symptoms, quality of life, diet, and activity level.

- f096: Followup medical history, detailed physical examination, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies, questionnaires on liver symptoms, quality of life, diet, and activity level

- f144: Followup medical history, detailed physical examination, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies, questionnaires on liver symptoms, quality of life, diet, and activity level

- f192: Followup medical history, detailed physical examination, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies, questionnaires on liver symptoms, quality of life, diet, and activity level

5.2. Visits, data forms, and procedures

Phase/Visit	Form abbr	Procedure
Screening		
s1	RG	Registration (document consent, sociodemographics, assign IDs)
	BG	Baseline history
	LQ/LP	Symptoms of liver disease, age 18 or older/age 8-17
	PE	Physical examination (detailed exam)
	SD/SE/SF	Liver biopsy materials documentation (if liver biopsy is reported on form BG)
	HE/HF/HG	Liver biopsy histology findings (reading at clinical center; if SD form says biopsy is adequate for scoring)
	LT	Liver tissue banking (if SD form says liver tissue was obtained for banking)
	AD	AUDIT, age 8 or older (alcohol questionnaire)
	LD	Lifetime drinking history, age 18 or older (Skinner)
	LS	Lab tests done only during screening (etiologic tests)
	PL	Patient location (patient contact information)
s2	BD	Food questionnaire, age 18 or older/age 2-17 and documentation of completion of the food questionnaire
	IR	Liver imaging studies report (ultrasound, MRI, or CT; if imaging study is reported on form BG)
	LR	Lab tests done during screening and followup (hematology, hepatic, clinical chemistry, HbA1c if needed)
	PA/MA	Physical activity, age 18 or older/age 8-17
	QF/PV/PW/PY	SF-36 questionnaire, age 18 or older/age appropriate pediatric QOL form
	PQ/PR/PS/PT	Parent pediatric QOL form, age appropriate version
	CG	Genetic consent documentation
	BC	Blood collection for DNA
	BP	Blood processing for serum and plasma
	ED	Database enrollment

Phase/Visit	Form abbr	Procedure
Followup phase		
24 week followup visit		
f024	HI	Followup medical history (interim history – includes info re: adverse effects, alcohol use)
	PF	Focused physical examination
	IR	Liver imaging studies report (ultrasound, MRI, or CT; if imaging study is reported on form HI)
	SD	Liver biopsy materials documentation (if liver biopsy is reported on form HI)
	LT	Liver tissue banking (if SD form says liver tissue was obtained for banking)
	PL	Patient location (update as needed)
48 week followup visit		
f048	HI	Followup medical history (interim history – includes info re: adverse effects, alcohol use)
	PE	Detailed physical examination
	LR	Lab tests done during screening and followup (hematology, hepatic, clinical chemistry, HbA1c if needed)
	BP	Blood processing for serum and plasma
	IR	Liver imaging studies report (ultrasound, MRI, or CT; if imaging study is reported on form HI)
	SD	Liver biopsy materials documentation (if liver biopsy is reported on form HI)
	LT	Liver tissue banking (if SD form says liver tissue was obtained for banking)
	LQ/LP	Symptoms of liver disease, age 18 or older/age 8-17
	BD	Food questionnaire, age 18 or older/age 2-17, food questionnaire documentation
	PA/MA	Physical activity, age 18 or older/age 8-17
	QF/PV/PW/PY	SF-36 questionnaire, age 18 or older/age appropriate pediatric QOL form
	PQ/PR/PS/PT	Parent pediatric QOL form, age appropriate version
	PL	Patient location (update as needed)
96 week followup visit		
f096		Same as 48 week followup visit

Phase/Visit	Form abbr	Procedure
144 week followup visit		
f144		Same as 48 week followup visit
192 week followup visit		
f192		Same as 48 week followup visit
As needed		
	FI	Family member identification (patient's relative(s) enrolled in NAFLD database)
	CO	Database closeout

5.3. Guide for visit s1

Procedures

- Obtain signed consent (and assent if child patient is of an age to assent) for the NAFLD Database
- Obtain permission to abstract data from patient's medical records
- Initiate data collection for screening and baseline values
  - Physical exam and anthropometry
  - Interview for baseline history (responses may be modified or expanded upon chart review)
  - Laboratory testing (etiologic tests)
  - Liver biopsy (pathologist should grade slides from most recent biopsy [and previous biopsies if most recent biopsy does not show NAFLD] and obtain 10 unstained slides for each biopsy if possible or arrange for standard of care biopsy if appropriate; if arranging for standard of care biopsy, prepare for collection of flash frozen liver tissue)
  - Alcohol use questionnaires
  - Liver symptom questionnaire
- Obtain patient location information
- If patient appears eligible at the close of visit s1
  - Schedule patient for visit s2
  - Schedule patient for any needed etiologic tests
  - Schedule patient for standard of care biopsy if appropriate
  - Schedule patient for standard of care imaging study if appropriate
  - Schedule patient for laboratory tests (hematology, hepatic clinical chemistry, HbA1c) if needed

Data collection forms

- Forms completed for all patients
  - RG - Registration
  - PE - Physical Examination
  - BG - Baseline History
  - LS - Laboratory Results – Tests Done Only During Screening
- Additional forms for patients age 18 or older
  - AD - AUDIT
  - LD - Lifetime Drinking History (Skinner)
  - LQ - Symptoms of Liver Disease
- Additional forms for patients age 8 through 17
  - AD - AUDIT
  - LP - Symptoms of Liver Disease (Children)
- Additional form for patients age 2 through 7
  - LP - Symptoms of Liver Disease (Children)

- Additional forms required under specific conditions
  - SD/SE/SF - Liver Biopsy Materials Documentation (if biopsy is reported on Form BG)
  - HE/HF/HG - Liver Biopsy Histology Findings (if liver biopsy is available and can be scored)
  - LT - Liver Tissue Banking (if liver tissue was obtained for banking)

Forms for clinical center use only

- PL - Patient Location
- Medical records release (use local form)

After the patient leaves the clinical center

- Register patient on clinic data system
- Apply labels to forms as needed
- Set up NAFLD Database chart for patient and file the materials generated at registration that will be used at visit s2
- Key completed data forms
- Package biopsy slides for sending to the DCC
- Ship flash frozen liver tissue specimen to NIDDK Biosample Repository by overnight delivery service

5.4. Guide for visit s2

Before the patient arrives for the visit

- Abstract data from patient's medical records as needed
- Gather the most recent prior liver biopsy slides and liver imaging studies as available
- Apply labels to forms as appropriate
- Gather specimen collection and mailing materials (labels, tubes, shippers)
- Remind patient to fast for 12 hours prior to visit
- If advisable, alert phlebotomy lab staff of need to obtain plasma and serum samples (45 or more samples per patient)
- Label specimen tubes and cryovials

Procedures

- Complete diet, activity, and quality of life questionnaires
- Obtain consent for genetic specimen banking
- Collect blood for laboratory test (hematology, liver panel, clinical chemistry, HbA1c, lipid profile, and glucose and insulin levels)
- Collect blood for genetic specimen banking (2 tubes) and biosample banking (2 tubes)
- Confirm eligibility (hand/eyeball review of unkeyed data)
- Re-affirm consent
- Explain to the patient that you will electronically confirm eligibility after keying the data collected at this visit but that since you believe the patient to be eligible, you would like to schedule the patient for visit f024

Data collection forms (form abbreviation)

- Forms completed for all patients
  - BD - Food Questionnaire Documentation
  - BP - Blood Processing for Serum and Plasma
  - CG - Genetic Consent Documentation (this form documents both consent and refusal)
  - ED - Database Enrollment
  - IR - Liver Imaging Studies Report (if a liver imaging study was reported on the Baseline Medical History (BG) form)
  - LR - Laboratory Results - Tests Done During Screening and Followup
- Additional forms for patients age 18 or older
  - Block 98 Food Questionnaire (use the printed booklet provided by the DCC)
  - PA - Physical Activity
  - QF - MOS 36-Item Short-Form Health Survey
- Additional forms for patients age 13-17
  - Brief Food Questionnaire (use the printed booklet provided by the DCC; do not print from the NASH CRN website)
  - MA - Modifiable Activity Questionnaire
  - PQ - Pediatric Quality of Life: Parent Report for Teens (age 13-17)

  – PY - Pediatric Quality of Life: Teen Report (age 13-17)
- Additional forms for patients age 8-12
  – Brief Food Questionnaire (use the printed booklet provided by the DCC)
  – MA - Modifiable Activity Questionnaire
  – PR - Pediatric Quality of Life: Parent Report for Children (age 8-12)
  – PW - Pediatric Quality of Life: Child Report (age 8-12)
- Additional forms for patients age 5-7
  – Brief Food Questionnaire (use the printed booklet provided by the DCC)
  – PS - Pediatric Quality of Life: Parent Report for Young Children (age 5-7)
  – PW - Pediatric Quality of Life: Young Child Report (age 5-7)
- Additional forms for patients age 2-4
  – Brief Food Questionnaire (use the printed booklet provided by the DCC)
  – PT - Pediatric Quality of Life: Parent Report for Toddlers (age 2-4)
- Additional forms for patients who consent/assent to blood draw for DNA extraction
  – BC - Blood Collection for DNA

Forms for clinical center use only

- Check for updates to Patient Location (PL)

After the patient leaves the clinical center

- Key data collection forms
- Run Enrollment Task and generate enrollment materials (appointment schedule for followup visits)
- Package whole blood tube for DNA banking for mailing and ship to Genetics Repository
- Process blood to serum and plasma and aliquot for banking; store in local freezer until batch sufficient for mailing accumulates
- Hold food questionnaires for batch mailing to the DCC

5.5. Visit windows: enrollment and followup

- **Enrollment** must occur within 120 days of initiating screening
- **f024**: window runs from 12 weeks through 36 weeks, ideal date is 24 weeks (168 days) after enrollment date
- **f048**: window runs from (36 weeks+1 day) through 72 weeks, must be at least 12 weeks after f024; ideal date is 48 weeks (336 days) after enrollment date
- **f096**: window runs from (72 weeks+1 day) through 120 weeks, must be at least 24 weeks after f048; ideal date is 96 weeks (672) after enrollment date
- **f0144**: window runs from (120 weeks+1 day) through 168 weeks, must be at least 24 weeks after f096; ideal date is 144 weeks (1008 days) after enrollment date
- **f0192**: window runs from (168 weeks+1 day) through 216 weeks, must be at least 24 weeks after f144; ideal date is 192 weeks (1344 days) after enrollment date

5.6. Interim (unscheduled) visits or telephone contacts

- Unscheduled visits or telephone contacts may occur as needed. No time windows or minimum time separations are imposed for such visits or contacts.

- Data are not collected at interim visits (i.e., data forms are not completed) unless reporting a death or a serious adverse event or liver biopsy

- If a liver biopsy is scheduled for a NAFLD Database patient between scheduled NAFLD Database visits, complete the forms related to liver biopsy (forms SD and LT) at the time of the liver biopsy and send any flash frozen tissue to the Biosample Repository and any unstained slides obtained to the DCC; the visit code for the forms will be the code for the followup visit that is open as of the date of the biopsy; if no visit window is open (ie, after enrollment but prior to opening of f024 window) use visit code 'n'.

6. Study procedures

6.1. Assignment of study identifiers

What

- The NASH CRN uses 2 identifiers for patients
  - ID number (4 digits)
  - ID code (3 alphabetic characters)
- These identifiers help assure confidentiality of patient identity

Materials

- ID number and code labels received from the Data Coordinating Center
- Registration (RG) form

When

- Eligibility evaluation visit (visit s1)

By whom

- Clinical Coordinator

Procedures

- Complete the Registration (RG) form; if the patient remains eligible at the close of the form, assign the ID number and code by peeling a label off the label sheet and affixing it to the specified item on form RG
- The patient will be known by these IDs for the duration of the NASH CRN, including participation in any other NASH CRN studies
- Key the Registration (RG) form into NAFLD Database data system; this must be the first form keyed and no other forms may pre-date the date of the RG form

Comments

- Once an ID number and its associated ID code are assigned, these IDs must be used by the patient for the duration of the NASH CRN and cannot be changed
- Do NOT reassign or reuse IDs assigned to patients found to be ineligible or who refuse enrollment

6.2. Baseline History (BG) Form

Who

- Complete for Database patients of all ages
- Study Physician and Clinical Coordinator sign the form

What

- The form queries:
  - Family history of liver disease
  - Information on initial diagnosis of NAFLD, NASH, or cryptogenic cirrhosis
  - Liver biopsy history
  - Weight history
  - Tobacco cigarette smoking history (for patients age 8 or older)
  - Menstrual history (female patients)
  - Medical history (answer items based on information from all sources available to you)
  - Medication use currently and in the past 6 months
- Flash Card # 17, Weight Pattern over Past 5 Years, is used with Form BG

When

- Visit s1 (but given that you need to do chart review, this may not be finished until visit s2)

How

- Mix of interview data and data obtained by chart review
- The smoking interview should be an interview with the patient, not the parent
- Other questions on the BG form can be answered by interview with the patient, parent, or both and in consultation with the patient's partner if available – ie, use all sources to get the most accurate information that you can

Definitions of hepatic events queried on Forms BG and HI

- The database will be tracking the occurrence of clinically significant hepatic events. These include variceal bleeding, ascites, edema, hepatic encephalopathy, hepatopulmonary syndrome, hepatorenal syndrome or liver cancer. For each of these, the presence at baseline should be documented on Form BG. Any recurrence should be documented on the HI form. The following guidelines may be useful in defining these events:

  - **Ascites.** The results of abdominal paracentesis and testing of ascetic fluid is the gold standard for the diagnosis of ascites. However, the appearance of ascites on imaging such as ultrasound or CT scan in the setting of portal hypertension is highly suggestive.
  - **Hepatic encephalopathy.** There should be sufficient documentation of a reversible decrease in neurologic function, in the absence of hypoxia, acidosis, drug toxicity, and other metabolic and toxic insults.

- **Hepatopulmonary syndrome.** The diagnosis rests on documenting the presence of arterial deoxygenation and intrapulmonary vasodilation in patients with liver disease. Most commonly the presence of symptoms, such as the insidious onset of dyspnea, or the clinical findings of clubbing, cyanosis, or decreased oxygen saturation on pulse oximetry prompt an evaluation. Arterial blood gases, contrast echocardiography, and lung perfusion scanning are used in further evaluation of hepatopulmonary syndrome.
- **Hepatorenal syndrome.** Occurs in patients with advanced liver disease and portal hypertension characterized by impaired renal function and marked abnormalities in the arterial circulation and activity of endogenous vasoactive systems. In the kidney there is marked renal vasoconstriction that results in low GFR, whereas in the extrarenal circulation there is predominance of arterial vasodilation, which results in reduction of total systemic vascular resistance and arterial hypotension.
- **Hepatocellular carcinoma.** Cirrhosis may predispose patients to develop hepatocellular carcinoma, the most common primary malignant hepatic neoplasm. Physical findings such as ascites, fever, portal hyptertension, and jaundice are associated with hepatocellular carcinoma. Rise in serum alkaline phosphatase and low alpha feto protein (AFP) level and imaging studies for evaluation of liver lesions (tumor) may lead to a liver biopsy for confirmatory diagnosis.
- **Pedal edema.** Accumulation of excessive watery fluid in lower extremities will be evaluated by the investigator during physical examination.
- **Variceal bleeding.** The results of diagnostic testing including EGD, radionuclide imaging, or angiography, in the setting of portal hypertension should be confirmed.

6.3. Followup Medical History (HI) form

Who

- Complete for Database patients of all ages
- Study Physician and Clinical Coordinator sign the form

What

- The form queries/reviews
  - Change in patient's NAFLD Database diagnostic category
  - Alcohol consumption since the last visit (age 8 and older)
  - Tobacco cigarette smoking since the last visit (age 8 and older)
  - Medical history diagnoses and procedures since the last visit
  - Medication use since the last visit

When

- Visits f024, f048, f096, f144, and f196

How

- Mix of interview data and data obtained by chart review
- The smoking and alcohol use interview should be an interview with the patient, not the parent or patient's partner
- Other questions on the HI form can be answered by interview with the patient, parent, or both and in consultation with the patient's partner if available – ie, use all sources to get the most accurate information that you can

6.4. Physical examination (PE and PF forms)

Who

- All Database patients

What

- Anthropometry
  - Height
  - Weight
  - Waist circumference
  - Hip circumference
- Vital signs
  - Temperature
  - Blood pressure
  - Resting radial pulse
  - Respiratory rate
- System review (limited review for focused physical examination (PF) form)
  - Skin, including grading of acanthosis nigricans
  - Head, eyes, ears, nose, throat
  - Neck
  - Lymphatic
  - Chest and lungs
  - Heart
  - Abdomen
  - Liver and spleen
  - Extremities
  - Geniturinary/pelvis (may be skipped)
  - Nervous system (may be skipped)
- Tanner staging
  - Completed on form PE only
  - Tanner staging stops once a patient reaches age 18 regardless of development
  - Tanner staging stops for those under age 18 if the patient is Tanner 5 in all areas at 2 consecutive visits

When

- Detailed physical (form PE) at visit s1 and annually thereafter (f048, f096, f144, and f192)
- Focused physical (form PF) at visit f024

How

- Ideally, use a stadiometer for height measurement
- Ideally, use the Gulick II tape measure for waist and hip measurement; this device may be obtained from www.fitnessmart.com (608-735-4718, model 67019, listed at $36); it is manufactured by Country Technology Inc: 608-735-4718
- See the sections that follow which detail the protocol for measurement of height, weight, waist circumference, and hip circumference

6.5. Height measurement

- Height may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a wall-mounted stadiometer with a horizontal measuring block (or fixed angle) is used; other height measuring devices are acceptable
- Follow the manufacturer's recommendation regarding method and frequency of calibration of the stadiometer
- The patient stands erect on the platform with his/her back parallel to the vertical mounted measure scale (but not touching the wall), looking straight ahead with his/her head in the Frankfort horizontal plane (the horizontal plane defined by the lower margin of the bony orbit (the bony socket containing the eye) and the most forward point in the supratragal notch (the notch just above the anterior cartilaginous projections of the external ear)
- The horizontal measuring block is brought down snugly, but not tightly, on the top of the head
- Record the height to the nearest tenth of the unit of measurement (1 decimal place)
- Ask the patient to step away, raise the measuring block, ask the patient to return, and repeat the measurement

6.6. Weight measurement

- Follow the manufacturer's recommendation regarding method and frequency of calibration of the scale
- Weight may be recorded in pounds or kilograms
- Two measurements are recorded
- Ideally, weight is measured in the morning after voiding and before breakfast; this should be possible in the Database since most followup visits are to be fasting; if this is not possible, try to measure the patient's weight at the same time of day and under the same conditions as the baseline measurements are obtained
- Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
- Patient should stand still in the middle of the scale platform with head erect and eyes looking straight ahead
- Record the weight to the nearest tenth of the unit of measurement (1 decimal place)
- Ask the patient to step away, zero the scale, ask the patient to return, and repeat the measurement
- Patients who have limb amputations or who are wearing casts should have weight measured, but note this on the form on the margin (the notes may be keyed at data entry in the General Comments area of the keying)

6.7. Waist circumference measurement

- Waist circumference may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
- If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
- Patient should stand with feet together
- Pull an appropriate amount of tape out of the housing
- Ask the patient to bare his/her waist
- Wrap the tape once around the waist: the measure should be taken around the abdomen horizontally at the midpoint between the highest point of the iliac crest and lowest part of the costal margin in the mid-axillary line
- Mark the midpoint on both sides of the patient using a washable marker
- Patient may be asked to assist in passing the tape around the abdomen by holding the end of the tape in position
- When the tape is positioned in the horizontal plane at the correct height, the patient should be asked to keep his/her arms at the sides and breath naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
- Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
- Record the measurement to the nearest tenth (one decimal place)
- Remove the tape, retract the tape, and repeat the procedure
- If the tape cannot be made horizontal across the waist markings, default to the right hip and note this in the margin of the form

6.8. Hip circumference measurement

- Hip circumference may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
- If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
- Patient should stand with feet together
- Pull an appropriate amount of tape out of the housing
- Ask the patient to adjust his/her clothing to allow measuring the hips over the patient's underwear
- Wrap the tape once around the hips over the underwear: the measure should be taken at fullest part of the hips (maximum extension of the buttocks)
- Patient may be asked to assist in passing the tape around the hips by holding the end of the tape in position
- When the tape is positioned correctly, the patient should be asked to keep his/her arms at the sides and breath naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
- Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
- Record the measurement to the nearest tenth (one decimal place)
- Remove the tape and repeat the procedure

6.9. Triceps skin fold measurement

The triceps skin fold measurement is to be performed on both children and adult participants.

Skinfold calipers

- Use a Harpenden skinfold caliper, manufactured by Holtrain, for triceps skinfold measurement.
- The Harpenden skinfold caliper can be purchased from Bodytrends at http://www.bodytrends.com/creharp.htm or 1-800-549-1667 item #FCH4, at a cost of $419.99.

Care and use

- Ensure that your caliper is clean; always clean the caliper before and after use on a patient.
- Ensure that your caliper opens freely and smoothly by opening the caliper to approximately 20 mm and allowing it to close several times.
- Do not open and shut the caliper rapidly or allow the caliper to snap shut - this can cause damage.
- Check to make sure the pointer is clearly reading zero before starting the skinfold measurement. If not, loosen the flat screw on top of the dial, turn the dial slowly and gently until the pointer reads zero and then turn the screw tight again.

Locating the triceps skinfold site

- Take all measurements on the right side of the body on bare, dry skin. An exception might be where a deformity or missing limb would necessitate using the left side.
- Instruct the patient to stand erect with feet together, shoulders relaxed, and arms hanging freely at the sides.
- Ask the patient to roll up his/her right sleeve above the shoulder and flex the right arm 90 degrees at the elbow with the palm facing up.
- Stand behind the patient's right side, palpate the shoulder and locate the upper most edge of the posterior border of the acromion process of the scapula. Mark this location.
- Hold the zero end of the measuring tape at this mark and extend the tape down the posterior surface of the upper arm over triceps muscle to the tip of the olecranon process of the ulna (the bony part of the elbow).
- Keep the tape in position and measure the distance from the acromion to the olecranon process over the posterior surface of the upper arm. The midpoint will be half this distance.
- Mark the midpoint using a water soluble pen. This is the site for the triceps skinfold measurement.
- Have the patient relax the elbow so that the right arm hangs freely to the side.

Triceps skinfold measurement

- Firmly grasp the fold of skin and underlying adipose tissue between the thumb and forefinger, about 2 cm above the marked site, and lift it away from the other body tissue.

The grasp should not be so firm as to be painful

- Place the jaws of the caliper approximately 2 cm below the pinched fold over the marked point, dial up, perpendicular to the length of the fold. Be sure the caliper is in the middle of the fold.
- Measure the triceps skinfold thickness to the nearest 0.5 mm while continuing to hold the skinfold with the fingers.
- Release the caliper tension and read the skinfold measurement from the caliper about 3 seconds after releasing the caliper tension.
- When taking measurements, do not allow the caliper to snap shut onto the patient as this could cause discomfort.

Recording the triceps skinfold measurements

- Remove the caliper and release the triceps skinfold,. Repeat skinfold measurement at marked point as described above.
- If the second measurement differs by more than 2 mm from the first triceps skinfold measurement, repeat the second measurement.
- If both differences between the second and third measurement AND the first and third measurement are greater than 2 mm, rest the patient for 5 minutes and repeat the session.
- Record two triceps skinfold measurements on the Physical Exam (PE) form as they are measured.

6.10. Mid-upper arm circumference

- Have patient stand erect with arms hanging freely at the sides of the trunk with palms facing the thighs
- Patient should be wearing loose clothing without sleeves to allow total exposure of shoulder area
- Locate midpoint of upper arm as in triceps skinfold measurement
- Position tape around upper arm at midpoint and perpendicular to the long axis of the arm
- Tape should be touching the skin but not compressing the soft tissue
- Record the circumference in inches or centimeters on the Physical Exam (PE) form

6.11. Liver biopsy

- Details of liver biopsy procedures, tissue banking, slide preparation, and shipment of slides to the DCC are discussed in the SOP Part IV, Liver Biopsy and NAFLD/NASH Histology Scoring System document

6.12. Alcohol use questionnaires (AD, LD, other forms)

What / Who

- AUDIT (AD) form (patients age 8 and older)
- Skinner Lifetime Drinking History (LD) form (patients age 18 or older)
- Summary question on Enrollment (ED) form
- Questions on interval alcohol consumption on Followup Medical History (HI) form
- Flash Card #15, Drink Equivalents, can be used with the alcohol questionnaires
- Flash Card #16, Patterns of alcohol intake, provides the interviewer with sample language for administering the LD form

Purpose

- At screening, obtain a detailed history of the patient's use of alcohol so as to be able to judge if the patient can be said to have NAFLD or cryptogenic cirrhosis
- Monitor alcohol use during followup

Who

- Patients age 8 or older
- Alcohol use is not assessed in patients age 2 through 7

How

- Form AD is self-administered for patients age 13 or older, without help from spouse or family
- Form AD is interviewer administered for patients age 8-12, parents may assist patients age 8-12
- Form LD is interviewer administered for patients age 18 or older

Comments

- At screening, the patient is never asked directly how much alcohol he/she drinks per week on average; clinic staff must judge this based on all information obtained from interview, chart review, discussions with those who know the patient etc; the clinic staff use their best judgement to answer the eligibility question on the Enrollment (ED) form
- The Clinical Coordinator should complete section A on page 1 of Form AD and apply labels to subsequent pages before asking the patient to complete the form

6.13. Liver symptom questionnaires (forms LP and LQ)

What / Who

- LQ - Symptoms of Liver Disease, patients age 18 and older
- LP - Symptoms of Liver Disease (Children), patients age 2 through 17

Purpose

- To obtain the patient's view of his/her liver disease symptoms

When

- Visit s1
- Annually during followup (ie, at f048, f096, f144, and f192)

How

- Self-administered, patients age 13 and older, without help from spouse or family
- Interview administered, patients age 2-12; parent may assist patients

6.14. Diet questionnaires (Block 98, Block Brief, and Form BD)

Who/What

- Block 98 Food Questionnaire (patients age 18 and older)
- Block Brief Food Questionnaire (patients age 2 through 17)
- Food Questionnaire Documentation (BD) form
- Portion size illustration (part of the Block Food Questionnaire booklet)

Purpose

- To determine patient's usual eating habits over the past year or so, including all meals or snacks, at home or in a restaurant or carry-out.
- To assess food frequency and quantity over the preceding year

When

- Visit s2
- Annual followup visits (ie, visits f048, f096, f144, and f192)

Procedure

- Use #2 pencil and fill in the bubble areas completely
- Before giving the booklet to the patient to complete, the Clinical Coordinator must:
  - Affix the Food Questionnaire ID label in the area where the patient is instructed to complete his/her name
  - Mark the patient's four digit ID # in the bubble area of the front page of the booklet
  - Mark the date in the bubble area of the front page of the booklet – THIS IS VERY IMPORTANT TO DO CORRECTLY – when the analysis is returned by the Block staff, date is the item which will distinguish screening from followup questionnaires
  - Mark the patient's gender in the bubble area of the front page of the booklet
  - Mark the patient's age at last birthday in the bubble area of the front page of the booklet
- Provide the patient with:
  - #2 pencil
  - Booklet
  - Portion size illustration
- Instruct the patient on completion of the booklet
  - Patient enters his/her best estimate of height and weight
  - Patient enters his/her best estimate of food eaten in the past year, frequency of eating a food, and portion size
  - Patient not to skip any foods and to mark "NEVER" if did not eat a certain food
- Remain available to answer questions as patient completes the booklet

- Review the completed booklet for completeness and consistency and color in any bubble areas that are partially or lightly completed or go over in #2 pencil any response marked in ink
- Complete the Food Questionnaire Documentation (BD) form
- Put the completed booklet in a box (or other collection site) at the clinical center to hold for batch mailing to the DCC

Mailing completed questionnaires to the DCC

- Batch mail monthly using a mailing service that tracks packages
- Use the Transmittal Log for Food Questionnaires (TB) as a shipping list for the batch shipment
- Address the batch to:

Food Questionnaire Coordinator
NASH CRN Data Coordinating Center
615 North Wolfe Street, Room 5010
Baltimore, MD 21205
410-955-8175

Comments

- The Block questionnaire booklets are obtained from the DCC; contact Rosemary Hollick (rhollick@jhsph.edu) to obtain additional copies
- The diet analysis provided by the Block group will be sent to the DCC

6.15. Physical activity questionnaires (forms MA and PA)

Purpose

- To evaluate correlations between level of activity and disease or mechanisms of disease

What / Who

- PA - Physical Activity, patients age 18 or older
- MA - Modifiable Activity Questionnaire, patients age 8 through 17
- Patients age 7 or younger will not complete an activity questionnaire

When

- Visit s2
- Annually during followup (ie, at f048, f096, f144, and f192)

How

- PA - self-administered, without help from spouse or family
- MA - self-administered or interview administered as judged best by interviewer

6.16. Quality of life questionnaires

Purpose

- To evaluate correlations between self-reported quality of life and disease severity

What / Who

- Patients age 18 or older
  - Complete form QF, MOS 36-Item Short-Form Health Survey
- Patients age 13-17
  - Patients complete form PY, the teen report version of the Pediatric Quality of Life questionnaire
  - Flash Card #9 (English) or Flash Card # 12 (Spanish) may be used with form PY
  - Parents of patients age 13-17 complete form PQ, the Pediatric Quality of Life parent report on adolescent child age 13-17
  - Flash Card #10 (English) or Flash Card # 13 (Spanish) may be used with form PQ
- Patients age 8-12
  - Patients complete form PW, the child version of the Pediatric Quality of Life questionnaire
  - Flash Card #9 (English) or Flash Card # 12 (Spanish) may be used with form PW
  - Parents of patients age 8-12 complete form PR, the Pediatric Quality of Life parent report on child age 8-12
  - Flash Card #10 (English) or Flash Card # 13 (Spanish) may be used with form PR
- Patients age 5-7
  - Patients complete form PV, the young child version of the Pediatric Quality of Life questionnaire
  - Flash Card #11 (English) or Flash Card # 14 (Spanish) may be used with form PV
  - Parents of patients age 5-7 complete form PS, the Pediatric Quality of Life parent report for young child age 5-7
  - Flash Card #10 (English) or Flash Card # 13 (Spanish) may be used with form PS
- Patients age 2-4
  - Parents of patients age 2-4 complete form PT, the Pediatric Quality of Life parent report for toddlers age 2-4
  - Flash Card #10 (English) or Flash Card # 13 (Spanish) may be used with Form PT

When

- Visit s2
- Annual followup visits (ie, visits f048, f096, f144, and f192)

Procedure

- Clinical Coordinator should complete Part A of each form and apply labels to subsequent pages as needed before giving the form to the patient/parent to complete
- Self administered for patients age 18 and older, without help from spouse or family

- Clinical Coordinator may assist patients age 5 through 17
- Self administered for parents
- Clinical Coordinator should check returned forms for completeness before the patient leaves the clinical center

Comment

- Spanish versions of the forms can be printed from the clinic data system or the NASH CRN web site (www.nashcrn.com)

6.17. Liver imaging studies (form IR)

Who/What

- Patients who have had a hepatic imaging examination (ultrasound, computerized tomography (CT), or magnetic resonance imaging (MRI) scan) within 12 months of the date of the first screening visit or following the first screening visit (as part of standard of care)
- Patients who do not meet the histological requirements for NAFLD or cryptogenic cirrhosis into the NAFLD Database must have a hepatic imaging examination (ultrasound, CT, or MRI scan) obtained within 12 months of the date of the first screening visit or following the first screening visit (as part of standard of care) that is suggestive of NAFLD or suspected cryptogenic cirrhosis

Form

- Liver Imaging Studies Report (IR) form
- Report results of most recent scan of each type done in year prior to screening (s2) or in the period since the last study visit.

When

- Visit s2 if a liver imaging study is reported on the Baseline Medical History (BG) form
- Visits f024, f048, f096, f0144, and f0192 if a liver imaging study is reported on the Followup Medical History (HI) form

Comments

- Required for eligibility if patient does not meet histologic criteria for definite NAFLD or definite cryptogenic cirrhosis
- Scan findings suggestive of NAFLD: fatty infiltration
- Scan findings suggestive of cryptogenic cirrhosis: cirrhosis, ascites
- Scan findings suggestive of hepatic tumors: hepatic mass, hepatic cysts

6.18. Laboratory measures (forms LS and LR)

Who

- All Database patients

What

- Form LS covers assessments collected only at screening:
  - Screening etiologic tests
  - Iron assessments
  - HFE gene analysis
  - Ceruloplasmin measurement
  - Alpha-1 antitrypsin assessment
  - Autoantibody studies
  - Immunoglobulin levels
  - Thyroid stimulating hormone and alpha feto protein assessment

- Form LR covers assessments collected during screening and followup
  - Hematology
  - Chemistries and HbA1c
  - Liver panel and alpha feto protein
  - Fasting lipids
  - Fasting glucose and insulin

When

- Form LS: Visit s1
- Form LR: Visit s2 and annually thereafter (f048, f096, f144, and f192)
- Given the extent of information recorded on form LS, you may still be completing it at visit s2 or drawing blood for tests at visit s2; use visit code s1 anyway
- Requirements for fasting – nothing by mouth except water for at least 12 hours before blood draw

Instructions for Form LS

- Most of the tests on Form LS are intended to be obtained by chart review; time windows for the allowed age of tests are specified on the form
- Serological assessment to exclude viral causes of chronic liver disease is required for all patients
- Iron overload screening is required for all patients; hepatic iron index is recorded if available, but is not required
- HFE gene analysis is required only if the patient has an abnormality in an iron overload screening test, a family history of iron overload or hemochromatosis, or histological iron of greater than 3+
- Ceruloplasmin is required for patients age 2 through 39

6.18. Laboratory measures (forms LS and LR)

- Alpha-1 antitrypsin assessment is required for all patients
- Autoantibody studies are required for all patients
- Immunoglobulin levels (IgA, IGG, IgM) are recorded if available
- Thyroid stimulating hormone is required for all patients

Instructions for form LR

- The measures on form LR can also be obtained by chart review, both at screening and during followup; the time window for each type of assessment is specified on the form
- During followup, the time window for the assessment is "in the time window for the followup visit (check the patient's Visit time window guide)" – eg, f048 has an acceptable calendar time period within which it may be done; if you can find a hematology assessment in the patient's chart that was done under the same conditions as required by the study and which provides the required values and was done within the time window for visit f048, you do not need to order another hematology at f048
- If the chart review tests are out of the time window or the test conditions can't be ascertained or differ from what is required, the measures have to be done at the study visit and can be charged to the study
- For baseline, the required time window is within 6 months of the screening visit date except for HbA1c; the required time window for HbA1c is within 3 months of the screening visit date

6.19. Plasma and serum collection for Biosample Repository

Purpose

- Collection of whole blood from NAFLD Database patients of all ages
- Separation of plasma and serum at clinical center: five or six 0.5 mL aliquots of plasma and forty 0.5 mL aliquots of serum are to be obtained in 2.0 mL cryogenic vials
- Store plasma and serum aliquots at -70° C prior to batch shipping to the NIDDK Biosample Repository at Fisher BioServices

Forms / Materials

- BP - Blood Processing for Plasma and Serum
- Labels for citrate CTAD (blue top) tube and serum separator SST tubes (red top)
- Labels for plasma and serum cryovials
- SS - Specimen Shipment log
- NIDDK Biosample Repository shipper

When

- Visit s2
- Annual followup visits (ie, f048, f096, f144 and f192)
- Batch shipments: Monthly or semi-monthly

By whom

- Phlebotomist
- Clinical Coordinator

Equipment

Blood tubes/aliquot vials

- One 4.5 mL CTAD (blue top) tube - *to be provided by the DCC*
- Four 10 mL SST (red top) tubes - *provided by clinical centers*
- 45 or more 2.0 mL cryogenic vials - *provided by clinical centers*
  - vials should be able to withstand -196 degrees C
  - vials should be self standing (flat bottom, not curved), externally threaded, 13.5 mm wide x 48.3 mm tall, with silicone washers

Labels

- Preprinted labels for whole blood collection tubes (4.5 mL CTAD tube and 10 mL SST tubes) and preprinted labels for Form BP – *labels are printed at the clinical center via web-based data management system; use MACO ML-5000 1" x 1 ½" labels, 50 labels/page*
- Preprinted polypropylene labels for 2.0 mL cryogenic vials – *provided by the DCC*

Equipment

- Centrifuge
- -70° C freezer
- Swing out rotor
- 5 mL pipettes

Blood processing procedures

- Patient instructed to fast 12 hours prior to blood draw
- Collect whole blood into one 4.5 mL CTAD (blue top; Becton-Dickinson) tube for plasma
- Collect whole blood into four 10 mL SST (red top) tubes for serum
- Blood for plasma and serum to be centrifuged, aliquoted, and frozen within one hour

Plasma

- Collect blood into CTAD (blue top; Becton-Dickinson) tube. Ensure that CTAD tubes have not expired. (*check that date shown above "Exp" in lower right corner of label is later than current month*)
- Completely fill vacutainer tube
- Mix gently by inversion 5 times
- Within 30 minutes, centrifuge at 1800 x g for 15 minutes at 4° C, preferably with a swing out rotor
- Immediately after centrifugation, insert a 5 mL pipette below surface of plasma
- Remove the clear plasma while avoiding blood cells
- Transfer plasma into aliquots of 0.5 mL each into 5-6 labeled 2.0 mL cryovials
- Freeze at -70° C immediately

Serum

- Collect blood into serum separator (red top) tubes. Ensure that SST tubes have not expired. (*check that date shown above "Exp" in lower right corner of label is later than current month*)
- After filling, invert each SST tube gently at least 5 times
- Allow blood to clot for **at least 30 minutes** at room temperature
- Centrifuge at 1800 x g for 15 minutes at 4° C, preferably with a swing out rotor
- Transfer 0.5 mL of serum into 40 labeled 2.0 mL cryovials
- Freeze at -70° C immediately

Note: Separated serum and plasma may be stored at -20° C for up to one day before transfer to -70° C while ensuring samples remain frozen during the transfer.

Blood Processing for Plasma and Serum (BP) form

- Complete the Blood Processing for Plasma and Serum (BP) form
- Affix labels for the CTAD plasma and the SST serum to the BP form
- Affix aliquot 00 cryovial labels to the BP form

Shipping samples to the NIDDK Biosample Repository

- Specimens are to be batch-shipped monthly to Fisher BioServices
- Aliquots will be stored locally at the clinical center at -70° C prior to shipping
- Ship specimens in the STP 320 Saf T Pak shipper (provided by the NIDDK Biosample Repository); each shipper can accommodate aliquots for up to 5 patient visits (230 aliquots)
- Complete the Specimen Shipment Log (form SS) and enclose a copy with each shipment of specimens.

6.19. Plasma and serum collection for Biosample Repository

- Keep a notebook of all completed Specimen Shipment Logs (Form SS) so that you have a record of all shipments to the Biosample Repository

6.20. Specimen collection for Genetics Repository

Purpose

- Collection of whole blood from NAFLD Database patients of all ages who consent for genetic research
- Shipment of whole blood to the NIDDK Genetics Repository at Rutgers University for DNA banking

Forms

- NAFLD Database consent and assent (if adolescent patient) for genetic research
- Genetic Consent Documentation (CG) form
- Blood Collection for DNA (BC) form
- NIDDK Genetics Initiative Phlebotomy (GP) form

When

- Visit s2
- Ship same day as whole blood collection

By whom

- Clinical Coordinator and Study Physician (to obtain consent)
- Phlebotomist (to obtain whole blood)
- Person responsible for shipping whole blood to NIDDK Genetics Repository

Equipment

- Two 10 mL NaEDTA vacutainer tubes (purple top) - *provided by NIDDK Genetics Repository*
- Preprinted whole blood tube labels and form BC labels - *provided by clinical centers (printed from web based data management system; clinical center provides MACO ML-5000 labels (1" x 1 ½ ", 50 labels per page, www.maco.com)*
- Shipper - *provided by NIDDK Genetics Repository*
  - One model 472 Thermosafe Safety Mailer (body and lid)
  - One 2 ½" x 9" pre-cut section of absorbent materials
  - One roll waterproof tape
  - One press-lock plastic bag
  - One corrugated shipping carton with locking tabs

Blood collection procedures

- Collect blood into two 10 mL NaEDTA (purple top) tubes
- Invert each tube gently 6 times to mix blood with additives
- Keep blood at room temperature
- Check that patient ID information on preprinted tube labels matches information recorded onto the NIDDK Genetics Initiative Phlebotomy form
- Phlebotomist to sign and date the section: To Be Completed by Phlebotomist on the NIDDK Genetics Initiative Phlebotomy form

6.20. Specimen collection for Genetics Repository

Packaging procedures

- Ship whole blood at ambient room temperature same day to the NIDDK Genetics Repository
- Package the whole blood tubes in the body of the Safety Mailer (Model 472 Thermosafe Safety Mailer)
- Tear off one section of absorbent materials along perforates and place it so it exactly covers cavity of the Safety Mailer
- Place lid of Safety Mailer over body and absorbent material and press down firmly so that lid closes properly. Reposition absorbent material so that it does not get caught between the lid and body
- Peel backing from two 18" long pieces of red waterproof tape and seal the Safety Mailer lid to the body; peel backing from second piece of tape and continue sealing the mailer, overlapping the first piece of tape about two inches on both ends
- Place the sealed Safety Mailer into the press-lock plastic bag. **Do not seal the bag**.
- Place the NIDDK Genetics Initiative Phlebotomy form in the mailer box outside the plastic bag
- Slide the Safety Mailer and open press-lock bag into the corrugated carton
- Seal the press-lock bag and close carton using the locking tabs
- Place sealing tape (not included) over them

Shipping procedures

- Use the preprinted Federal Express shipping label, marked for *Priority Overnight Delivery*, to ship whole blood to the NIDDK Genetics Repository
- Outside cardboard box must have stamped "Diagnostic Specimen Packed in Compliance with IATA Packing Instructions 650"
- Call Federal Express, 1-800-Go-FEDEX (1-800-463-3339) for courier*
- Notify Dana Witt or Elva Peralta at the NIDDK Genetics Repository that blood is being shipped and provide the Federal Express tracking numbers and the NIDDK ID numbers. Notification may be via:
  - email: witt@biology.utgers.edu
    peralta@ biology.rutgers.edu
  - Fax: 1-732-445-1149
  - Telephone: 1-732-445-1498
- Ship whole blood to:
  Rutgers University/Cell Repository/NIDDK
  604 Allison Rd., Room C120A
  Nelson Laboratory
  Piscataway, New Jersey 08854-8000

*Due to inconsistent Federal Express delivery around holidays and likelihood of closure of the Genetics Repository on holidays, do not schedule deliveries on the day before or the day of a national holiday. Check with Federal Express and with the Genetics Repository if there is any question about delivery availability or closure.

6.21. Adverse event reporting

Definitions

- **Adverse event** is defined as any unfavorable sign, symptom, state, condition, or laboratory finding in a Database patient. Adverse events may result from appropriate application of the protocol in relation to the processes of enrolling, studying, or following Database participants, as well as from mistake or misadventure.
- **Associated with study participation** means that there is a reasonable possibility that the event may have been caused by participation in the study.
- **Serious adverse event** is defined as any event that suggests a significant hazard, contraindication, side effect, or precaution. Serious adverse event includes any event that is fatal or life-threatening, is permanently disabling, requires or prolongs inpatient hospitalization, or is a congenital anomaly, cancer, or overdose.
- **Unexpected adverse event** is defined as any adverse event that is not identified in nature, severity, or frequency in the risk information included in the Database protocol.

Reportable Database events

- Any serious and unexpected adverse event thought to be associated with a Database procedure is reportable to the Database.
- Any event threatening the integrity or well-being of the Database (eg, suspected fraud) is a reportable Database event. We recognize that this category is not well-defined; however, it is included as a reminder that reportable events can have a broader scope than events that happen to a patient.
- Deciding whether an event is reportable to the Database (ie, is in either of these categories) will be the responsibility of the Principal Investigator (PI) of the center. The study chair, the NIDDK project officer, and staff at the Data Coordinating Center are available for consultation.

CTCAE v3.0

- Events are reported on the Adverse Event (AN) form
- The NASH CRN uses the Common Terminology Criteria for Adverse Events, (CTCAE v3.0) to specify and grade adverse events.
- This document is posted on the NASH CRN website (www.nashcrn.com – click on Documents and then click on General Documents)
- Use the CTCAE v3.0 to specify the Short Name for the adverse event and the severity grade for the adverse event.

Clinical center responsibilities regarding reportable Database events that occur at your clinical center

- You must notify the Data Coordinating Center about occurrence of events judged reportable to the Database as follows: If an event has occurred that you judge is reportable to the

Database, complete the Adverse Event (AN) form. Key this form to the Database. Also send it to the Data Coordinating Center with a narrative description of the event and your subsequent course of action -- describe what happened, the actions taken in response to the event, and the relationship of the event to the Database protocol. Please refer to the patient by his/her NASH CRN patient ID number and code; do not use the patient's name.

- The Data Coordinating Center should receive copies of all correspondence with a clinic's IRB regarding events judged reportable to the Database. Because the Database is a multicenter study, events judged reportable to the Database at one clinical center must be reported to the IRB of each participating center.

Data Coordinating Center responsibilities

- The Data Coordinating Center will send a copy of each report received about an event judged reportable to the Database to all clinical centers, with instructions for each to forward the report to their IRB. Copies of the report will also be sent to the NIDDK, Chairman's Office, and chair of the Data and Safety Monitoring Board (DSMB) for the Database.

- The Data Coordinating Center will maintain a list of such events for reporting and review at Steering Committee meetings and DSMB meetings.

Clinical center responsibilities with respect to events that occur at other clinical centers

- When you receive a report from the Data Coordinating Center regarding occurrence of an event reportable to the Database at another NASH CRN clinical center, you must forward that report to your IRB. It may be that your IRB has no comment on events occurring elsewhere; nevertheless, the notification of your IRB is still a Database requirement.

Local reporting requirements

- Your institution's IRB has reporting requirements of its own regarding events occurring in the course of conduct of a study. These reporting requirements may be more stringent than those adopted by the Database. Regardless of what the Database requires, you must continue to meet your local IRB's requirements. If the local requirements are more stringent than the Database's, you may report events locally that you do not report to Database.

6.22. Adverse event reporting management

- Since the Database is an observational cohort study, few adverse events related to the study are expected. Potential adverse events are those related to blood draws for the study, such as hematoma, cellulitis, phlebitis, and arterial puncture.

- If such an event occurs, appropriate medical care should be provided immediately in the clinic.

- If a suspected adverse event is reported by telephone several days later, the participant should be evaluated in the clinic by medical staff (preferable) or referred to an appropriate facility for evaluation and management.

- All such events should be documented in the study chart.

- Since patients with cirrhosis at baseline may be entered into the Database, it is likely that some will develop significant liver-related morbidity or mortality during the course of followup. While this information is important and should be documented on the Followup Medical History (HI) form, it would only be considered a reportable adverse event if it is related to the study in some way.

6.23. Procedures for missed or incomplete visits

Purpose

- Record data about missed or incomplete visits

Form

- Missed or Incomplete Visit (MV) form

When

- At close of a visit window for any missed followup visit or for any followup visit with specific forms not completed

By whom

- Clinical Coordinator

Procedures for missed or incomplete in-person visits

- For a missed visit:
  - Date of missed visit is the last date of the visit window
  - Indicate reason(s) for missed visit
- For an incomplete visit:
  - Date of incomplete visit is the date on which a partial set of procedures was performed
  - Indicate reason(s) for missed procedures

6.24. Procedures for patients lost to followup

Purpose

- Ascertain vital status of patient
- Document reason(s) patient did not attend visit
- Ascertain if patient is lost to followup

When

- Whenever patient misses a study visit and is difficult to contact

By whom

- Clinical coordinator

Search strategies

- Contact all persons identified on the Patient Location (PL) form
  - Telephone different times during the day/evening
  - Send letter via regular or certified registered mail to determine if patient is still at listed address
- Check current telephone directory for listings both for the patient and the patient's contacts specified on the PL form, eg., next of kin, health care professionals
- Check post office for forwarding address; ask patient's contacts for forwarding address
- Check obituaries
- Check state vital records

6.25. Procedures for mortality closeout

Purpose

- Record participant death

Forms

- Complete the Death Report (DR) form

By whom

- Study Physician and Clinical Coordinator

6.26. Medical management of patients (standard of care)

- NAFLD Database participants are to be closed out if they are being enrolled into PIVENS, TONIC, or another NASH CRN main study, but not at their final f192 visit.

- For purposes of prolonging the NAFLD Database and keeping the NAFLD Database participants accessible to clinical center staff, a closeout (CO) form is not to be filled out or keyed upon completion of the participant's last scheduled visit.

- An explanation or sample dialogue to be discussed with the NAFLD Database participant is provided below:

  "On behalf of the NASH Clinical Research Network, we appreciate your participation in the NAFLD Database over the past four years. Your involvement has contributed invaluable information and data that will help us better understand Nonalcoholic Fatty Liver Disease or Nonalcoholic Steatohepatitis. The NASH CRN will be applying for additional funds to extend this study for several more years. If we obtain additional funding, we will be in touch to ask your interest in re-enrolling in the NAFLD Database study. This would likely be in 2009. Again, thank you for your participation, and we look forward to seeing you in the near future."

6.27. Study closeout (CO form)

- NAFLD Database participants are to be closed out if they are being enrolled into PIVENS, TONIC, or another NASH CRN main study, but not at their final f192 visit.

- For purposes of prolonging the NAFLD Database and keeping the NAFLD Database participants accessible to clinical center staff, a closeout (CO) form is not to be filled out or keyed upon completion of the participant's last scheduled visit.

- An explanation or sample dialogue to be discussed with the NAFLD Database participant is provided below:

  "On behalf of the NASH Clinical Research Network, we appreciate your participation in the NAFLD Database over the past four years. Your involvement has contributed invaluable information and data that will help us better understand Nonalcoholic Fatty Liver Disease or Nonalcoholic Steatohepatitis. The NASH CRN will be applying for additional funds to extend this study for several more years. If we obtain additional funding, we will be in touch to ask your interest in re-enrolling in the NAFLD Database study. This would likely be in 2009. Again, thank you for your participation, and we look forward to seeing you in the near future."

7. Forms management

7.1. Clinical center ID codes

Alphabetic IDs

- Alphabetic clinic IDs are used on forms, lists, and tables
- Alphabetic clinical center IDs are based on the name of the institution with which the clinical center is affiliated
- Assigned IDs

Case Western Reserve University	CWRU
Duke University	DUKE
Indiana University	IU
Saint Louis University	SLU
University of California, San Diego	UCSD
University of California, San Francisco	UCSF
University of Washington	UW
Virginia Commonwealth University	VCU

Numeric site IDs

- The NIDDK Genetics and Biosample Repositories use numeric IDs to identify the NASH CRN clinical centers
- These will be used on the specimens (whole blood and plasma, serum, and liver tissue samples sent to the Genetic and Biosample Repositories, respectively)
- Assigned IDs

Case Western Reserve University	220
Duke University	222
Indiana University	221
Saint Louis University	223
University of California, San Diego	224
University of California, San Francisco	225
University of Washington	226
Virginia Commonwealth University	227

7.2. Patient identifiers

What

- Patient ID number
- Patient code

Patient ID number

- 4 characters, all numeric
- ID number labels will be distributed to clinics by the Data Coordinating Center
- The ID number for a patient will remain the same for the duration of the NASH CRN, even if the patient enters another NASH CRN study or if the patient fails screening and is subsequently re-evaluated – the ID never changes

Ranges of patient IDs assigned to clinics

Case Western Reserve University	CWRU	1001	-	1999
Duke University	DUKE	2001	-	2999
Indiana University	IU	3001	-	3999
Saint Louis University	SLU	4001	-	4999
University of California, San Diego	UCSD	5001	-	5999
University of California, San Francisco	UCSF	6001	-	6999
University of Washington	UW	7001	-	7999
Virginia Commonwealth University	VCU	8001	-	8999

Patient code

- 3 character alpha code assigned by the Data Coordinating Center and printed on the ID number label
- Each patient code is unique across the NASH CRN

7.3. Visit ID code

- 2 to 4 character alpha-numeric code
- Determined by purpose of visit and timing with respect to visit windows
- Visit ID codes

s1	Screening
s2	Screening, baseline data collection, and enrollment
f024	24 weeks followup visit (approximately 6 months)
f048	48 weeks followup visit (approximately 1 year)
f096	96 weeks followup visit (approximately 2 years)
f144	144 weeks followup visit (approximately 3 years)
f192	192 weeks followup visit (approximately 4 years)
n	Unscheduled visit

7.4. General guidelines for forms completion

Ink

- Forms should be completed in ink that is dark enough to photocopy legibly; do not use pencil or colors (e.g., red, green, light blue, or purple) that do not photocopy well

Changing responses on forms

- If an error is made on the form, correct the response by marking through the response with one or two lines and writing the correct response next to or above the original response. The staff member making the correction should put their initials and the date in the margin by the correction. A brief explanation for the change should also be written in the margin; e.g., 'error', 'pt changed mind', 'wrong response checked'.
- Do not obliterate, erase, or white-out incorrect responses
- The idea is to preserve an audit trail of the original response and subsequent changes to it

Multipage forms

- The patient ID number should be written on the top right of every page of every form in the space provided -- protect yourself against ineffective staples and photocopying mishaps

Miscellaneous

- All written responses should be printed legibly so the responses can be keyed to the database
- Do not use abbreviations or short-hand that may not be easily understood or keyed in the written responses
- Numeric data should be recorded in the units prescribed on the form and to the level of precision (number of digits) indicated on the form
- All numbers should be right justified and leading and trailing zeroes should be recorded on the form where applicable (e.g., an age of 8 would be written and keyed as "08").
- All letter codes should be left justified with the remaining spaces left blank (e.g., a visit ID for the s1 visit code would be completed and keyed as "s1 ").
- The clinical coordinator should review all responses for completeness and accuracy before signing off on the form
- Wherever possible, forms should be completed in real time, not retroactively. Interviews and questionnaires should be completed on the actual data form.
- The data on some forms, such as the Laboratory results form, will be transcribed from worksheets or lab reports, but the visit date on the form should correspond to the date the testing took place.
- Staple relevant lab reports and worksheets to the data form; if your lab reports are transferred to you electronically, print a paper copy of the report and staple the copy to the Database form.

Calculations

- All calculations should be performed using a calculator
- Values should be rounded according to the NASH CRN data rounding rule (see section on data rounding rule, later in this chapter of the SOP)

7.5. Instruction box

- Each form includes an instruction box at the top of the first page. This instruction box gives the purpose of the form, when it should be completed, who administers the form, the respondent, and specific instructions for the form

7.6. Form skips, stops, ineligibility symbols

Skip pattern

- Form navigation (skip pattern) instructions are indicated in **boldface**. Skips are designated by an arrow from that response to a box with the number of the next item to be completed.

Stop sign

- Stops are indicated with an arrow from the response to a stop sign – instructions are given that must be fulfilled in order to continue with the form. For example, Form RG (Registration) asks if the patient has signed the consent form; if the response is "no", the form is stopped with the instructions that *'the consent form must be signed prior to continuing with screening'*.

Ineligibility sign

- Ineligible conditions are designated by an arrow from the response to the symbol:

Other 

- Other special instructions are indicated on the form in *italics*. Some examples are:
  – *check only one*: only one of the listed responses should be checked
  – *check all that apply*: one or more of the listed responses may be checked
  – *specify*: a response should be printed on the line(s) provided

7.7. Headers and footers

- Each page of each form includes headers and footers which identify the form and the patient. The top right of the first page of each form has a space to check when the form is keyed [( )keyed]. The top right of subsequent pages is reserved for the patient ID number. The footers include the form abbreviation, form revision number and date, the form name, and the page number. For example:

NAFLD Database Patient ID: __ __ __ __

Form RG
Revision 0 (17 Feb 04) RG - Registration Page 2 of 3

- The keyed box should be √ed when the form is keyed; the person keying the form should also date and initial the form by the keyed box
- The patient ID number should be written on each page of the form

7.8. Key fields

- The first 7 items of each form include the key fields which identify the clinical center, patient, visit and study

A. Clinical center, patient and visit identification
   1. Center ID: __ __ __ __
   2. Patient ID: __ __ __ __
   3. Patient code: __ __ __
   4. Date form completed: __ __ - __ __ __ - __ __
      day mon year
   5. Visit code: __ __ __ __
   6. Form & revision: __ __ __
   7. Study: NAFLD DATABASE 1

- The form and revision number will be printed on the forms in item 6; if a form is only used for one specific visit, the visit code will also be printed on the forms
- When a form revision affects the data that are collected, the form revision number and date will change; if this occurs, older revisions of that form should no longer be used for data collection
- If the form is revised without affecting the data collection - i.e., the wording of an item is revised - only the revision date of the form will be changed.

7.9. Missing data

- If a data item is missing and cannot be obtained when the form is completed or reviewed, write the appropriate code in the first left hand space of the empty data field:

  ? = data temporarily missing or inconsistent; to be collected or resolved in the near future; items keyed with a ? will need to be followed up on and resolved
  d = patient does not know the answer
  n = not applicable in this situation
  m = data missing
  r = patient refused

- When using any of the above codes, the entire data field does not need to be filled with the code (e.g., a missing height would be completed as m _ _._).

- If data are missing on the form, an explanation for the missing values should be written on the form and keyed to the database in the General Comments section of the keying.

- It is very important to keep the number of missing data items at a minimum, especially at baseline, since many future papers will depend on having a good set of baseline values. If an item is missing at the time the form is filled out, but is expected to be collected in the near future, use a '?' rather than the 'm' code for the item on the form. The 'm' missing code is for items that are truly missing. Coordinators are discouraged from using the 'm' code as a way to get through the data entry checks and enroll a patient; the screening windows should be broad enough to allow you to collect all data within the allotted time window. Also, if the data system will not accept a value because it is out of range, please contact the DCC, so we can make a determination as to whether the range checks need to be adjusted. In the meantime, use a '?' rather than an 'm' on the form. If there is a valid reason that a required baseline laboratory value is missing, please fax the LR or LS form to the DCC along with the reason for the missing value.

7.10. Administrative sign off

- Each form contains a section for administrative sign off
- These items include the Clinical Coordinator PIN and signature and the date the form was reviewed.
- Depending on the form, they may also include the PIN and signature of other staff

7.11. Handling forms

Form duplication

- The individual forms and form sets specific to a particular visit, patient age, and language (English and Spanish) are available on the NASH CRN website and on the data system (using the Print task)
- You can print master copies from the website or data system and then photocopy as needed or print as needed from the website or data system – if you print copies ahead of time, do not print huge quantities as forms may be revised, especially in the early days of a study
- If a master copy gets frayed or faded, print a new master — always use clear copies for reproduction masters.

Form storage

- Forms for patients registered but not enrolled in the NAFLD Database should be kept in a single folder in a locked room or locked filing cabinet.
- Each patient who is enrolled in the NAFLD Database will have a patient file – either a notebook or file folder which is kept in a locked room or locked filing cabinet. The patient file should contain all NAFLD Database documents for the patient – consents, forms, appointment schedule, labels, randomization materials. The forms should be arranged in the notebook or folder chronologically by visit. Tabs can be used to separate the visits.

7.12. Data rounding rules

To round data, examine the digits following the last position required on the form:

- If the first digit following the last data position required for the response is less than 5, leave the digit in the last data position required for the response unchanged, e.g., if you need to round to _._, then 4.73 rounds to 4.7 and 1.44 rounds to 1.4
- If the first digit following the last data position required for the response is 5 or more, round up the digit in the last data position required for the response, e.g., if you need to round to _._, then 4.78 rounds to 4.8 and 4.75 rounds to 4.8

When completing a calculation for the NAFLD Database, apply the rounding rule only at the last step, when required to record a quantity on the NAFLD Database form.

7.13. Data audits and edits

Data audits

- The Data Coordinating Center will serve as the site monitor
- The Data Coordinating Center will conduct periodic data audits as a quality control measure
- Audits may be done by mail or on-site
- During an audit, the forms will be reviewed to see if they were completed and keyed correctly; the forms will also be checked against the source documents to be sure that values were transcribed correctly.

Source documents include but are not limited to:

- Upper abdominal imaging study reports
- Laboratory test result reports
- Medical records for archival information
- There are no source documents for questionnaires (the questionnaires are the original documents for the data collection)

Data edits

- Computerized data edits will be sent to the clinics periodically
- The data edits check for consistency and questionable values in the database.

Changes resulting from audits or edits

- Changes made to the forms as a result of an audit or an edit should be marked "per audit" or "per edit" and should be dated and initialed.

8. Quality assurance

8.1. Site visits

Purpose

- Conduct an audit of selected patient data
- Review documentation and procedures for the NAFLD Database
- Tour facilities
- Discuss with clinical center personnel any problems that have occurred or that are expected to occur in conducting the study

The following regulatory and study documents should be available or accessible:

- IRB communications organized with original approval letters, revision approvals, annual renewals, serious adverse event forms, and any communications regarding concerns or special requests from clinical center review board
- Signed and dated consent and assent forms for all participants including the date and signature of a witness
- Documents including NAFLD Database Protocol, PPMs, and SOPs
- Study forms for participants should be available for data audit

Participants

- At least two DCC personnel will attend the site visit. At least one person from another NAFLD Database clinical center will also attend. Representatives from other resource centers associated with the NASH CRN may also attend
- NASH CRN certified staff from the clinical center

Reviewed during site visit

- IRB documentation
  - Original approval
  - Annual renewals (if applicable)
  - IRB submissions
  - Approval for updated consent and assent forms and protocol

- Documents
  - Directory
  - SOPs
  - Forms Book
  - PPMs
  - Protocol

- Enrollment and retention
  - Status
  - Recruitment and retention strategies
  - Problems
  - Losses to followup

- Personnel
  - Certification status
  - Personnel changes
  - Backup plans for personnel in event of absence

- Clinical management
  - Adverse event reporting procedures
  - Study procedures
  - Clinical center coordination
  - Scheduling
  - Clinical center concerns or problems

- Participant files
  - Security
  - Organization
  - Consent statements

- Specimen shipment
  - Comparison of specimens expected and received
  - Shipping procedures and problems
  - Shipping supplies

- Protocol performance
  - Protocol deviations

- Forms and data management
  - Monthly form status reports
  - Source documentation
  - Data audit (selected patients)
  - Eligibility criteria
  - Adverse events
  - Death reports

- Previous site visit report
  - Action items followup
  - Data audit followup

Site visit followup

- A list of action items is compiled at the end of the site visit to identify items which require further action. The procedure for site visit action item followup is:
  - Action items will be listed at the end of the site visit report
  - Clinical centers will be required to respond to action items within 30 days of receipt of the site visit report. Responses should be in writing and sent to the CC.
  - The DCC will be required to respond to the action items within 30 days of the completion of the site visit report. The DCC will send a written report to the clinical center.

8.2. Performance monitoring

- The DCC will generate enrollment reports that will provide a count of participants enrolled at each clinical center

- On approximately a quarterly basis, the DCC will generate reports summarizing the performance of all clinical centers. These reports will include information on enrollment and the percentage of expected visits for which documentation has been entered into the NAFLD Database data system. Also, for those visits for which data have been entered, the report will show the percentage of missed visits, the completeness of data collection, the timeliness of data entry, and protocol deviations. Performance reports will be reviewed by the Steering Committee, and the Steering Committee will make decisions regarding actions to be taken in the event that a clinical center is performing poorly.

8.3. Data quality surveillance

General procedures

- Quality assurance of data accuracy will occur routinely through three main procedures: data entry checks, monthly checks for completeness and edits, and form audits

- In addition, detection of problems may occur during data analysis. For example, in preparing reports for Steering Committee meetings, problems may be discovered. Outliers and unusual variations or patterns in the data are examined and may reveal problems.

- Quality assurance of data analysis is achieved by independent replication of key analyses within the DCC and review of reports by multiple individuals before distribution

Data entry checks

- The data system will contain checks during the data entry process of range, logic, and consistency of items within forms

- The data system will perform checks between forms to ensure that the same fields entered on different forms match

- A double data entry system will be used for all forms

Monthly check for completeness and edits

- On a monthly basis, DCC will generate a database report of:
  - number of participants enrolled
  - missed visits
  - incomplete visits (missing or pending forms)
  - missed specimen collection or shipment
  - edits (see below)

- Edits are run on the database of the keyed forms monthly. Checks for missing, out-of-range, unusual and inconsistent values, cross-form checks and arithmetic errors are some of the types of checks performed. A listing of edits is sent to each clinical center for resolution. The clinical center must respond to each edit on the listing, make appropriate changes to the forms and database, compute documentation of each change, and file the documentation with the edited data collection form. Items that cannot be corrected (e.g., missing values, unusual measures) are entered into a database at the DCC. These items are excluded from future edits. A hard copy of the edits, with each resolution should be kept in a notebook located at the clinical center.

Forms audits

On a periodic (approximately monthly) basis the DCC selects and requests copies of forms for specific participants be sent by each clinical center to the DCC for auditing

- Audited forms are compared with the database; discrepancies are noted and queried
- Audited paper forms are also inspected for other problems, which are noted and queried
- Each clinical center will be required to resolve discrepancies from the audit report and fax the resolutions to the DCC within 5 days
- The DCC will generate a summary report of the audit discrepancies by clinical center to be distributed to all NAFLD Database centers
- Discrepancy rates over time by clinical center are reported to the Steering Committee

NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

NAFLD Database

Standard Operating Procedures

Part IV:

Liver Biopsy
and
NAFLD/NASH Histology Scoring System

April 2005

NAFLD Database SOP IV: Biopsy and Histology Scoring

Contents

1.Overview

1.1. Philosophy

Nonalcoholic fatty liver disease (NAFLD) is defined by hepatic steatosis in the absence of known causes of liver disease and significant alcohol consumption. Nonalcoholic steatohepatitis (NASH) is a subset of NAFLD defined histologically by steatosis, spotty lobular inflammation, and distinctive pattern of zone 3 injury characterized by hepatocellular ballooning; Mallory's hyaline and perisinusoidal fibrosis (psf) may also be present. In some as yet poorly defined fraction of patients, there is progression of disease to cirrhosis and hepatocellular carcinoma. The NASH Clinical Research Network (NASH CRN) is a clinical research network sponsored by the NIDDK to study the natural history of NAFLD in both adult and pediatric patients and to carry out (1) a treatment trial of NASH in adult patients and (2) a treatment trial of NAFLD in pediatric patients. This document specifies the procedures for liver biopsy and histology scoring for the NAFLD Database. Procedures for other NASH CRN studies, including the PIVENS and TONIC trials, are specified in separate manuals specific to the particular study.

The procedures specified by the NASH CRN in their studies are based on two concepts: one is the principle that a primary goal of the NASH CRN is to collect a database of specimens along with clinical and laboratory data, so that this information is available for research even after the NASH CRN has disbanded. The other concept behind these procedures is the necessity of a system of evaluation of NASH that can be utilized in evaluating efficacy in treatment trials. Because there are no serum markers or other diagnostic modalities that can accurately stage or grade the liver disease in these patients, a liver biopsy remains the standard against which all other methods will be compared. Every effort should be made to build a liver tissue archive and slide archive that are as complete as possible, so that what is accomplished will have value for the future as well as the present. With this principle in mind, consents should include language that permits such approved uses of liver biopsy materials without the need for re-consent at a later date; patients who refuse access to their liver biopsies or who refuse to provide specimens for banking should not be enrolled in the NASH CRN.

Ideally, the NAFLD Database will obtain a piece of liver tissue for banking and 10 unstained slides for archiving from each biopsy evaluated for the NAFLD Database. However, because some of the biopsies evaluated for the NAFLD Database will not provide these materials (eg, not enough tissue is obtained or the paraffin block is exhausted), contingency plans have been developed, including requesting to borrow the institutional slides and returning them after central scoring has been completed for satisfying inclusion/exclusion criteria related to liver histology..

It should be emphasized that a surgical pathology report alone is not sufficient for comparison with other biopsy data nor for satisfying inclusion/exclusion criteria related to liver histology. Tissue slides must be available for review and must be judged by the NAFLD Database pathologist to be adequate for scoring for the slides to be used to satisfy NAFLD Database liver histology criteria. A copy of the surgical pathology report must be obtained for all slides. The surgical pathology report serves two purposes: (1) to verify and document the identity of the patient whose tissue is on the slides that are being evaluated and (2) to provide the date of the biopsy.

1.2. Tasks and forms related to liver biopsy

Occurrence of liver biopsy(s) done before screening and occurrence of liver biopsy during screening are queried on the Baseline Medical History (BG) form. After enrollment in the NAFLD Database, biopsies should occur prospectively under the care of the NAFLD Database investigator, and the forms and materials related to biopsy should be dealt with when the biopsy occurs, which may be outside of the context of a NAFLD Database visit. However, as a check to be sure that all biopsies that occur are caught, occurrence of a biopsy since the previous NAFLD Database visit is queried on the Followup Medical History (HI) form.

Up to two biopsies done prior to screening may be included in the NAFLD Database slide archive, as well as a biopsy done during screening (ie, up to 3 pre-enrollment biopsies may be included).

Use the SE form (Most Recent Prior Liver Biopsy Materials Documentation) to document the outcome of the most recent biopsy done before screening for the NAFLD Database began (and that you want evaluated in the NAFLD Database) with regard to availability of stained and unstained slides for scoring and archiving. Use the HE form (Histology Findings for Most Recent Liver Biopsy Done Prior to Database Registration) to score the slides from this biopsy.

If a patient has another biopsy done prior to screening that you want evaluated for the NAFLD Database, use the SF form (Next Most Recent Prior Liver Biopsy Materials Documentation) to document the outcome of that biopsy with regard to availability of stained and unstained slider for scoring and archiving. Use the HG form (Histology Findings for Next Most Recent Liver Biopsy Done Prior to Database Registration) to score the slides from this biopsy.

It is assumed that flash frozen liver tissue will not be available from biopsies done prior to Database registration.

If a biopsy is done anytime after Database registration (ie, during screening or followup), the Liver Biopsy Materials Documentation (SD) form must be completed to document the outcome of that biopsy with regard to availability of tissue for banking and stained and unstained slides for scoring and archiving. If tissue was obtained for banking, the Liver Tissue Banking (LT) form must be completed. If the biopsy was done during screening, then the local NAFLD Database Study Pathologist must complete the Liver Biopsy Histology Findings (HF) form.

Other forms that the NAFLD Database uses to document activities and materials related to liver biopsy are the Central Histology Review (CR) form and logs for shipping frozen tissue and slides (forms SS and TS). In summary, these ten forms (SD, SE, SF, LT, HE, HF, HG, CR, TS, SS) are used to:

- For a biopsy done during screening or followup, document what slides, if any, are available for scoring and sending to the DCC and whether liver tissue was obtained for banking (form SD)
- For the most recent liver biopsy done prior to screening, document what slides, if any are available for scoring and sending is the DCC (form SE)
- For the next most recent liver biopsy done prior to screening , document what slides, if any are available in scoring and sending to the DCC (form SF)

1.2. Tasks and forms related to liver biopsy

- If liver tissue was obtained for banking for a biopsy done during screening or followup, document how that tissue was obtained and frozen (form LT)
- Document local scoring of a biopsy done during screening or followup (form HF)
- Document local scoring of most recent biopsy done prior to screening (form HE)
- Document local scoring of next most recent liver biopsy done prior to screening (Form HG)
- Document central scoring of biopsies (form CR)
- Document shipment of slides to the DCC (form TS)
- Document shipment of flash frozen liver tissue to the Biosample Repository (Form SS)

Note that forms SE and HE are used together, forms SF and HG are used together, and forms SD, LT and HF are used together. Form SF should not be completed unless form SE has been completed. Forms SD, SE, and SF are not interchangeable.

The adult hepatologist and his/her pediatric counterpart, the Study Pathologist, and the Clinical Coordinator must work together to accomplish these tasks and complete the required forms. Considerable cooperation and close communication will be required to complete these tasks successfully.

2. Obtaining liver biopsy materials for scoring for the NAFLD Database

2.1. Overview

Baseline liver biopsy specimens can be divided into two categories: (1) those performed prior to consent for screening, and (2) those performed as part of standard of care during the screening process, but after consent for screening and enrollment in the NAFLD Database has been obtained. In the case of (1), we will try to obtain 10 unstained slides for NAFLD Database exclusive purposes, but limited biopsy materials may require that the NAFLD Database borrow the institution's biopsy slides. In the case of (2), sufficient tissue should be collected to provide for the institutional pathology slides AND 10 unstained slides for NAFLD Database exclusive use AND for banking at the Biosample Repository. Followup liver biopsies should all be in category (2).

Baseline biopsies will be scored by the local NAFLD Database Study Pathologist (to determine eligibility) and also centrally, by the Pathology Committee. Biopsies done after enrollment will be read locally for standard of care and will also be scored centrally by the pathology committee.

2.2. Baseline biopsies performed prior to consent for screening

Because these biopsies were obtained prior to consent for NAFLD Database screening, investigators may have little control over variables such as fixation or biopsy size, but these considerations should not prevent investigators from trying to obtain unstained slides for NAFLD Database purposes. However, it is an unfortunate fact that the amount of tissue available in archived paraffin blocks may be very limited.

Upon learning that a patient had a biopsy prior to screening (queried on the Baseline Medical History (BG) form), the Clinical Coordinator should obtain the original pathology materials: the surgical pathology report, the institution's H&E and Masson's trichrome slides, and the paraffin block so that 10 unstained slides may be obtained (see below for how to prepare and label those slides).

Upon receipt of the original pathology materials, the NAFLD Database Clinical Coordinator should verify that all materials pertain to the NAFLD Database patient they are said to and should annotate the surgical pathology report with the patient's NASH CRN ID number and ID code and black out the patient's name. The annotated report should be attached to the Liver Biopsy Slide Documentation (SD) form and a copy of the annotated report must be sent to the DCC when the biopsy slides are sent to the DCC. The Clinical Coordinator and/or Study Pathologist must determine the following so that Form SD may be completed:

- Determine if the biopsy is adequate for scoring for the NAFLD Database
- Determine the number and type of the institution's stained slides available for the local review of the biopsy and determine if any stained slides need to be sent to the DCC; if fewer than 2 unstained slides are available for sending to the DCC, the DCC must be sent the institution's stained slides

2.2. Baseline biopsies performed prior to consent for screening

- If the DCC will be sent stained slides, label those stained slides with NASH CRN slide labels for stained slides (which are not the same as the labels for unstained slides); transcribe the slide sequence numbers (printed on the slide label) to form SD
- If the DCC will be sent stained slides, determine if the NAFLD Database is borrowing the stained slides from the institution or if the NAFLD Database is taking possession of the stained slides; if borrowing the stained slides, determine the date by which the slides need to be returned to the institution and note the source of the slides; record this information on form SD
- If unstained slides are obtained, label them with the NASH CRN slide labels for unstained slides (which are not the same as the labels for stained slides); transcribe the slide sequence numbers from the slide labels to form SD

If the biopsy is adequate for scoring for the NAFLD Database, the Study Pathologist should complete the NAFLD Database Liver Biopsy Histology Findings (HF) form using the institution's H&E stained slide and Masson's trichrome stained slide, as described later in this document.

If there is no H&E stained slide in the original materials and an additional slide cannot be obtained from the paraffin block (i.e., the paraffin block has been exhausted), the biopsy is insufficient for evaluation for the NAFLD Database. However, the patient may be eligible for the NAFLD Database under criteria that do not require histologic evidence of disease (e.g., the NAFLD Database includes patients with suspected disease as well as those with histologically confirmed disease).

If only a single H&E slide or if only the H&E and Masson's trichrome slides are available, these should be reviewed locally and forwarded to the DCC for central review. If only a single H&E slide is available, the biopsy is sufficient for evaluation for the NAFLD Database, but be aware that the estimation of fibrosis will not be optimal. A single H&E slide is the minimum requirement for scoring histology for the NAFLD Database; other NASH CRN studies, such as the PIVENS trial, will have more rigorous requirements.

The NAFLD Database should request that the slides be provided outright, with no arrangements to return the slides at the end of the NAFLD Database. In the event that any of the slides from a prior biopsy need to be returned to the original laboratory, the first plan should be for those slides to be returned at the end of the NAFLD Database. If that is not acceptable to the original pathology laboratory, the slides will be returned to the original pathology laboratory after the central review is completed.

Slides for patients who do not enroll in the NAFLD Database should be returned upon determination that the patient will not enroll.

2.3. Baseline and followup biopsies performed after consent for screening - biopsy procedure

Each clinical center investigator will notify their NAFLD Database pathologist (e.g., via local institutional requisition sheet) when a biopsy is performed on a NAFLD Database patient so that when the block is initially cut for the local institution's requirements, the NAFLD Database's additional 10 unstained slides can be cut at the same time and the chances of loss of tissue with refacing the block are minimized and so that liver tissue can be flash frozen for banking.

In order to insure adequate material for thorough histologic review, investigators should obtain needle core biopsies of at least 2.0 cm length using a 16 or greater gauge needle. If there is adequate tissue beyond 2.0 cm, the extra tissue may be flash frozen and banked at the Biosample Repository.

2.4. Preparation of slides

Biopsy tissue for slides should be placed in buffered formalin as soon as possible; investigators should use whatever formaldehyde solution is used by the site's pathology laboratory.

If this is a baseline biopsy, each clinical center will obtain locally stained H&E and Masson's trichrome slides as part of their standard institutional pathology materials; these will be read by the local NAFLD Database Study Pathologist for the local evaluation (i.e., for completion of Form HF).

For both baseline and followup biopsies, the local laboratory should cut 10 additional slides (additional to what is cut for the institutional pathology needs) at the same time as the initial sections are obtained for the institutional slides in order to decrease the chances of loss of tissue with refacing the block. The local NASH CRN Study Pathologist should advise on the selection of a maximum of 10 unstained slides to be submitted to the Histology Review Center at the DCC for banking if there are (1) more than 10 unstained slides available or (2) if the unstained slides were cut from different tissue blocks obtained from the same liver biopsy core(s). The NASH CRN Pathology Committee's recommendation is that all unstained slides submitted come from the same, single liver biopsy core when possible. Centers should be careful not to submit unstained slides with insufficient or fragmented biopsy tissue since it is not possible for DCC staff to sort those out before sending the slides for central staining.

Tissue should be placed on pre-cleaned, "charged" slides to allow for possible future use with immunohistochemistry. The specifications for the recommended brand of slides are:

SuperFrost Plus slides, Precleaned

Distributor:	Fisher Scientific
Catalog No.:	#12-550-15
Size:	25/75/1.0 mm
Estimated cost:	$65.96 per gross (144 slides/gross); $660.78 per case of 10 gross
Tele:	1-800-766-7000

Coverslips should not be placed on the slides. Slides should be labeled with the patient specific preprinted NASH CRN labels for stained slides. The 10 (or as many that can be obtained up to 10) uncoverslipped, unstained slides will be forwarded to the DCC for the purposes of central staining, review, and archiving.

2.5. Labeling stained and unstained slides at the clinical center

Each of the slides (stained and unstained) sent to the DCC must be labeled with a NASH CRN slide label . Two types of slide labels are used in the NASH CRN:

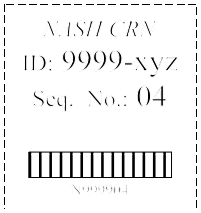
(1) permanent labels are used for unstained slides; these labels can withstand the chemicals used during staining for unstained slides.
(2) removable labels (overlabels) are used for stained slides that are borrowed from an institution

The requirements for the labeling scheme for slides are:

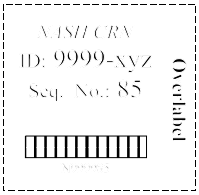
- Each slide must be uniquely identifiable
- The unique identifier for the slide has to be built from information that can be specified when the label is printed
- The slide label must be able to be read by human eye
- Labels for unstained slides must be resistant to solvents used in the staining process and to over-labeling by interim processes (e.g., when sent for central staining)
- Labels for borrowed stained slides must be removable since those slides must be returned to the original pathology laboratory

The solution to these requirements is to use Brady labels in the format shown below: (1) labels for unstrained slides and (2) for stained slides which are borrowed.

(1)



(2)



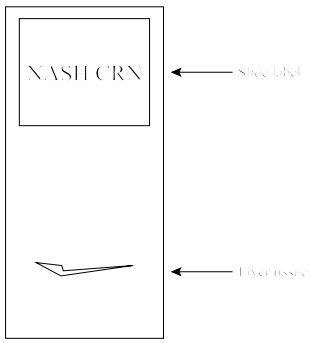
Slide labels are provided to each clinical center in preprinted form, 1 set of labels per patient ID, by the DCC. Each set includes labels for unstained slides and labels for stained slides.

The unique identifier for a slide will consist of the patient ID number and a sequential number. The sequence numbers on the labels for unstained slides run from 01 to 60 (60 is the maximum number of unstained slides that we think we will ever get for any one patient over the life time of all NASH CRN studies). The sequence numbers on the labels for stained slides run from 81 to 90 (10 is the maximum number of stained slides that we think we will ever borrow for any one patient over the lifetime of all NASH CRN studies).

The slide labels include the following information:

- NASH CRN (printed on label)
- Patient ID number (4 digit number printed on label) and patient code (3 character alphabetic code printed on label)
- Slide sequence number (printed on label)
- Bar code constructed from N (for NASH CRN), 4 digit ID number, and 2 digit slide sequence number
- Labels for borrowed slides also have "Overlabel" printed on the label

Permanent labels should be positioned squarely at the top end of the slide, on the same side that the liver tissue is on and over the white part of the glass, with no edges hanging off the slide. Overlabels should be placed on the back of the slide, directly on the glass and opposite the existing label. Both permanent labels and overlabels should be positioned so that the print is oriented vertically with the narrow end of the slide (see diagram below).



2.6. Liver tissue for banking at Biosample Repository

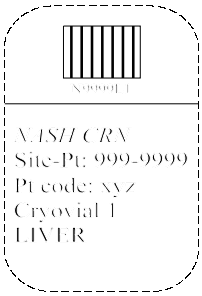
The extra piece of liver tissue (minimum 1-2 mm or greater) will be flash frozen as follows:

- Place extra liver tissue in a 2.0 mL cryogenic vial which is made of polypropylene and self-standing with externally threaded vials and silicone washers (13.5 mm wide x 48.3 mm tall); this vial is designed for ultra low temperatures (-196 ° C, liquid nitrogen vapor) (supplier: CIC, catalog number 5012-0020; phone 877-738-8247)

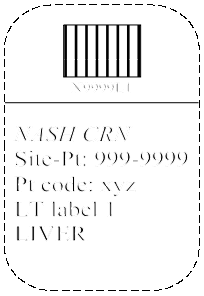
- Apply a pre-printed white tab, "Cryovial" label provided by DCC to the cryogenic vial according to the following steps:

  - Attach the label to the vial when the vial is at room temperature
  - Leave the cap on the vial when labeling; the inside of the vial is sterile and should remain so
  - Apply the label to the vial so that the long edge of the label is parallel to the floor when the vial is held in an upright position; the label should not trail off the bottom of the vial or over the cap

- While holding the vial in an upright position, affix the colored portion of the label to the vial first
- Wrap the clear tail around the perimeter of the vial; the end of the clear tail should overlap the colored portion of the label
- Press firmly on the entire label; verify that all edges of the label adhere to the vial
- When possible, allow newly labeled vials to set at room temperature for several hours prior to subjecting them to colder temperatures (24 - 48 hours is optimal)
- The liver vial label has the following format:



- Within 5 minutes of obtaining, place the liver tissue into the vial; place the capped vial into the portable Barnstead/Thermolyne Thermo stainless steel Dewar liquid nitrogen flask (supplier: Fisher Scientific, catalog number 11-670-25C, 22.9 cm high; 800-766-7000)
- Complete the Liver Tissue Banking (LT) form; apply the corresponding “LT form” label to the LT form



2.6 Liver tissue for banking at Biosample Repository

- Complete the Specimen Shipment Log (SS) form
- Ship cryovials to the Biosample Repository on Monday, Tuesday or Wednesday; store temporarily in -70° C freezer or in a liquid nitrogen freezer at the clinical center until the next batch
  shipment to McKesson.
- Make sure you use the “Cryovial” and “LT form” labels from the same set (ie, with the same sequence number)

3. Development of the histology scoring system

3.1. Background

The NASH CRN has designed and validated a feature scoring system that can be used for the spectrum of NAFLD, both to correlate histologic features with clinical and physiologic measurements and to document changes in liver tissue observed over time in patients in natural history studies and clinical trials.

3.2. Methods and validation

Pathologists from the NIH and from the 8 clinical centers participating in the NASH CRN met in March 2002 to discuss the features to monitor and the criteria for scoring these features. Each study pathologist was asked to contribute cases of adult and pediatric liver biopsies. The cases were chosen from patients referred for consideration of NASH who had been histologically diagnosed as either diagnostic, borderline/suspicious, or not diagnostic of NASH. The cases were to have no confounding liver diseases. Subsequent to the March 2002 meeting and after criteria for the scoring system were established based on the discussions at the meeting, additional cases were requested from each center's pathologist. The original and newly received cases were anonymized and were assembled into what is called the anonymized study set. This set consists of 50 cases (32 adult, 18 pediatric) with H&E and Masson stains for each case.

During the summer and fall of 2002, the anonymized study set was circulated to each study pathologist for review to establish agreement for features and for diagnosis according to the three categories of definite, borderline, and not NASH. The adult cases were scored by each pathologist twice and the pediatric cases once.

Weighted group and pair-wise Kappa statistics were used to evaluate inter- and intra-rater agreement. Inter-observer agreement was assessed for both adult and pediatric cases, and intra-observer agreement was determined using the adult cases.

Cochran's Chi-square test for trend and GEE logistic regression were used to analyze univariate and multivariate relationships between feature scores and the diagnosis of NASH. Adult cases were analyzed separately from pediatric cases.

Fourteen features of NAFLD were evaluated in the scoring system. Four of the features were evaluated semi-quantitatively:

- Steatosis percent (0-3)
- Modified Brunt criteria for fibrosis (0-4); the modification, specifically, was subdivision of stage 1 into 1a, 1b, and 1c:
  - 1a: zone 3 perisinusoidal fibrosis that does not require a trichrome stain
  - 1b: zone 3 perisinusoidal fibrosis that does require a trichrome stain
  - 1c: portal fibrosis only

  The remainder of the fibrosis scoring used Brunt criteria.
- Lobular inflammatory foci/20x (0-3)
- Hepatocellular ballooning (0-2).

Location of steatosis was recorded but not scaled. An additional 9 features were recorded as either present or absent.

Weighted group kappa statistics for the inter-rater agreement on adult cases were 0.84 for fibrosis, 0.79 for steatosis, 0.56 for hepatocellular ballooning and 0.45 for lobular inflammation. Agreement on the diagnosis of NASH had a kappa of 0.61. Kappa values for intra-rater agreement were higher for all features, while inter-rater agreement on pediatric cases was generally lower. By multivariate analysis 5 features independently correlated with the diagnosis of NASH in adults: hepatocellular ballooning ($P<0.0001$), perisinusoidal fibrosis ($P=0.0009$), lobular inflammation ($P=0.002$), steatosis ($P=0.004$) and acidophil bodies ($P=0.02$).

In summary, we have devised a feature scoring system for NAFLD and NASH that has inter- and intra-rater agreement similar to other semiquantitative scoring systems for chronic liver disease. The pediatric NAFLD features were not as reproducible as those of adults, possibly due to the presence of varied patterns of injury. In accordance with the idea that NASH is a pattern of injury comprised of several features, this scoring system demonstrates that a firm diagnosis of NASH correlates with the degree of steatosis, characteristic pattern of fibrosis, lobular inflammation, and hepatocellular ballooning. Based on this evaluation, the NASH CRN will use this system to evaluate liver biopsies for features of NAFLD.

4. Evaluation at the clinical center (for Form HF)

4.1. Introduction

The local site NAFLD Database Study Pathologist will evaluate baseline biopsies only, for eligibility determination only. In the case of the NAFLD Database, the evaluation determines what category the patient is in (histologically confirmed NAFLD or histologically confirmed cryptogenic cirrhosis). Patients who do not meet histologic criteria for NAFLD nor for cryptogenic cirrhosis may be eligible for the Database in the categories of suspected NAFLD or suspected cryptogenic cirrhosis.

The local site NAFLD Database Study Pathologist should make his/her evaluation using the locally stained H&E slide (all features except fibrosis) and Masson's trichrome slide (fibrosis only) and should complete the Liver Biopsy Histology Findings (HF) form. A copy of the HR form is included at the end of this document.

4.2. Guidelines for features scored in the local evaluation

The following guidelines are provided for uniformity of reading among the NAFLD Database pathologists using this system. The system is based entirely on H&E stain for all lesions except fibrosis, and on Masson's trichrome stain for fibrosis.

4.2.1. Steatosis grade (0-3; 4x or 10x)

0: <5%
1: 5 -33%
2: 34 - 66%
3: > 66%

In the recognition that (1) deciding on less than 5% can be challenging and (2) that this really is a significant cut-off for allowing patients into long-term follow-up studies such as the NASH CRN is conducting, we think it is best to err on the side of grade 1 rather than grade 0 when faced with a borderline case. Evaluation is best at 4x or 10x at most.

4.2.2. Steatosis location

Steatosis location has 4 choices for characterization:

Zone 3: distribution means not panacinar and not strictly zone 1
Zone 1
Azonal: this pattern is the random scattered macrosteatosis
Panacinar: implies severe steatosis that appears to involve the whole acinus equally, rather than the more random azonal pattern

4.2.3. Fibrosis stage (0-4; requires trichrome slide)

0: None
1a: Zone 3, perisinusoidal fibrosis (requires trichrome)
1b: Zone 3, perisinusoidal (easily seen on H&E; verified on trichrome)
1c: Portal/periportal only
2: Zone 3 and periportal, any combination
3: Bridging
4: Cirrhosis

4.2.4. Lobular inflammation (0-3; 20x)

Amount of lobular inflammation combines mononuclear, fat granulomas, and polymorphonuclear (pmn) foci.

0: None
1: < 2 / 20x mag
2: 2-4 / 20x mag
3: >4 / 20x mag

4.2.5. Portal chronic inflammation (0-1)

0: None to minimal
1: Greater than minimal

4.2.6. Hepatocellular ballooning (0-2)

0: None
1: Few
2: Many

4.2.7. Steatohepatitis diagnosis

At some point in your evaluation of the case, decide how you would sign this case at your institution, using whatever criteria you currently use to make a diagnosis of steatohepatitis. The question asked is "Is steatohepatitis present?" and the choices for response are:

0: No
1: Suspicious/borderline/indeterminate
2: Yes, definite steatohepatitis

This choice has to be made based on the H&E and Masson's trichrome slides, even if you have access to other stains (such as ubiquitin immunohistochemistry).

4.2.8. Exclusion of other liver disease and other features

- Primary biliary cirrhosis
- Wilson's disease
- Chronic cholestatic liver disease
- Vascular lesions of ALD/B-C/VOD
- Inflammation suggestive of AIH, HCV
- Pigment suggestive of HH
- Globules suggestive of A1AT
- Hepatocellular changes suggestive of HBV
- Granulomas suggestive of sarcoid, PBC, infection
- Evidence of cirrhosis
- Other features
  - Mallory's hyaline (rule out cholate stasis)
  - Perisinusoidal fibrosus away from septa
  - Hepatocyte ballooning
  - Megamitochondria

Diagnosis of primary biliary cirrhosis, Wilson's disease, bile duct obstructions, primary sclerosing cholangitis, autoimmune liver disease, HCV, hemochromatosis, A1AT deficiency, and HBV by the Study Physician are exclusionary; these diagnoses are marked with caution symbols on the HE, HF, and HG forms.

4.2.9. Evaluation of cryptogenic cirrhosis

You must answer the question "is cirrhosis present?" (yes or no) and if present, you must answer the question "in your opinion, is this cryptogenic cirrhosis?" (yes or no). The criterion for a yes answer to cryptogenic cirrhosis is cirrhosis that fails to meet criteria for NAFLD (including NASH) and without evidence of other forms of chronic liver disease.

4.2.10. Comments

This item is for any comments you want to share with the rest of the group on the case such as comments on unusual features or on a difficult scoring choice.

4.3. NAS score (0-8)

The NASH Activity Score (NAS) is a composite score that is calculated from the steatosis grade (0-3), lobular inflammation grade (0-3), and the hepatocellular ballooning score (0-2) – the scores for these 3 components are summed. The NAS may range from 0 through 8. Since this score is not used in evaluation for eligibility for the NAFLD Database, it will be calculated as needed during analysis and is not included on the scoring form.

4.4. Unscheduled liver biopsy

Unscheduled biopsies (i.e., biopsies done after screening and at a followup time other than 96 weeks after randomization) will be evaluated locally for standard of care and also centrally by the Pathology Committee. Form HF will not be completed for unscheduled liver biopsies. Form CR will be completed upon central review by the Pathology Committee. The CR form will be use visit code n.

5. Central pathology evaluation (for Form CR)

5.1. Procedures

Each pathology review session will include attendance by at least 3 Pathology Committee members and will include at least one of the co-chairs. Central reviews are planned for every 3-4 months. Review sessions will last 1-2 days. One or 2 DCC members will attend each central review to complete the Central Pathology Review (CR) form for each biopsy reviewed. These completed data forms will be keyed at the DCC.

Following central review, slides will be returned to storage at the DCC. Slides that are only loaned to the NAFLD Database for a specified time will be returned to the clinical center at the required time. Slides will be packed and shipped for return to the clinical center as specified later in this manual.

5.2. Documentation of which slides were used for evaluation

The sequence numbers of the slides reviewed will be recorded on the CR form for each case.

5.3. Guidelines for features scored in the central evaluation

The following features are evaluated both centrally and locally, and the guidelines to be used for the central evaluation of these features are the same as the guidelines used for the local evaluation:

- Steatosis grade
- Steatosis location
- Fibrosis stage
- Lobular inflammation
- Hepatocellular ballooning
- Cirrhosis diagnosis
- Other features (Mallory's hyaline, perisinusoidal fibrosis away from septa, hepatocyte ballooning, megamitochondria)
- Features to suggest pre-existing steatohepatitis

Steatohepatitis diagnosis and amount of portal inflammation is scored both centrally and locally, but the scoring of this feature for the central evaluation differs from the scoring for the local evaluation.

Guidelines for features scored only in the central evaluation or scored differently in the central evaluation from the local evaluation are described below.

5.3.1. Length of biopsy

The tissue is measured (mm) on the glass slide prior to reading the slide.

5.3.2. Microvesicular steatosis, contiguous patches

0: Not present
1: Present

The definition agreed on was the pathologists' definition of microsteatosis: foamy appearance to the cytoplasm, nucleus centrally located. Remember, this is not the small droplet type of "macro" steatosis that doesn't completely fill the cell. Microsteatosis typically needs ORO for confirmation in routine practice.

5.3.3. Microgranulomas seen (yes/no)

5.3.4. Large lipogranulomas seen (yes/no)

5.3.5. Portal chronic inflammation

0: None
1a: Mild
1b: More than mild

5.3.6. Acidophil bodies

0: Rare/absent
1: Many

5.3.7. Pigmented macrophages (Kupffer cells)

0: Rare/absent
1: Many

5.3.8. Megamitochondria

0: Rare/absent
1: Many

5.3.9. Mallory's hyaline

0: Rare/absent
1: Many

5.3.10. Glyogen nuclei

0: Rare/absent
1: Many

5.3.11. Iron: hepatocellular grade (0-4)

0: Absent or barely discernible, 40x (skip hepatocellular iron distribution)
1: Barely discernible granules, 20x
2: Discrete granules resolved, 10x
3: Discrete granules resolved, 4x
4: Masses visible by naked eye

5.3.12. Iron: hepatocellular distribution

0: Periportal
1: Periportal and midzonal
2: Panacinar
3: Zone 3 or nonzonal

5.3.13. Nonhepatocellular iron grade (0-2)

0: None (skip nonhepatocellular iron distribution)
1: Mild
2: More than mild

5.3.14. Nonhepatocellular iron distribution

0: Large vessel endothelium only
1: Portal/fibrosis bands only, but more than just in large vessel endothelium
2: Intraparenchymal only
3: Both portal and intraparenchymal

5.3.15. Steatohepatitis diagnosis

0: No
1a: Suspicious/borderline/ indeterminate: Zone 3 pattern
1b: Suspicious/borderline/ indeterminate: Zone 1, periportal pattern
2: Yes, definite

5.3.16. Comments

General comments on the biopsy can be recorded by the Pathology Committee.

6. Shipping slides to the Data Coordinating Center

6.1. Packing and shipping slides

The steps in shipping slides are:

- Complete the Histology Slide Transmittal Log (Form TS), specifying the number of stained and unstained slides for each patient included in the shipment; specify the date and visit code for the slides for each patient included in the shipment; make a copy of the completed TS form for your clinic records and include the original in the shipment
- Attach a copy of the surgical pathology report for each slide set included in the shipment; make sure that each report is annotated with the patient's NASH CRN ID number and ID code and that the patient's name has been blacked out
- Packing
  - Pack slides in standard slide shipping boxes, which hold 25 slides (VWR International catalog number 82003-418, HS15989A (blue), 82003-420 (green) ; phone 800-932-5000)
  - Use tissue paper or something similar, inside the box, to prevent the slides from moving during shipment
  - Seal the box shut with tape
  - Wrap the box in bubble wrap
  - Place the bubble wrapped box inside a padded jiffy bag. Place the jiffy bag and pathology reports and Form TS in a Federal Express box; stuff the Federal Express box as needed with newspaper or other packing material so that the bubble wrapped box does not move around
  - Ship second day arrival to

    Pat Belt
    NASH CRN Data Coordinating Center
    615 North Wolfe Street, Room W5010
    Baltimore, MD 21205
    410-955-8175

    You may bill the shipment to the DCC's slide shipment Federal Express account (#2991625081)
  - Notify Pat Belt to expect the shipment (email pbelt@jhsph.edu or fax 410-955-0932)

6.2. Receipt of slides at the Data Coordinating Center

When slides are received, staff at the Data Coordinating Center will:

- Notify the clinic that the shipment was received
- Review the shipment for consistency with the accompanying shipping log, for damage, for appropriate labeling and other quality control items
- Send slides for central staining as appropriate (currently, 3 stains are planned)
- Log the stained slides into the DCC slide library system and designate a storage location
- If the received slides are not adequate for evaluation, the institution's slides may be requested and used to evaluate a biopsy

6.3. Returning slides to the clinical center

- Log the slides out of the DCC slide library system
- Complete a shipping log for return of slides to the clinical center
- Pack up slides for return to the clinical center
- Notify the clinical center of the impending return of the slides
- Obtain confirmation of receipt of the returned slides from the clinical center

7. Appendices

Central Histology Review

Purpose: Record results of the NASH CRN Pathology Committee review of liver biopsy slides archived at the Histology Review Center.
When: Quarterly after the start of patient enrollment or more often as determined by the Pathology Committee.
By whom: Data Coordinating Center staff.
Instructions: Upon review of the liver biopsy slides by the NASH CRN Pathology Committee, the designated Data Coordinating Center staff member should complete the CR form. The CR form will be keyed by the Data Coordinating Center personnel.

A. Clinic, patient and visit identification

__ __ __ __ 1. Center ID

__ __ __ __ 2. Patient ID

__ __ __ 3. Patient code

__ __ / __ __ __ / __ __ 4. Date of central reading

__ __ __ __ 5. Visit code

c r 1 6. Form and revision

__ 7. Study: **1**=Database; **2**=PIVENS; **3**=TONIC

__ __ / __ __ __ / __ __ 8. Date of biopsy

B. Slide sequence number

9. Sequence number for

__ __ . . . a. H & E stained slide

__ __ . . . b. Masson's trichrome stained slide

__ __ . . . c. Iron stained slide

__ __ . . . d. Other slide

_____ . . . . . Specify type of stain for other slide

C. Administrative information

__ __ __ 10. CC Initials

_____ 11. CC Signature

__ __ / __ __ __ / __ __ 12. Date form reviewed

__ 13. Tissue adequate: **0**=No ➜ Request original slides from submitting clinic; **1**=Yes

14. Followup with clinic (*Specify*):

_____

_____ 15. Biopsy length (mm)

H & E stain

16. Steatosis (assume macro, e.g., large and small droplet)

_____ . . . a. Grade: **0**=<5%; **1**=5-33%; **2**=34-66%; **3**=>66%

_____ . . . b. Location: **0**=Zone 3 (*central*); **1**=Zone 1 (*periportal*); **2**=Azonal; **3**=Panacinar

_____ . . . c. Microvesicular steatosis, contiguous patches: **0**=Absent; **1**=Present

17. Inflammation

_____ . . . a. Amount of lobular inflammation: combines mononuclear, fat granulomas, and pmn foci:
0=0; **1**=<2 under 20x mag; **2**=2-4 under 20 mag; **3**=>4 under 20 mag

_____ . . . b. Microgranulomas seen: **0**=No; **1**=Yes

_____ . . . c. Large lipogranulomas seen: **0**=No; **1**=Yes

_____ . . . d. Amount of portal, chronic inflammation: **0**=None; **1**=Mild; **2**=More than mild

18. Liver cell injury

_____ . . . a. Ballooning: **0**=None; **1**=Few; **2**=Many

_____ . . . b. Acidophil bodies: **0**=Rare/absent; **1**=Many

_____ . . . c. Pigmented macrophages (*Kupffer cells*): **0**=Rare/absent; **1**=Many

_____ . . . d. Megamitochondria: **0**=Rare/absent; **1**=Many

_____ 19. Mallory's hyaline: **0**=Rare/absent; **1**=Many

_____ 20. Glycogen nuclei: **0**=Rare/absent; **1**=Many

Masson's trichrome stain

_____ 21. Fibrosis stage: **0**=None; **1a**=Mild, zone 3 perisinusoidal (*requires trichrome*);
1b=Moderate, zone 3, perisinusoidal (*does not require trichrome*); **1c**=Portal/periportal only;
2=Zone 3 and periportal, any combination; **3**=Bridging; **4**=Cirrhosis

22. Iron stain

_____ . . . a. Hepatocellular iron grade: **0**=Absent or barely discernible, 40x ➜ **GOTO item 22c**;
1=Barely discernable granules, 20x; **2**=Discrete granules resolved, 10x; **3**=Discrete granules resolved, 4x;
4=Masses visible by naked eye

_____ . . . b. Hepatocellular iron distribution: **0**=Periportal; **1**=Periportal and midzonal; **2**=Panacinar; **3**=Zone 3 or azonal

_____ . . . c. Nonhepatocellular iron grade: **0**=None ➜ **GOTO item 23**; **1**=Mild; **2**=More than mild

_____ . . . d. Nonhepatocellular iron distribution: **0**=Large vessel endothelium only; **1**=Portal/fibrosis bands only, but more than just in large vessel endothelium; **2**=Intraparenchymal only; **3**=Both portal and intraparenchymal

_____ 23. Is this steatohepatitis? **0**=No; **1a**=Suspicious/borderline/indeterminate: Zone 3 pattern;
1b=Suspicious/borderline/indeterminate: Zone 1, periportal pattern; **2**=Yes, definite

_____ 24. Is cirrhosis present? **0**=No ➜ **GOTO item 27**; **1**=Yes

_____ 25. Is this cryptogenic cirrhosis: **0**=No ➜ **GOTO item 27**; **1**=Yes

26. Features suggestive of steatohepatitis etiology for cryptogenic cirrhosis:

_____ . . . a. Mallory's hyaline (*rule out cholate stasis*): **0**=Absent; **1**=Present

_____ . . . b. Perisinusoidal fibrosis away from septa: **0**=Absent; **1**=Present

_____ . . . c. Hepatocyte ballooning: **0**=Absent; **1**=Present

_____ . . . d. Megamitochondria: **0**=Absent; **1**=Present

_____ . . . e. Other notable findings: **0**=Absent; **1**=Present; Specify: _____

27. Other comments: _____

Keyed: ( )

NAFLD Database

HE - Histology Findings for Most Recent Liver Biopsy Done Prior to Database Registration

Purpose: Record results of histologic evaluation of slides from **most recent liver biopsy done prior to Database registration.**

When: Visit s1.

By whom: Study Pathologist at the NASH CRN clinical center (this is not the form used for central reading) and Clinical Coordinator.

Instructions: The Study Pathologist should complete this form using the institution's H & E slide and if available, the institution's Masson's trichrome slide. Upon completion of this form, the Study Pathologist should give the original HF form to the Clinical Coordinator. If fewer than 2 unstained slides are available for the biopsy, the institution's H & E and Masson's trichrome slides must be sent to the DCC for central pathology review. If 2 or more unstained slides are available for the biopsy, only the unstained slides need to be sent to the DCC. The Study Pathologist should forward the stained slides (if needed) and up to 10 unstained slides to the Clinical Coordinator for forwarding to the Data Coordinating Center.

A. Center, patient and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date of reading:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code: s 1 ____ ____

6. Form & revision: h e 1

7. Study: NAFLD Database 1

B. Biopsy information

8. Date this biopsy was performed *(obtained from surgical pathology report):*

____ ____–____ ____ ____–____ ____
day mon year

9. What slides are to be used in this evaluation *(check all that apply)*

a. H & E: (1)

b. Masson's trichrome: (1)

C. NAFLD evaluation (use H & E and Masson's trichrome slides only)

10. Steatosis *(assume macro, e.g., large and small droplet)*

a. Grade:

< 5% (0)
5-33% (1)
34-66% (2)
> 66% (3)

b. Location:

Zone 3 (0)
Zone 1 (1)
Azonal (2)
Panacinar (3)

11. Fibrosis stage *(Masson's trichrome stain)*

0: None (0)
1a: Zone 3, perisinusoidal (requires trichome) (1)
1b: Zone 3, perisinusoidal (easily seen on H&E) (2)
1c: Portal/periportal only (3)
2: Zone 3 and periportal, any combination (4)
3: Bridging (5)
4: Cirrhosis (6)

Patient ID: ____ ____ ____ ____

12. Inflammation

a. Amount of lobular inflammation: combines mononuclear, fat granulomas, and pmn foci:

0 (0)
< 2 / 20x mag (1)
2-4 / 20x mag (2)
> 4 / 20x mag (3)

b. Amount of portal, chronic inflammation:

None to minimal (0)
Greater than minimal (1)

13. Hepatocellular ballooning:

None (0)
Few (1)
Many (2)

14. Is steatohepatitis present:

No (1)
Suspicious/borderline/indeterminate (2)
Yes, definite (3)

D. Exclusion of other liver disease

15. Is there evidence of primary biliary cirrhosis:

Yes (1) No (2)

16. Is there evidence of Wilson's disease:

Yes (* 1) No (2)

** Caution: Wilson's disease is exclusionary*

17. Features of chronic cholestatic liver disease *(check all that apply)*

a. Bile duct loss/infiltration/sclerosis: (1)
b. Florid duct lesions: (1)
c. Cholate stasis: (1)
d. Copper deposition: (1)
e. Other *(specify):* (1)

_____

f. None: (1)

18. Features of other forms of chronic liver disease *(check all that apply)*

a. Vascular lesions of ALD/B-C/OVD: (1)
b. Inflammation suggestive of AIH, HCV: (1)
c. Pigment suggestive of HH: (1)
d. Globules suggestive of A1AT: (1)
e. Hepatocellular changes suggestive of HBV: (1)
f. Granulomas suggestive of sarcoid, PBC, infection: (1)
g. Other *(specify):* (1)

_____

h. None: (1)

E. Evaluation of cryptogenic cirrhosis

19. Is cirrhosis present:

Yes (1) No (2)

21. ____

20. In your opinion, is this **cryptogenic cirrhosis**
(cirrhosis that fails to meet criteria for NAFLD and without evidence of other form(s) of chronic liver disease):

Yes (1) No (2)

F. Other features

21. Other features *(check all that apply)*

a. Mallory's hyaline (r/o cholate stasis): (1)
b. Perisinusoidal fibrosis away from septa: (1)
c. Hepatocyte ballooning: (1)
d. Megamitochondria: (1)
e. Other *(specify):* (1)

_____

f. None: (1)

Patient ID: ___ ___ ___ ___

G. Other comments

22. Other comments:

_____

_____

_____

_____

_____

H. Administrative information

23. Study Pathologist PIN: ____ ____ ____

24. Study Pathologist signature:

_____

25. Clinical Coordinator PIN: ____ ____ ____

26. Clinical Coordinator signature:

_____

27. Date form reviewed:

____ ____–____ ____ ____–____ ____
day mon year

Keyed: ( )

NAFLD Database

HF - Liver Biopsy Histology Findings

Purpose: Record results of histologic evaluation of slides from liver **biopsy done after registration in Database and before enrollment in Database.**

When: Baseline visit s1 if biopsy slides are available and adequate for scoring.

By whom: Study Pathologist at the NASH CRN clinical center (this is not the form used for central reading) and Clinical Coordinator.

Instructions: The Study Pathologist should complete this form using the institution's H & E slide and if available, the institution's Masson's trichrome slide. Upon completion of this form, the Study Pathologist should give the original HF form to the Clinical Coordinator. If fewer than 2 unstained slides are available for the biopsy, the institution's H & E and Masson's trichrome slides must be sent to the DCC for central pathology review. If 2 or more unstained slides are available for the biopsy, only the unstained slides need to be sent to the DCC. The Study Pathologist should forward the stained slides (if needed) and up to 10 unstained slides to the Clinical Coordinator for forwarding to the Data Coordinating Center.

A. Center, patient and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date of reading:

____ ____ – ____ ____ ____ – ____ ____
day mon year

5. Visit code: s 1 ____ ____

6. Form & revision: h f 1

7. Study: NAFLD Database 1

B. Biopsy information

8. Date this biopsy was performed *(obtained from surgical pathology report):*

____ ____ – ____ ____ ____ – ____ ____
day mon year

9. What slides are to be used in this evaluation *(check all that apply)*

a. H & E: (1)

b. Masson's trichrome: (1)

C. NAFLD evaluation (use H & E and Masson's trichrome slides only)

10. Steatosis *(assume macro, e.g., large and small droplet)*

a. Grade:

< 5%	(0)
5-33%	(1)
34-66%	(2)
> 66%	(3)

b. Location:

Zone 3	(0)
Zone 1	(1)
Azonal	(2)
Panacinar	(3)

11. Fibrosis stage *(Masson's trichrome stain)*

0: None	(0)
1a: Zone 3, perisinusoidal (requires trichome)	(1)
1b: Zone 3, perisinusoidal (easily seen on H&E)	(2)
1c: Portal/periportal only	(3)
2: Zone 3 and periportal, any combination	(4)
3: Bridging	(5)
4: Cirrhosis	(6)

Patient ID: ___ ___ ___ ___

12. Inflammation

a. Amount of lobular inflammation: combines mononuclear, fat granulomas, and pmn foci:

0 (0)
< 2 / 20x mag (1)
2-4 / 20x mag (2)
> 4 / 20x mag (3)

b. Amount of portal, chronic inflammation:

None to minimal (0)
Greater than minimal (1)

13. Hepatocellular ballooning:

None (0)
Few (1)
Many (2)

14. Is steatohepatitis present:

No (1)
Suspicious/borderline/indeterminate (2)
Yes, definite (3)

D. Exclusion of other liver disease

15. Is there evidence of primary biliary cirrhosis:

Yes (1) No (2)

16. Is there evidence of Wilson's disease:

Yes (* 1) No (2)

** Caution: Wilson's disease is exclusionary*

17. Features of chronic cholestatic liver disease *(check all that apply)*

a. Bile duct loss/infiltration/sclerosis: (1)
b. Florid duct lesions: (1)
c. Cholate stasis: (1)
d. Copper deposition: (1)
e. Other *(specify):* (1)

_____

f. None: (1)

18. Features of other forms of chronic liver disease *(check all that apply)*

a. Vascular lesions of ALD/B-C/OVD: (1)
b. Inflammation suggestive of AIH, HCV: (1)
c. Pigment suggestive of HH: (1)
d. Globules suggestive of A1AT: (1)
e. Hepatocellular changes suggestive of HBV: (1)
f. Granulomas suggestive of sarcoid, PBC, infection: (1)
g. Other *(specify):* (1)

_____

h. None: (1)

E. Evaluation of cryptogenic cirrhosis

19. Is cirrhosis present:

Yes (1) No (2)

21.

20. In your opinion, is this **cryptogenic cirrhosis**
(cirrhosis that fails to meet criteria for NAFLD and without evidence of other form(s) of chronic liver disease):

Yes (1) No (2)

F. Other features

21. Other features *(check all that apply)*

a. Mallory's hyaline (r/o cholate stasis): (1)
b. Perisinusoidal fibrosis away from septa: (1)
c. Hepatocyte ballooning: (1)
d. Megamitochondria: (1)
e. Other *(specify):* (1)

_____

f. None: (1)

G. Other comments

22. Other comments:

_____

_____

_____

_____

_____

H. Administrative information

23. Study Pathologist PIN: ____ ____ ____

24. Study Pathologist signature:

_____

25. Clinical Coordinator PIN: ____ ____ ____

26. Clinical Coordinator signature:

_____

27. Date form reviewed:

____ ____–____ ____ ____–____ ____

day mon year

Keyed: ( )

NAFLD Database

HG - Histology Findings for Next Most Recent Liver Biopsy Done Prior to Database Registration

Purpose: Record results of histologic evaluation of slides from **next most recent liver biopsy done prior to Database registration.**

When: Visit s1.

By whom: Study Pathologist at the NASH CRN clinical center (this is not the form used for central reading) and Clinical Coordinator.

Instructions: The Study Pathologist should complete this form using the institution's H & E slide and if available, the institution's Masson's trichrome slide. Upon completion of this form, the Study Pathologist should give the original HF form to the Clinical Coordinator. If fewer than 2 unstained slides are available for the biopsy, the institution's H & E and Masson's trichrome slides must be sent to the DCC for central pathology review. If 2 or more unstained slides are available for the biopsy, only the unstained slides need to be sent to the DCC. The Study Pathologist should forward the stained slides (if needed) and up to 10 unstained slides to the Clinical Coordinator for forwarding to the Data Coordinating Center.

A. Center, patient and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date of reading:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code: s 1 ____ ____

6. Form & revision: h g 1

7. Study: NAFLD Database 1

B. Biopsy information

8. Date this biopsy was performed *(obtained from surgical pathology report):*

____ ____–____ ____ ____–____ ____
day mon year

9. What slides are to be used in this evaluation *(check all that apply)*

a. H & E: (1)

b. Masson's trichrome: (1)

C. NAFLD evaluation (use H & E and Masson's trichrome slides only)

10. Steatosis *(assume macro, e.g., large and small droplet)*

a. Grade:

< 5%	(0)
5-33%	(1)
34-66%	(2)
> 66%	(3)

b. Location:

Zone 3	(0)
Zone 1	(1)
Azonal	(2)
Panacinar	(3)

11. Fibrosis stage *(Masson's trichrome stain)*

0: None	(0)
1a: Zone 3, perisinusoidal (requires trichome)	(1)
1b: Zone 3, perisinusoidal (easily seen on H&E)	(2)
1c: Portal/periportal only	(3)
2: Zone 3 and periportal, any combination	(4)
3: Bridging	(5)
4: Cirrhosis	(6)

12. Inflammation

a. Amount of lobular inflammation: combines mononuclear, fat granulomas, and pmn foci:

0 (0)
< 2 / 20x mag (1)
2-4 / 20x mag (2)
> 4 / 20x mag (3)

b. Amount of portal, chronic inflammation:

None to minimal (0)
Greater than minimal (1)

13. Hepatocellular ballooning:

None (0)
Few (1)
Many (2)

14. Is steatohepatitis present:

No (1)
Suspicious/borderline/indeterminate (2)
Yes, definite (3)

D. Exclusion of other liver disease

15. Is there evidence of primary biliary cirrhosis:

Yes (1) No (2)

16. Is there evidence of Wilson's disease:

Yes (* 1) No (2)

** Caution: Wilson's disease is exclusionary*

17. Features of chronic cholestatic liver disease *(check all that apply)*

a. Bile duct loss/infiltration/sclerosis: (1)
b. Florid duct lesions: (1)
c. Cholate stasis: (1)
d. Copper deposition: (1)
e. Other *(specify):* (1)

_____

f. None: (1)

18. Features of other forms of chronic liver disease *(check all that apply)*

a. Vascular lesions of ALD/B-C/OVD: (1)
b. Inflammation suggestive of AIH, HCV: (1)
c. Pigment suggestive of HH: (1)
d. Globules suggestive of A1AT: (1)
e. Hepatocellular changes suggestive of HBV: (1)
f. Granulomas suggestive of sarcoid, PBC, infection: (1)
g. Other *(specify):* (1)

_____

h. None: (1)

E. Evaluation of cryptogenic cirrhosis

19. Is cirrhosis present:

Yes (1) No (2)

21. ____

20. In your opinion, is this **cryptogenic cirrhosis**
(cirrhosis that fails to meet criteria for NAFLD and without evidence of other form(s) of chronic liver disease):

Yes (1) No (2)

F. Other features

21. Other features *(check all that apply)*

a. Mallory's hyaline (r/o cholate stasis): (1)
b. Perisinusoidal fibrosis away from septa: (1)
c. Hepatocyte ballooning: (1)
d. Megamitochondria: (1)
e. Other *(specify):* (1)

_____

f. None: (1)

Patient ID: ___ ___ ___ ___

G. Other comments

22. Other comments:

_____

_____

_____

_____

_____

H. Administrative information

23. Study Pathologist PIN: ____ ____ ____

24. Study Pathologist signature:

_____

25. Clinical Coordinator PIN: ____ ____ ____

26. Clinical Coordinator signature:

_____

27. Date form reviewed:

____ ____–____ ____ ____–____ ____
day mon year

Keyed: ( )

NAFLD Database

LT - Liver Tissue Banking

Purpose: To document collection of extra liver tissue and flash freeze procedures for liver specimen banking.

When: Whenever more than 2 cm of liver tissue are obtained during a biopsy. If you have more than one pre-enrollment biopsy with flash frozen liver tissue available, contact the Data Coordinating Center. Only one LT form may be completed prior to enrollment in the Database. Use visit code s1, f024, f048, f096, f144, f192, or in followup, use the code for the followup visit that is currently open (check the patient's visit time window guide). If after enrollment and before the f024 window is open, use visit code "n". This form is expected whenever the Liver Biopsy Materials Documentation (SD) form says liver tissue was obtained for banking.

By whom: Clinical Coordinator.

Instructions: Liver biopsy tissue should be obtained by a needle core biopsy (as opposed to a wedge biopsy) using a 16 or greater gauge needle. Whenever more than 2 cm of tissue are obtained during biopsy, place a 1-2 mm segment of liver tissue into a 2.0 mL polypropylene cryovial with preprinted label attached. Flash freeze liver tissue immediately (within 5 minutes following biopsy) by placing labeled cryovial containing liver tissue into a portable liquid nitrogen container. Store the cyrovial locally in -70° C (or colder) freezer temporarily and batch ship cryovials on dry ice monthly to the NIDDK Biosample Repository located at McKesson Bioservices.

A. Center, patient and visit identification

1. Center code: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date form initiated:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code
(s1, n, or code for followup visit that is open):

____ ____ ____ ____

6. Form & revision: 1 t 1

7. Study: NAFLD Database 1

B. Liver biopsy

8. Date of biopsy:

____ ____–____ ____ ____–____ ____
day mon year

9. Was the liver tissue obtained using a 16-gauge or greater needle:

Yes (1) No (2)

10. Was liver tissue obtained via a second pass:

Yes (1) No (2)

11. Was the liver tissue obtained from a needle core biopsy *(as opposed to a wedge biopsy):*

Yes (1) No (2)

C. Cryovial label

12. Attach duplicate cryovial label:

D. Flash freeze procedures

13. Was tissue flash frozen within 5 minutes of biopsy by placing in portable liquid nitrogen container:

Yes (1) No (2)

15. ____

14. Explain what was done and why protocol was not followed:

_____

_____

_____

15. Was tissue shipped on dry ice to the Biosample Repository on same day as biopsy:

Yes (1) No (2)

17. ___

16. Describe conditions of local storage prior to shipment to the Biosample Repository *(e.g., temperature, date and time placed in freezer):*

_____

_____

_____

E. Administrative information

17. Clinical Coordinator PIN: ___ ___ ___

18. Clinical Coordinator signature:

_____

19. Date form reviewed:

___ ___–___ ___ ___–___ ___
day mon year

Keyed: ( )

NAFLD Database

SD - Liver Biopsy Materials Documentation

Purpose: This form is used **only for biopsies done after Database registration** (i.e., during baseline or followup). Use forms SE and SF for biopsies done prior to registration in the Database. To document whether liver tissue was obtained for banking and whether the biopsy is adequate for scoring; if adequate for scoring, the number and type of slides available for archival at the DCC is noted. If slides cannot be archived at the DCC, the source of the slides and the date by which the slides that must be returned to the clinical center are recorded.

When: As needed during baseline (visit s1) and followup (visits f024, f048, f096, f144, f192). During followup, specify the code for the followup visit that is currently open (check the patient's visit time window guide). If no window is open (i.e., right after enrollment), use visit code "n".

By whom: Clinical Coordinator in consultation with the Study Pathologist.

Instructions: This form is used to document acquisition of tissue and slides from liver biopsies done after Database registration (during screening and followup). The SD form provides information about the tissue and slides from the reported biopsy and alerts the DCC to expect completion of the Liver Tissue Banking (LT) form, receipt of tissue at the Biosample Repository, and receipt of slides from the biopsy at the DCC. It also provides a record of the source of the slides, the number and type of stained slides available for review at the clinical center, the need (if any) to borrow those stained slides or provision of those stained slides to the NASH CRN without requiring return of the slides, and the number of unstained slides to be provided to the NASH CRN. A copy of the original surgical pathology report for the biopsy must be obtained; the patient's name should be blacked out, the report should be annotated with the patient's NASH CRN ID number and ID code (use labels provided - PATH or PRpt), and the annotated report should be stapled to the back of this form. The surgical pathology report documents the date of biopsy. The slides should be labeled using the labels provided by the DCC. For unstained slides use permanent labels with sequence numbers 01-60; the borrowed slides should be labeled on the back of the slide with removable labels with sequence numbers 81-90.

A. Center, patient and visit identification

1. Center code: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date form initiated:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code *(s1 or code for followup visit that is currently open):*

____ ____ ____ ____

6. Form & revision: s d 2

7. Study: NAFLD Database 1

B. Surgical pathology report

8. Was a copy of the surgical pathology report for the biopsy obtained:

Yes (+ 1) No (* 2)

26. ____

+ Annotate the report with the patient's NASH CRN ID number and code (you may use one of the pathology labels), black out the patient's name, and attach the report to this form.

** This biopsy cannot be used for the NAFLD Database.*

9. Date of biopsy specified on the surgical pathology report:

____ ____–____ ____ ____–____ ____
day mon year

C. Biopsy specimens and stained slides at the clinical center

10. Was a sample of liver tissue obtained for banking:

Yes (* 1) No (2)

** If Yes, complete the Liver Tissue Banking (LT) form*

11. Is this visit s1:

Yes (1) No (2)

14. ____

12. Were you able to obtain stained slides from this biopsy for local evaluation and were they adequate for scoring:

Yes (+ 1) No (* 2)

26. ____

+ Continue with this form and also complete form HF.

** This biopsy cannot be used for the NAFLD Database.*

13. What stained slides from the biopsy are available for local evaluation *(check all that apply)*

a. H & E stain: (1)

b. Masson's trichrome stain: (1)

D. Unstained slides to be sent to the DCC

14. Are unstained slides available for sending to the DCC:

Yes (1) No (2)

17. ____

15. How many unstained slides will be sent to the DCC: ____ ____

16. What are the slide sequence numbers for those slides *(from the NASH CRN labels on each slide - use permanent labels, sequence numbers 01-60)*

a. Slide sequence number: ____ ____ 01-60

b. Slide sequence number: ____ ____ 01-60

c. Slide sequence number: ____ ____ 01-60

d. Slide sequence number: ____ ____ 01-60

e. Slide sequence number: ____ ____ 01-60

f. Slide sequence number: ____ ____ 01-60

g. Slide sequence number: ____ ____ 01-60

h. Slide sequence number: ____ ____ 01-60

i. Slide sequence number: ____ ____ 01-60

j. Slide sequence number: ____ ____ 01-60

E. Stained slides to be sent to the DCC

17. Is the institution's H & E stained slide to be sent to the DCC:

Yes (1) No (2)

20. ____

18. Slide sequence number for this slide *(from the NASH CRN label on the slide - use removable labels, sequence numbers 81-90):*

____ ____ 81-90

19. Is the H & E stained slide to be returned to the clinical center:

Yes (1) No (2)

20. Is the institution's Masson's trichrome stained slide to be sent to the DCC:

Yes (1) No (2)

23. ____

21. Slide sequence number for slide *(from the NASH CRN label on the slide - use removable labels, sequence numbers 81-90):*

____ ____ 81-90

22. Is the Masson's trichrome slide to be returned to the clinical center:

Yes (1) No (2)

23. Is at least one of the stained slides to be returned to the clinical center *(i.e., either item 19 = yes or item 22 = yes):*

Yes (1) No (2)

26. ____

24. When do the stained slides need to be returned to the clinical center *(check only one):*

Immediately after central review (1)

At the end of the NASH CRN funding period (2)

25. Which pathology department did these slides come from *(check only one):*

NASH CRN clinical center's pathology department (1)

26. ___

Other, *(specify):* (2)

_____
name

_____
address

_____
address

_____
address

_____
phone

Note: this is the Database's record of the source of the slides i.e., where the clinical center should send the slides when they are received back from the DCC.

F. Administrative information

26. Clinical Coordinator PIN: ___ ___ ___

27. Clinical Coordinator signature:

_____

28. Date form reviewed:

___ ___–___ ___ ___–___ ___
day mon year

Keyed: ( )

NAFLD Database

SE - Most Recent Prior Liver Biopsy Materials Documentation

Purpose: To document whether the **most recent biopsy done prior to Database registation** is adequate for scoring; if adequate for scoring, the number and type of slides available for archival at the DCC is noted. If slides cannot be archived at the DCC, the source of the slides and the date by which the slides that must be returned to the clinical center are recorded.

When: As needed during baseline.

By whom: Clinical Coordinator in consultation with the Study Pathologist.

Instructions: This form is used to document acquisition of slides from the most recent liver biopsy done prior to Database registration and reported on the Baseline Medical History (BG) form. The SE form provides information about slides from the reported biopsy and alerts the DCC to expect receipt of slides from the biopsy. It also provides a record of the source of the slides, the number and type of stained slides available for review at the clinical center, the need (if any) to borrow those stained slides or provision of those stained slides to the NASH CRN without requiring return of the slides, and the number of unstained slides to be provided to the NASH CRN. A copy of the original surgical pathology report for the biopsy must be obtained; the patient's name should be blacked out, the report should be annotated with the patient's NASH CRN ID number and ID code (use labels provided - PATH or PRpt), and the annotated report should be stapled to the back of this form. The surgical pathology report documents the date of biopsy. The slides should be labeled using the labels provided by the DCC. For unstained slides use permanent labels with sequence numbers 01-60; the borrowed slides should be labeled on the back of the slide with removable labels with sequence numbers 81-90.

A. Center, patient and visit identification

1. Center code: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date form initiated:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code s 1 ____ ____

6. Form & revision: s e 1

7. Study: NAFLD Database 1

B. Surgical pathology report

8. Was a copy of the surgical pathology report for the biopsy obtained:

Yes (+1) No (*2)

24. ____

+ Annotate the report with the patient's NASH CRN ID number and code (you may use one of the pathology labels), black out the patient's name, and attach the report to this form.

** This biopsy cannot be used for the NAFLD Database.*

9. Date of biopsy specified on the surgical pathology report:

____ ____–____ ____ ____–____ ____
day mon year

C. Stained slides at the clinical center

10. Were you able to obtain stained slides from this biopsy for local evaluation and were they adequate for scoring:

Yes (+1) No (*2)

24. ____

+ Continue with this form and also complete form HE.

** This biopsy cannot be used for the NAFLD Database.*

11. What stained slides from the biopsy are available for local evaluation *(check all that apply)*

a. H & E stain: (1)

b. Masson's trichrome stain: (1)

Patient ID: ____ ____ ____ ____

D. Unstained slides to be sent to the DCC

12. Are unstained slides available for sending to the DCC:

Yes (1) No (2)

15. ____

13. How many unstained slides will be sent to the DCC: ____ ____

14. What are the slide sequence numbers for those slides *(from the NASH CRN labels on each slide - use permanent labels, sequence numbers 01-60)*

a. Slide sequence number: ____ ____ 01-60

b. Slide sequence number: ____ ____ 01-60

c. Slide sequence number: ____ ____ 01-60

d. Slide sequence number: ____ ____ 01-60

e. Slide sequence number: ____ ____ 01-60

f. Slide sequence number: ____ ____ 01-60

g. Slide sequence number: ____ ____ 01-60

h. Slide sequence number: ____ ____ 01-60

i. Slide sequence number: ____ ____ 01-60

j. Slide sequence number: ____ ____ 01-60

E. Stained slides to be sent to the DCC

15. Is the institution's H & E stained slide to be sent to the DCC:

Yes (1) No (2)

18. ____

16. Slide sequence number for this slide *(from the NASH CRN label on the slide - use removable labels, sequence numbers 81-90):*

____ ____ 81-90

17. Is the H & E stained slide to be returned to the clinical center:

Yes (1) No (2)

18. Is the institution's Masson's trichrome stained slide to be sent to the DCC:

Yes (1) No (2)

21. ____

19. Slide sequence number for slide *(from the NASH CRN label on the slide - use removable labels, sequence numbers 81-90):*

____ ____ 81-90

20. Is the Masson's trichrome slide to be returned to the clinical center:

Yes (1) No (2)

21. Is at least one of the stained slides to be returned to the clinical center *(i.e., either item 17 = yes or item 20 = yes):*

Yes (1) No (2)

24. ____

22. When do the stained slides need to be returned to the clinical center *(check only one):*

Immediately after central review (1)

At the end of the NASH CRN funding period (2)

23. Which pathology department did these slides come from *(check only one):*

NASH CRN clinical center's pathology department (1)

24. ____

Other, *(specify):* (2)

_____ name

_____ address

_____ address

_____ address

_____ phone

Note: this is the Database's record of the source of the slides i.e., where the clinical center should send the slides when they are received back from the DCC.

Patient ID: ___ ___ ___ ___

F. Administrative information

24. Clinical Coordinator PIN: ____ ____ ____

25. Clinical Coordinator signature:

_____

26. Date form reviewed:

____ ____–____ ____ ____–____ ____
day mon year

Keyed: ( )

NAFLD Database

SF - Next Most Recent Prior Liver Biopsy Materials Documentation

Purpose: To document whether the **next most recent biopsy done prior to Database registation** is adequate for scoring; if adequate for scoring, the number and type of slides available for archival at the DCC is noted. If slides cannot be archived at the DCC, the source of the slides and the date by which the slides that must be returned to the clinical center are recorded.

When: As needed during baseline.

By whom: Clinical Coordinator in consultation with the Study Pathologist.

Instructions: This form is used to document acquisition of slides from the next most recent liver biopsy done prior to Database registration and reported on the Baseline Medical History (BG) form. The SF form provides information about slides from the next more recent prior biopsy and alerts the DCC to expect receipt of slides from the biopsy. It also provides a record of the source of the slides, the number and type of stained slides available for review at the clinical center, the need (if any) to borrow those stained slides or provision of those stained slides to the NASH CRN without requiring return of the slides, and the number of unstained slides to be provided to the NASH CRN. A copy of the original surgical pathology report for the biopsy must be obtained; the patient's name should be blacked out, the report should be annotated with the patient's NASH CRN ID number and ID code (use labels provided - PATH or PRpt), and the annotated report should be stapled to the back of this form. The surgical pathology report documents the date of biopsy. The slides should be labeled using the labels provided by the DCC. For unstained slides use permanent labels with sequence numbers 01-60; the borrowed slides should be labeled on the back of the slide with removable labels with sequence numbers 81-90.

A. Center, patient and visit identification

1. Center code: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date form initiated:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code s 1 ____ ____

6. Form & revision: s f 1

7. Study: NAFLD Database 1

B. Surgical pathology report

8. Was a copy of the surgical pathology report for the biopsy obtained:

Yes (+1) No (*2)

24.

+ Annotate the report with the patient's NASH CRN ID number and code (you may use one of the pathology labels), black out the patient's name, and attach the report to this form.

** This biopsy cannot be used for the NAFLD Database.*

9. Date of biopsy specified on the surgical pathology report:

____ ____–____ ____ ____–____ ____
day mon year

C. Stained slides at the clinical center

10. Were you able to obtain stained slides from this biopsy for local evaluation and were they adequate for scoring:

Yes (+1) No (*2)

24.

+ Continue with this form and also complete form HG.

** This biopsy cannot be used for the NAFLD Database.*

11. What stained slides from the biopsy are available for local evaluation *(check all that apply)*

a. H & E stain: (1)

b. Masson's trichrome stain: (1)

Patient ID: ____ ____ ____ ____

D. Unstained slides to be sent to the DCC

12. Are unstained slides available for sending to the DCC:

Yes (1) No (2)

15. ____

13. How many unstained slides will be sent to the DCC: ____ ____

14. What are the slide sequence numbers for those slides *(from the NASH CRN labels on each slide - use permanent labels, sequence numbers 01-60)*

a. Slide sequence number: ____ ____ 01-60

b. Slide sequence number: ____ ____ 01-60

c. Slide sequence number: ____ ____ 01-60

d. Slide sequence number: ____ ____ 01-60

e. Slide sequence number: ____ ____ 01-60

f. Slide sequence number: ____ ____ 01-60

g. Slide sequence number: ____ ____ 01-60

h. Slide sequence number: ____ ____ 01-60

i. Slide sequence number: ____ ____ 01-60

j. Slide sequence number: ____ ____ 01-60

E. Stained slides to be sent to the DCC

15. Is the institution's H & E stained slide to be sent to the DCC:

Yes (1) No (2)

18. ____

16. Slide sequence number for this slide *(from the NASH CRN label on the slide - use removable labels, sequence numbers 81-90):*

____ ____ 81-90

17. Is the H & E stained slide to be returned to the clinical center:

Yes (1) No (2)

18. Is the institution's Masson's trichrome stained slide to be sent to the DCC:

Yes (1) No (2)

21. ____

19. Slide sequence number for slide *(from the NASH CRN label on the slide - use removable labels, sequence numbers 81-90):*

____ ____ 81-90

20. Is the Masson's trichrome slide to be returned to the clinical center:

Yes (1) No (2)

21. Is at least one of the stained slides to be returned to the clinical center *(i.e., either item 17 = yes or item 20 = yes):*

Yes (1) No (2)

24. ____

22. When do the stained slides need to be returned to the clinical center *(check only one):*

Immediately after central review (1)

At the end of the NASH CRN funding period (2)

23. Which pathology department did these slides come from *(check only one):*

NASH CRN clinical center's pathology department (1)

24. ____

Other, *(specify):* (2)

_____ name

_____ address

_____ address

_____ address

_____ phone

Note: this is the Database's record of the source of the slides i.e., where the clinical center should send the slides when they are received back from the DCC.

Patient ID: ___ ___ ___ ___

F. Administrative information

24. Clinical Coordinator PIN: ____ ____ ____

25. Clinical Coordinator signature:

_____

26. Date form reviewed:

____ ____–____ ____ ____–____ ____
day mon year

NAFLD Database

SS - Specimen Shipment Log

Purpose: To record information about contents of specimen shipment and receipt of specimens at NIDDK Biosample Repository, McKesson BioServices.

When: Monthly. Ship on Monday, Tuesday, or Wednesday. Avoid shipments 2 days prior to weekends and holidays.

By whom: Clinical Coordinator or laboratory personnel responsible for shipping.

Instructions to shipper: Complete one Specimen Shipment Log for every shipment of specimens sent to NIDDK Biosample Repository (McKesson). Clinical Coordinator/laboratory personnel should record Federal Express airbill number at top of page 2, and record date and number of specimens shipped. Place a copy of the Specimen Shipment Log on top of the styrofoam cooler lid. **(Do not place the log inside of box with the dry ice).** Close and seal the cardboard box. Keep original Specimen Shipment Log in files. All shipments should be sent via Federal Express priority service (next day, AM delivery). Notify the Biosample Repository of the shipment via fax (301-515-4049) or email (niddkrepository@mckessonbio.com) on the day package is picked up by Federal Express. Include the tracking number in the notification.

Packing instructions:

Check that 1 absorbent pad is in the Saf T Pak Biohazard plastic bag.
Insert frozen cryovials into small cardboard boxes with dividers. Place only one tube into each cardboard cell. Each cardboard box may hold 81 aliquot tubes (1.5 mL).
Insert each cardboard box with cryovials into its own plastic bag and seal.
Place each plastic bag with specimen box into its own STP-710 Tyvek envelop and seal.
Place each Tyvek envelope into STP-111 inner brown cardboard box. No more than 3 Tyvek envelopes containing boxes with cryovials can be placed into the STP-111 inner brown cardboard box. If shipping only 1 or 2 specimen boxes, fill the rest of the space inside the cardboard inner box with bubble wrap to prevent movement.
Tape the inner cardboard box closed before placing in the styrofoam cooler.
Place cardboard box in upright position in bottom of styrofoam cooler.
Surround the STP-111 inner brown cardboard box with about 8 kg of 2" blocks or nuggets of Dry Ice.
Fill excessive room left in the insulated freezer box with bubble wrap to stabilize specimens in transit.
Place the polystyrene lid onto the freezer box.
Place a completed Specimen Shipment Log on top of the cooler lid.
Secure fiberboard box with packing tape.

Labeling Shipper:

Place Dry Ice label on side of the box in upper left corner. Record the weight of Dry Ice as 8 kg. Place address label on side of box in lower left corner. Do not cover the printed words, “Diagnostic Specimen.”
Use the preprinted Federal Express air bill to ship specimens to McKesson/NIDDK Biosample Repository. Complete return address (leave “Sender Federal Express account number” blank). Section 6, Special Handling: Check “Yes, Shippers Declaration not required,” check “Dry Ice” block and enter “1" x “8"kg. Section 7, Enter “1" under “Total Packages” and the total weight of 24 lbs. Place completed Federal Express Airbill on side of box. Call Federal Express at 1-800-463-3339; give them the account number in section 7 of the Airbill.
Do not write on exterior of box.

Ship to: Heather Higgins (NIDDK)
McKesson BioServices Corporation
20301 Century Blvd, Bldg 6, Suite 400
Germantown, MD 20874
Telephone: (240) 686-4703

Federal Express Airbill Tracking Number: __ __ __ __ __ __ __ __ __ __ __ __ __

A. Center ID, shipment, and study information

1. Center ID: __ __ __ __

2. Sequential shipment number: __ __

3. Date specimens shipped:

__ __ - __ __ __ - __ __
day month year

4. Total number of plasma aliquots: __ __

5. Total number of serum aliquots: __ __

6. Total number of liver tissue cryovials: __ __

7. Study: NAFLD Database 1

B. Clinic administrative information

8. Print information of person preparing shipment:

_____
name

_____
telephone number

_____
Email address

C. Specimen shipment information

Record specified information about plasma and serum and liver tissue cryovials shipped in items 9 thru 16. Indicate number of cryogenic vials containing plasma, serum, and liver tissue. McKesson will fill in the Comments section (column h).

	a. Site ID - Patient ID	b. Patient code	c. Visit code	d. Date specimens separated (day-month-year)	e. # of plasma aliquots	f. # of serum aliquots	g. # of liver tissue cryovials	h. Comments (McKesson use only)
9.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
10.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
11.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
12.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
13.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
14.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
15.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
16.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				

D. Comments (to be completed by clinical center)

E. Comments (to be completed by Biosample Repository)

NAFLD Database

TS - Histology Slide Transmittal Log

Purpose: To inform the Data Coordinating Center of the shipment of histology slides (stained and unstained), and to record information about contents of slide shippers and status of slides received at the Data Coordinating Center. This form is also used by the Data Coordinating Center to inform clinical centers of the shipment of slides back to the clinical center.
When: Ship slides monthly (or more often, as needed) on Monday through Wednesday only.
By Whom: Clinical Coordinator responsible for slide shipping.
Instructions to Shipper:

- Complete one Histology Slide Transmittal Log for every shipment of slides
- Make a copy of the Histology Slide Transmittal Log for the clinical center's notebook of slide shipping logs
- Attach a copy of the surgical pathology report for each slide set included in the shipment. Make sure the report is annotated with the patient's NASH CRN ID number and patient code and that the patient identifiers are blacked out
- Place slides in interior slide box which holds up to 25 slides
- Place 1-2 sheets of tissue over the slides to help prevent shifting
- Surround slide box with bubble wrap
- Place the slide box wrapped with bubble wrap into a card board shipping box (eg., DHL box, FedEx box)
- Insert a copy of the Histology Slide Transmittal Log into the shipping box (file a copy of the TS log at the Clinical Center/DCC)
- Secure the shipping box with tape
- Place the consignee and return address labels on the exterior shipping box
- Fax (410 955-0932) a copy of the Histology Slide Transmittal Log to the Data Coordinating Center/clinical center
- Ship by two day delivery service **with ability to track the shipment to:**
  NASH CRN Slide Coordinator, NASH CRN Data Coordinating Center, 615 North Wolfe Street, Room W5010, Baltimore, MD 21205, (410) 955-8175 (phone)

A. Center, date, shipping and study identification

1. Center ID: ___ ___ ___ ___

2. Date form completed:

___ ___ - ___ ___ ___ - ___ ___
day month year

3. Study: NAFLD Database 1

4. Shipping destination *(check only one)*:

Data Coordinating Center (1)
Return to clinic (2)

5. Shipping service used *(check only one)*:

DHL (1)
FedEx (2)
Other, *(specify)* (3)

_____

6. Shipment tracking number:

_____

7. Person preparing shipment *(please print)*:

_____
please print

8. Comments *(to be completed by staff responsible for shipping slides. If applicable, record reason(s) for discrepancies between number of slides recorded for a patient on the SD form and the number of slides recorded for that patient on the TS form)*:

_____

_____

_____

Center ID: __ __ __ __

B. Slide shipment information

Record specified information about slides shipped in items 9 through 25. Indicate number of slides that are stained or unstained. Personnel receiving the shipment will fill in the Receipt code (column h) with all codes that apply.
Codes for column h. are as follows: A=Satisfactory, B=Missing surgical pathology report, C=Slide(s) broken, D=Slide(s) not with shipment, E=Slide(s) not labeled, F=Slide(s) mislabeled, G=Other.

	a. Patient ID	**b.** Patient code	**c.** Visit code	**d.** Date of biopsy (day-month-year)	**e.** surgical pathology report (y/n)	**f.** # of stained slides	**g.** # of unstained slides	**h.** Receipt codes (completed by staff member receiving and reviewing the shipment contents)
9.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
10.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
11.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
12.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
13.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
14.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
15.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
16.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
17.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
18.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
19.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
20.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
21.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
22.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
23.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
24.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	
25.	__ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __	__	__	__ __	

C. Shipment review (to be completed by staff receiving shipment)

26. Other comments regarding contents of shipment received:

_____

_____

_____

27. Person receiving shipment (please print):

_____

28. Date shipment received and reviewed:

___ ___ - ___ ___ ___ - ___ ___
day month year

NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

NAFLD Database
Standard Operating Procedures

Part V:
Standards of Care for Adult Patients with Fatty Liver Disorders

27 July 04

Contents

1. Introduction

The purpose of this document is to articulate a uniform set of practices to be applied by investigators in the NASH CRN to the care of patients with fatty liver disorders. As directed by the NASH CRN Steering Committee, these standards were developed so that patients with fatty liver disorders will receive uniform treatment across study sites, thereby reducing the extent to which care at a particular site will independently influence outcomes. These standards of care were derived by expert opinion, specifically the opinions of the NASH CRN investigators, and as such cannot and should not be construed to mean that they should be applied outside of the NASH CRN study sites or that the failure to apply these standards is a breach of community standards of care.

The NASH CRN Standards of Care Committee developed these standards in their initial draft form after the first meeting of the Committee on July 29, 2002 in Baltimore, MD. After review and revision, it was submitted to the CRN Steering Committee and approved in principal at its meeting on September 22, 2002 in Atlanta, GA. Committee members are aware that application of the standards described here could be viewed as an intervention in itself. However, the committee also felt that these standards are essential to fulfill our obligation to provide the best care to the participating patients.

Standards of care are based on the best available information at the time they are developed. Because the understanding of fatty liver disease is rapidly evolving, the Committee anticipates the need to review and revise the guidelines on an annual basis. In July and August each year, the Standards of Care Committee chairperson shall solicit recommendations for revisions from the Committee members and from the Steering Committee members. A revised document shall be presented to the Steering Committee for approval before October 1 of each year. Following approval, the necessary materials for implementation will be prepared by the Data Coordinating Center (DCC) and the new document will go into effect on January 1.

2. Specific recommendations

2.1 Dietary intake

a. Patients without diabetes will be instructed to follow NCEP Step 1 recommendations (Appendix 1). These recommendations will include specific discussions on total caloric intake, the amount and type of fat consumed, the amount of carbohydrate consumed.
b. Patients with known or newly discovered type 2 diabetes will receive specific recommendations as promulgated by the ADA (Appendix 2).
c. Recommendations regarding the use of specific nutritional supplements are addressed below.
d. Dietary guidelines may not apply to all persons or situations.

2.2 Weight loss

a. Overweight subjects (BMI > 25 kg/m^2) will be given a goal of losing and sustaining the loss of 5-10% of body weight. This weight loss should be achieved at a rate of 1-2 lbs per week per NHLBI guidelines (Appendix 3).
b. Patients will be instructed not to fast as a means of achieving weight loss.
c. Alternative diet plans intended to promote weight loss will be considered individually based on nutritional completeness.

2.3 Alcohol consumption

Patients will be instructed that total abstinence from alcohol is advisable. The Committee acknowledges the paucity of data regarding a minimal safe dose of alcohol in individuals with liver disease and consumption limited to "ceremonial use" or even amounts up to 10 g per week (1 oz 80 proof liquor, 3.5 oz non-fortified wine, 8 oz beer) may be safe.

2.4 Exercise

Patients will be instructed to engage in a lifestyle that includes regular moderate exercise. The recommendations of the Institute of Medicine will be used: regular physical activity of at least one hour daily.

2.5 Preventive medicine

a. Vaccination for viral hepatitis. The Committee did not reach a consensus on recommendations regarding vaccination against hepatitis A and B. While arguments could be made in favor of both, local practices are variable and the failure to vaccinate is unlikely to bias the study. Therefore disparities in site-specific practices would have no impact on the studies of the NASH CRN.

b. Hepatocellular carcinoma (HCC) screening. Because recent data suggest that cirrhosis caused by NASH is associated with a similar risk of developing hepatocellular carcinoma as other major causes of cirrhosis, patients with cirrhosis should undergo regular surveillance testing for HCC. In view of the lack of consensus in the field regarding an optimal cost-effective screening strategy, screening methods will not be standardized across sites but will be in accordance with local standards.

2.6 Management of coexisting morbidities

a. Type 2 diabetes
    i. Some patients will not have a pre-existing diagnosis of type 2 diabetes but meet diagnostic criteria as a result of testing performed for NASH CRN studies. These patients will be referred to their primary physicians for appropriate management. The use of an insulin-sensitizing agent will be suggested as initial therapy instead of a sulfonylurea or insulin if the primary physician elects to begin pharmacologic therapy.
    ii. Patients with controlled diabetes (Hgb $A_{1C} < 7\%$) will be continued on their current treatment regimens.
    iii. Patients with suboptimally controlled diabetes (Hgb $A_{1C} \geq 7\%$) will receive a recommendation for followup with their primary physician for improved glycemic control.

b. Hypertriglyceridemia

    Patients with fasting triglycerides > 200 mg/dL will be referred to their primary physicians for specific recommendations.

c. Hypercholesterolemia
    i. Nondiabetic patients with fasting LDL cholesterol levels > 130 mg/dL will be referred to their primary physicians for specific recommendations.
    ii. Diabetic patients with fasting LDL cholesterol levels > 100 mg/dL will be referred to their primary physicians for specific recommendations.

d. Hypertension
    i. Nondiabetic patients with repeated systolic blood pressure > 140 mm Hg or diastolic blood pressure > 90 mm Hg will be referred to their primary physicians for specific recommendations.
    ii. Diabetic patients with repeated systolic blood pressure > 130 mm Hg or diastolic blood pressure > 85 mm Hg will be referred to their primary physicians for specific recommendations.

e. Angina

    Patients will not be specifically evaluated for coronary heart disease (CHD). A review of systems will be obtained and if symptoms suggestive of angina are elicited, patients will be referred to their primary physicians for specific recommendations.

f. Sleep apnea

    Symptoms suggestive of sleep apnea (snoring, observed periods of apnea, disruptive sleep disturbances) will be specifically sought as part of the review of symptoms. If these symptoms are present, patients will be referred to their primary physicians for a possible sleep study. The rationale for this assessment is that disrupted sleep will contribute to fatigue and the role of periods of hypoxia in the pathogenesis of liver injury is unknown.

g. Hyperandrogenism and polycystic ovary syndrome (PCOS)

    Women with hirsutism (facial and/or chest hair) and non-menopausal menstrual irregularity (< 9 menstrual cycles in the past year) will be referred to their primary physicians or gynecologists to be evaluated for PCOS.

h. Occupational exposure to hepatotoxins

    A history of ongoing exposure to volatile hydrocarbons will be sought. Patients with ongoing occupational exposure to hydrocarbons will be instructed to verify workplace compliance with OSHA regulations.

2.7 Possibly helpful concomitant medication use

a. Ursodeoxycholic acid (UDCA; Actigall, Urso)
    i. UDCA will generally be stopped unless new data are published to indicate a significant benefit for patients with NASH.
    ii. A UDCA washout period of 3 months prior to liver biopsy or 3 months prior to randomization will be needed before entry into treatment trials.
    iii. UDCA may be continued in patients who have shown a significant improvement associated with treatment in liver histology or surrogate markers for NAFL such as ALT or imaging studies.

b. Metformin
    i. Patient receiving metformin as a treatment for diabetes may remain on the drug.
    ii. Patients treated with metformin for a diagnosis of NAFL or NASH may remain on the drug.
    iii. Patients receiving metformin as a treatment of other disorders of insulin resistance (e.g., PCOS) may remain on the drug.

c. Fibrates

Fibrates used to treat hypertriglyceridemia may be continued with dose escalations as clinically indicated.

d. Statins

Statins used to treat hypercholestereolemia may be continued with dose escalations as clinically indicated.

e. Thiazolidinediones (TZDs; pioglitazone, rosiglitazone)
   i. Patients receiving a TZD as a treatment for diabetes may remain on the drug.
   ii. Use of TZDs for NASH (non trial) should be discouraged, but their use will be at the discretion of physicians.

2.8 Possibly harmful concomitant medication use

a. Acetaminophen
   i. Acetaminophen should be restricted to < 3 grams in any given day.
   ii. Repeated use of > 1.5 grams daily for more than 3 consecutive days should be discouraged.
   iii. A history of using over-the-counter medications that may contain acetaminophen will be obtained at each visit.

b. Tamoxifen

A history suggesting the onset of NASH during tamoxifen use should lead to a discussion among the hepatologist, oncologist and patient regarding the risks associated with its continuation versus discontinuation. Additional options include use of an alternative estrogen receptor antagonist, although the risk of NASH posed by these agents is unknown.

c. Estrogens (OCP, HRT)

Estrogen use as oral contraception and hormone replacement therapy will not be discouraged.

d. Amiodarone
   i. Amiodarone can be continued for life-threatening arrythmias.
   ii. The continued use of amiodarone for non-lifethreatening arrythmias (e.g., atrial fibrillation) will be discussed with the patient's primary physician or cardiologist.

e. Iron supplements
   i. Patients with iron deficiency will be allowed to continue the use of iron supplements until iron stores are sufficient.
   ii. In the case of ongoing blood loss (e.g., meno/metrorrhagia, refractory GERD, portal hypertensive gastropathy), iron supplements will be continued as long as the transferrin saturation remains < 50%.
   iii. Patients having normal iron status (serum ferritin > 15 ng/mL or transferrin saturation > 20%) and taking iron supplements will be requested to discontinue the supplements.

2.9 Possibly helpful concomitant dietary supplement use

a. Vitamin E. Patients will be allowed vitamin E in doses not to exceed 800 IU daily.

b. Multivitamins. A daily multivitamin with iron content < 20 mg daily will be allowed. (Many commonly used multivitamins contain small amounts of iron, typically < 20 mg each.)

c. Betaine use will neither be recommended nor discouraged.
d. S-adenosylmethionine use will neither be recommended nor discouraged.
e. Herbal supplements: Milk thistle use will neither be recommended nor discouraged.

2.10 Possibly harmful concomitant dietary supplement use

a. Vitamin A supplements in excess of that contained in a daily multiple vitamin (5000 IU) should not be used.
b. Glucosamine use will be recorded but patients will not be given specific recommendations. Although hexosamines may have a role in causing insulin resistance, the effect of oral glucosamine on insulin sensitivity is unknown.
c. Herbal supplements
    i. St John's Wort has been associated with CYP 3A4 induction and should be discontinued if used and avoided if not used.
    ii. Ephedrin-containing products marketed for weight loss will be strongly discouraged because of potential adverse effects.
    iii. Other herbal remedies should be viewed as possible causes of liver injury and should be discontinued or avoided.

3. Implementation

The intention of the NASH CRN is to implement these standards of care immediately in the patients followed at all study sites. For new patients without a biopsy-established diagnosis of NAFL, the standards may be implemented at the discretion of the hepatologist depending on the index of suspicion of NASH. There will be no standardized lead-in period during which these standards will be applied before patients are enrolled in NASH CRN studies. Once a liver biopsy establishes a diagnosis of NASH, the standards should be implemented. Implementing the standards in patients with only steatosis on a liver biopsy will be at the discretion of the hepatologist. The Committee believes that implementing these standards of care constitutes good clinical practice and does not constitute an intervention; moreover, data will not be collected regarding outcomes after implementation of these standards. As such, their implementation is not under the purview of local Institutional Review Boards.

Responsibility for implementation: The NASH CRN will develop uniform teaching materials to provide patients with the information detailed above. Local sites will be responsible for printing costs. The materials will be distributed to patients by their hepatologists or other health care worker such as nurses and nutritionists. Distribution of materials to patients will be documented in their medical records.

Compliance and reinforcement: Patients' adherence to the above guidelines will be established by the study personnel. The practices will be reviewed by the hepatologist or associated health care worker with the patient during the clinic visit. Patients will be given the opportunity to take the teaching materials outlining the above recommendations at each hepatology clinic visit.

Frequency of follow-up: Patients will be seen at least twice yearly by the hepatologist. The visit should include an interim history, review of symptoms, updated medication list, physical exam, lab work, and discussion of adherence to the standard of care recommendations.

4. Preparation and dissemination of materials needed to implement the standards of care

4.1 Physician summary of guidelines

a. Physicians at the study sites will need ongoing reminders of the specifics of the standards of care
b. Pocket card and a small poster for patient care areas (Pocket guidelines are posted on the NASH CRN website: http://www.jhucct.com/nash/closed/docs/sop/database/sop.htm)

4.2 Patient brochures

a. What brochures are needed
    i. Healthy eating
    ii. Healthy weight loss
        1. BMI formula
        2. Goals
    iii. General NASH CRN brochure to cover most other recommendations (The NASH fact sheet is posted on the NASH CRN website at http://www.jhucct.com/nash/closed/docs/sop/database/sop.htm)
        1. Alcohol use
        2. Acetaminophen use
            a. Allowable amounts
            b. List of medications containing acetaminophen
        3. Supplemental iron use
        4. Vitamins
            a. Allowable vitamin E
            b. Allowable vitamin A
            c. MVI daily
        5. Warnings about herbal remedies
        6. Symptoms to report
            a. Angina
            b. Sleep apnea
            c. Irregular menstruation, facial hair
b. Brochure development
    i. Content: Standards of Care Committee
    ii. Design: Need a professional, aesthetically pleasing design
    iii. Printing: Local centers to arrange for printing, distribution, cost recovery
c. Updates to the brochures
    i. Content to be reviewed annually and discussed at Steering Committee meetings
    ii. Revised content and design to be prepared within 4 weeks of review at Steering Committee. Revisions to be distributed to the Steering Committee members for final approval.

4.3 Referring physician information

a. Implementation of the standards of care will occasionally require communication of findings such as newly diagnosed diabetes, hypertension, or hyperlipidemia. This communication will be in the form of a letter from the NASH CRN physician as part of standard medical care.

4.4 NASH CRN website

A public website will be considered in the future, but will not be implemented during the first year of the trial.

Appendix 1: NCEP Step 1 diet (standard recommendation)

The general dietary recommendations developed by the Institute of Medicine with the goal of promoting a healthy lifestyle will be reviewed for possible substitution for the NCEP guidelines below.

The following dietary recommendations were stated by the National Cholesterol Education Program (NCEP) in their monograph entitled *The Third Report of the Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)*, or ATP III (NHLBI, 2001). The primary goal of these recommendations is to provide a diet that would reduce the risk of coronary heart disease in individuals with high LDL cholesterol levels. A secondary target of risk reduction, which was new to this version of the report, was the metabolic syndrome or insulin resistance.

Nutrient Composition of the Therapeutic Lifestyle Change Diet

Nutrient	Recommended Intake
Saturated fat[1]	< 7% of total calories
Polyunsaturated fat	≤ 10% of total calories
Monounsaturated fat	≤ 20% of total calories
Total fat	25 - 35% of total calories
Carbohydrate[2]	50 - 60% of total calories
Fiber	20 - 30 g daily
Protein	Approximately 15% of total calories
Cholesterol	< 200 mg/day
Total calories[3]	Balance energy intake and expenditure to maintain desirable body weight/prevent weight gain

[1]Trans fatty acids are another LDL-raising fat that should be kept at a low intake.
[2]Carbohydrates should be derived predominantly from foods rich in complex carbohydrates including grains, especially whole grains, fruits, and vegetables.
[3]Daily energy expenditure should include at least moderate physical activity (contributing approximately 200 Kcal per day).

Appendix 2: ADA diet (for patients with type 2 diabetes)

Current evidence-based recommendations developed by the ADA are summarized below.

Nutritional Principles & Recommendations in Diabetes
American Diabetes Association
Diabetes Care 2004; 27:S36-S46
(Summary of A-level evidence for NASH CRN Appendix)

Carbohydrates
Choose whole grains, fruits, vegetables, low-fat milk
Amount of carbohydrate is more important than source
Non-nutritive sweeteners in usual doses

Fats
Limit to 10% of less of total calorie intake
Limit cholesterol to <300 mg per day

Obesity and Weight Loss
Modest weight loss by reduced energy intake improves insulin resistance
Structured programs of lifestyle change can produce weight loss of 5-7%
Exercise and behavior modification are useful adjuncts to reduction of energy ijntake

Older Adults
Energy requirements decline with age
Encourage physical activity

Hypoglycemia
Glucose is preferred treatment

Hypertension
Reduced sodium intake reduces blood pressure
Modest weight loss reduces blood pressure

Appendix 3: NHLBI Step 1 diet (for weight reduction)

Source: The Practice Guide. Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. NHLBI, 2000, p 27. URL: http://www.nhlbi.nih.gov/guidelines/obesity/practgde.htm

Nutrient	Recommended intake
Calories[1]	Approximately 500 - 1,000 kcal/day reduction from usual state
Total fat[2]	30% or less of total calories
Saturated fatty acids[3]	8 - 10% of total calories
Monounsaturated fatty acids	Up to 15% of total calories
Polyunsaturated fatty acids	Up to 10% of total calories
Cholesterol[3]	< 300 mg/day
Protein[4]	Approximately 15% of total calories
Carbohydrate[5]	55% or more of total calories
Sodium chloride	No more than 100 mmol/day (approximately 2.4 g of sodium or approximately 6 g of sodium chloride)
Calcium[6]	1,000 to 1,500 mg/day
Fiber[5]	20 - 30 g/day

1. A reduction in calories of 500 to 1,000 kcal/day will help achieve a weight loss of 1 to 2 pounds/week. Alcohol provides unneeded calories and displaces more nutritious foods. Alcohol consumption not only increases the number of calories in a diet but has been associated with obesity in epidemiologic studies as well as in experimental studies. The impact of alcohol calories on a person's overall caloric intake needs to be assessed and appropriately controlled.
2. Fat-modified foods may provide a helpful strategy for lowering total fat intake but will only be effective if they are also low in calories and if there is no compensation by calories from other foods.
3. Patients with high blood cholesterol levels may need to use the Step II diet to achieve further reductions in LDL-cholesterol levels; in the Step II diet, saturated fats are reduced to less than 7 percent of total calories, and cholesterol levels to less than 200 mg/day. All of the other nutrients are the same as in Step I.
4. Protein should be derived from plant sources and lean sources of animal protein.
5. Complex carbohydrates from different vegetables, fruits, and whole grains are good sources of vitamins, minerals, and fiber. A diet rich in soluble fiber, including oat bran, legumes, barley, and most fruits and vegetables, may be effective in reducing blood cholesterol levels. A diet high in all types of fiber may also aid in weight management by promoting satiety at lower levels of calorie and fat intake. Some authorities recommend 20 to 30 grams of fiber daily, with an upper limit of 35 grams.
6. During weight loss, attention should be given to maintaining an adequate intake of vitamins and minerals. Maintenance of the recommended calcium intake of 1,000 to 1,500 mg/day is especially important for women who may be at risk of osteoporosis.

Appendix 4: Common acetaminophen-containing over-the-counter medications

The number of acetaminophen-containing preparations is quite large and an updated list of such preparations could not be found. The FDA is currently considering ways to educate the public on the dangers of acetaminophen in combination medications. See their talking points for a meeting held September 19, 2002:
http://www.fda.gov/ohrms/dockets/ac/02/questions/3882Q1_Discussion%20Points%20Final.doc

The best approach to this issue will be to inquire about over-the-counter medication use as part of the medical history. If the FDA or other agencies develop written teaching materials regarding the use of acetaminophen containing products, these will be reviewed by the Standards of Care committee for possible inclusion in subsequent versions.