NASH CRN

Nonalcoholic Steatohepatitis

Clinical Research Network

NAFLD Adult Database 2

Standard Operating Procedures

Part I: Clinical Center Operations

6 April 2018

NAFLD Adult Database 2 SOP I: Center Operations

Contents

NAFLD Adult Database 2 SOP I: Center Operations

1. Design overview

1.1. Design synopsis

Objectives

- To continue to investigate the etiology, pathogenesis, natural history, diagnosis, treatment, and prevention of nonalcoholic fatty liver disease (NAFLD)
- To provide a resource for clinical trials and ancillary studies of the pathogenesis, natural history, diagnosis or diagnostic biomarker development and treatment of NAFLD, NASH, or NASH-related cirrhosis
- To continue the development of histopathological methods for diagnosis and assessment of NAFLD, NASH, and NASH-related cirrhosis
- To maintain and expand the centralized histopathological NAFLD repository and reading center
- To develop imaging methods for non-invasive diagnosis and assessment of NAFLD, NASH, and NASH-related cirrhosis and to develop a NAFLD digital imaging repository and analysis center
- To add to and expand the specimen bank comprising liver tissue, serum, plasma, and DNA obtained from participants with biopsy confirmed NAFLD

Type of study

- Prospective follow-up

Population

- Participants at least 18 years of age with known or suspected NAFLD or NASH-related cirrhosis

Inclusion criteria

- At least 18 years of age at time of initial screening
- Written informed consent to participate
- Willingness to be followed for up to 4 years
- **For continuing participants:** Previously enrolled in the NAFLD Database study, PIVENS, or TONIC trials
- **For new participants**:
  - Recent ($\leq$120 days before enrollment) liver biopsy
  - Collection of serum and plasma within 90 days of enrollment and up to 90 days before or 4-90 days after standard of care liver biopsy
  - Absence of regular or excessive use of alcohol within 2 years prior to initial screening

Exclusion criteria

- **For continuing participants:** Any conditions or circumstances likely to interfere with follow-up visits and procedures (per investigator's opinion)
- **For new participants:**
  - Clinical or histologic evidence of alcoholic liver disease
  - Evidence of other causes of chronic liver disease

- History of prolonged (> 1 month) total parenteral nutrition within a 6 month period before baseline liver biopsy
- Short bowel syndrome
- History of biliopancreatic diversion
- History of bariatric surgery (Participants expecting to undergo bariatric surgery can be enrolled prior to the procedure)
- Known HIV positive
- Other condition that is likely to interfere with study follow-up

Recruitment

- Target recruitment period: December 2009 to April 2019
- **Continuing participants**: 1,000 (300 with standard of care liver biopsy within the target recruitment period, 700 continuing participants without liver biopsy at enrollment)
- **New participants**: 600 participants with liver biopsy within 90 days of specimen collection
- Target for new liver biopsies: 900 (300 from continuing participants and 600 from new participants)
- Total sample size for adult participants in NAFLD Database 2: 2,500

Duration of follow-up

- 1 to 10 years (minimum of one year of follow-up until 31 December 2019)

Outcome measures

- Liver histology scores (derived from central reading of liver biopsy at entry, standard of care biopsy done during screening or follow-up, or liver biopsy obtained for PIVENS or TONIC trials)
- ALT, AST levels
- Glucose, insulin levels
- Lipid profile
- Body mass index and anthropometric data
- Alcohol consumption
- Medication use
- Beverage intake

Visit schedule

- Screening and enrollment into NAFLD Database 2: screening (t0) must be completed within 90 days of the signing of consent. Enrollment marks the successful completion of the screening process and initiates the yearly (with a target of every 48 weeks) study visits
- Follow-up visits at t048, t096, t144, t192, t240, t288, t336, t384, t432 and t480.

1.2. Data collection schedule

			Follow-up visits: weeks from enrollment									
	Screening	**Enrollment**	**t048**	**t096**	**t144**	**t192**	**t240**	**t288**	**t336**	**t384**	**t432**	**t480**
Consent and HIPAA authorization	X	-	-	-	-	-	-	-	-	-	-	-
Baseline medical history	X	-	-	-	-	-	-	-	-	-	-	-
Follow-up medical history (including interim drinking history, medication)	-	-	X	X	X	X	X	X	X	X	X	X
Beverage Intake	X	-	X	X	X	X	X	X	X	X	X	X
Physical examination	X	-	X	X	X	X	X	X	X	X	X	X
Liver biopsy review†	A	-	A	A	A	A	A	A	A	A	A	A
Provision of standard of care materials	X	-	-	-	-	-	-	-	-	-	-	-
Database eligibility confirmation	-	X	-	-	-	-	-	-	-	-	-	-
Alcohol use questionnaires												
AUDIT	X	-	-	-	-	-	-	-	-	-	-	-
Lifetime drinking history (Skinner)	X	-	-	-	-	-	-	-	-	-	-	-
Hematology	X	-	X	X	X	X	X	X	X	X	X	X
Hepatic panel	X	-	X	X	X	X	X	X	X	X	X	X
Clinical chemistry	X	-	X	X	X	X	X	X	X	X	X	X
HbAlc	X	-	X	X	X	X	X	X	X	X	X	X
Lipid profile (fasting)	X	-	X	X	X	X	X	X	X	X	X	X
Glucose and insulin levels (fasting)	X	-	X	X	X	X	X	X	X	X	X	X
Etiologic tests‡	X	-	-	-	-	-	-	-	-	-	-	-
Specimens for banking§	X	-	X	X	X	X	X	X	X	X	X	X

A = as available

† Liver biopsy required for new patients; as available for continuing patients

Hepatic panel: Total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma glutamyltransferase (GGT), albumin, prothrombin time (PT), INR.

Hematology: Hemoglobin, hematocrit, mean corpuscular volume (MCV), white blood cell count (WBC), red blood cell count (RBC), and platelet count.

Clinical chemistries: Creatinine, total protein, BUN, uric acid, and HbA1c Lipid profile: triglycerides, total cholesterol, LDL and HDL

‡ Etiologic tests: Hepatitis B surface antigen, hepatitis C antibody. Serum iron, total iron binding capacity, ferritin, and hepatic iron index (if liver iron measurement available). Ceruloplasmin (obtain if age 18-40 and record ceruloplasmin value, if available, for patients >40), alpha-1-antitrypsin level.

Autoantibody studies (ANA, ASMA, AMA). TSH

§ Specimens for banking include: serum, plasma, DNA, liver tissue when available

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

		Study visit (week)										
Procedure	**Screening**	**t048**	**t096**	**t144**	**t192**	**t240**	**t288**	**t336**	**t384**	**t432**	**t480**	**Total**
Fasting glucose and insulin	5	5	5	5	5	5	5	5	5	5	5	55
Fasting lipid	5	5	5	5	5	5	5	5	5	5	5	55
Complete blood count	5	5	5	5	5	5	5	5	5	5	5	55
Clinical chemistry	5	5	5	5	5	5	5	5	5	5	5	55
Hepatic panel	5	5	5	5	5	5	5	5	5	5	5	55
HbA1c	5	5	5	5	5	5	5	5	5	5	5	55
Plasma	10	10	10	10	10	10	10	10	10	10	10	110
Serum	30	20	20	20	20	20	20	20	20	20	20	230
Genetics	10	-	-	-	-	-	-	-	-	-	-	10
Other screening*	20	-	-	-	-	-	-	-	-	-	-	20
Total	**100**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**700**

All Database 2 study visits 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.

* Etiologic tests as needed

1.4. Study population composition

- 2,500 participants 18 years of age or older with:
  - NAFLD
  - NASH-related cirrhosis

- The target composition is:
  - New adult participants 1,500
  - Continuing adult participants,
    Biopsied at enrollment 300
  - Continuing adult participants
    Not biopsied at enrollment 700

2. Eligibility and enrollment

2.1. Inclusion and exclusion criteria

Inclusion criteria

Continuing participants previously met inclusion (and exclusion) criteria for the NAFLD Database study or PIVENS or TONIC trials, and these criteria are not listed here, but are in the applicable protocols for these NASH CRN studies. Both continuing and new participants must meet all of the inclusion criteria below, which are listed separately for continuing and new participants.

Continuing participants:

- Previously enrolled in the NAFLD Database study, PIVENS or TONIC trials
- Age at least 18 years during the consent process
- Willingness to continue to be followed for up to 4 years
- Ability and willingness to give written, informed consent to be enrolled into the Database 2 study

New participants:

- Age at least 18 years during the consent process
- Willingness to be followed for up to 4 years
- Ability and willingness to give written, informed consent to be screened for and, if eligible, to be enrolled into the Adult Database 2 study
- Minimal or no alcohol use history consistent with NAFLD (see exclusion criteria)
- Collection of a standard of care liver biopsy that is obtained within 120 days of enrollment
- Collection of biosamples (serum, plasma, DNA, and, if available, liver tissue) within 90 days prior to enrollment and 0-90 days before or 4-90 days after the standard of care liver biopsy

Exclusion criteria

Continuing participants who meet the following criterion will not be eligible:

- Any condition or circumstances, which, in the opinion of the investigator, would interfere with completion of scheduled follow-up visits and procedures for the duration of the Adult Database 2 study

New participants who meet any of the following criteria will not be eligible:

- Clinical or histological 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 for more than 1 month within a 6 month period before baseline liver biopsy
- Short bowel syndrome
- History of gastric or jejunoileal bypass preceding the diagnosis of NAFLD. Bariatric surgery performed following enrollment is not exclusionary. Liver biopsies obtained during bariatric surgery cannot be used for enrollment because of the associated surgical or anesthetic acute changes and the weight loss efforts that precede bariatric surgery
- History of biliopancreatic diversion
- Evidence of advanced liver disease defined as a Child-Pugh-Turcotte score equal to or greater than 10
- Evidence of chronic hepatitis B as marked by the presence of HBsAg in serum (participants with isolated antibody to hepatitis B core antigen, anti-HBc total, 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 4 g of iron by phlebotomy)
- 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 or congenital liver disease
- Evidence of systemic infectious disease
- Known HIV positive
- Disseminated or advanced malignancy
- Concomitant severe underlying systemic illness that in the opinion of the investigator would interfere with completion of follow-up
- Active drug use or dependence that, in the opinion of the study investigator, would interfere with adherence to study requirements
- Any other condition, which in the opinion of the investigator would impede compliance or hinder completion of study
- Inability to provide informed consent

2.2. Calculation of Child-Pugh-Turcotte score

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

1. Serum albumin (g/dL; recorded on the LR form)

	Points
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. International normalized ratio (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. Metabolic syndrome definition

Metabolic syndrome† is defined by three or more of the following:

1. Waist circumference
   40 in (102 cm) in males
   35 in (88 cm) 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

† Detection Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), publication date September 2002. Accessed at http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3_rpt.htm

2.4. 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:

- Unwilling to participate – the participant may be rescreened after 3 months at the discretion of the investigator
- TPN within 6 months of biopsy – the participant may be rescreened if he/she has been off TPN for at least 9 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

2.5. Co-enrollment in FLINT Trial

- When a NAFLD Adult Database 2 patient enrolls into the FLINT Trial, the visit schedule and requirements of the main treatment trial take precedence over the requirements for the NAFLD Adult Database 2. Adult Database 2 requirements are suspended for the duration of the participant's time in the FLINT Trial. The NAFLD Adult Database 2 Database Closeout (CO) form should be completed to suspend the Adult Database 2 visits while the patient is enrolled in the FLINT Trial. The FLINT 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 feasibility study

2.6. Guidelines for transferring NAFLD Adult Database 2 and FLINT patients

Patients enrolled in NAFLD Adult Database 2 who want to enroll in FLINT

- Transferring Adult Database 2 patients into FLINT necessitates that the screening procedures are conducted within the designated eligibility time window (e.g., liver biopsy and complete blood work must be obtained within 90 days prior to randomization in FLINT). Because the eligibility time windows in the NAFLD Adult Database 2 study and FLINT are different, Adult Database 2 procedures may not be compatible for FLINT screening purposes, with the possible exceptions of blood work obtained for the LS form and for DNA.
- Adult Database 2 patients **without** liver biopsy or a liver biopsy obtained ≥1 year prior to FLINT registration are good candidates for screening in FLINT.
- Physician discretion is recommended for Adult Database 2 patients **with** a recent liver biopsy as to whether the patient should register for FLINT; in this scenario, it may be reasonable to wait until the patient has completed their Adult Database 2 annual t048 visit.
- Recent liver biopsies obtained in the Adult Database 2 study and within the FLINT eligibility window (within 90 days prior to randomization) may be used for FLINT screening to determine eligibility.
- Have the patient sign the FLINT consent form.
- Complete and key the FLINT RG form but do NOT issue a new patient ID number and code.
- Blood for serum and plasma repository must be collected even if already banked for NAFLD Adult Database 2.
- Regarding blood for genetics repository:
    - If not already collected, have patient sign the FLINT genetic consent, collect a sample, and complete the FLINT CG form.
    - If blood for genetics testing 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 Adult Database 2 was satisfactory, leave the Adult Database 2 CG form in the data system and complete the FLINT CG answering 'yes' to question about prior blood draw for the Adult Database 2; the patient does not need to sign the FLINT genetic consent.
        - If the yield on the sample drawn when the patient screened for the Adult Database 2 was unsatisfactory, have the patient sign the FLINT genetic consent form and complete the FLINT CG form; the Adult Database 2 CG form should remain in the data system.

2.6. Guidelines for transferring NAFLD Adult Database 2 and FLINT patients

- Lab results reported on the Adult Database 2 LR and LS forms may be used on the FLINT 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, and quality of life) must be completed anew for FLINT.
- The physical exam (PE) form must be completed anew for FLINT.
- If the biopsy used for FLINT is the same one that was used for the NAFLD Adult Database 2, the local pathologist should not review the slides again. A generic document, the Liver Biopsy Histology Worksheet (HW form), should be completed whenever a biopsy is evaluated for any study in NASH CRN. The Clinic Coordinator should transcribe the information from the HW form to the Liver Biopsy Histology Findings (HF) form for FLINT. The HF form will not need to be signed by the Study Pathologist since the signature on the HW form is sufficient as long as the HW form is attached to the FLINT HF form.
- The FLINT SD form must be completed; transcribe information from the Adult Database 2 SD form. For items relating to slide sequence numbers, transcribe the slide numbers for the slides that were previously sent for the Adult Database 2 (i.e., only 10 unstained slides need to be sent from a single biopsy). If liver tissue preservation using RNA *later*® Solution was obtained, the FLINT LT form must be completed; transcribe information from the Adult Database 2 LT form. Where the FLINT LT form asks for the duplicate LT label to be affixed to the LT form, write in the label information from the Adult Database 2 form and write in the margin "see Adult Database 2 LT form". There will be more than 1 form in the data system pointing to the same numbered slides and liver tissue vials (Adult Database 2 SD/LT and FLINT SD/LT forms), but this is okay since the patient enrolled in the Adult Database 2.
- If the patient is eventually randomized in FLINT, have the patient complete FLINT visits and forms; you do not need to complete the MV form for the missed Adult Database 2 visits, but you do need to complete the Adult Database 2 Closeout (CO) form to suspend the patient's participation in the NAFLD Adult Database 2. The CO form can be completed prior to or after randomization in FLINT, but our advice is to complete it upon randomization in FLINT. The patient remains enrolled in NAFLD Adult Database 2 while participating in FLINT, but the patient is not subject to completion of NAFLD Adult Database 2 visits.
- Retain all NAFLD Adult Database 2 forms completed for the patient in the patient's NASH CRN file.
- Retain the patient's Adult Database 2 visit windows schedule since it will be needed once FLINT is completed.

2.6. Guidelines for transferring NAFLD Adult Database 2 and FLINT patients

Patients registered in NAFLD Adult Database 2 but never enrolled, now wants to register in FLINT

- The patient should be closed out of the NAFLD Adult Database 2 by completing and keying the Adult Database 2 EN form to document the reason(s) why the patient did not enroll in the Adult Database 2. Answer as many of the questions in sections B, C, D, E, and F of the form EN as you can, coding an item as 'm' if you do not know the answer; if the patient is eligible for the Adult Database 2 but is opting to go directly into FLINT, answer 'no' to item 22 (no longer consents) and check item 23a and 'Other reason' in item 23c and write in 'opted to go directly into FLINT.'
- Have the patient sign the FLINT consent form.
- Complete and key the FLINT RG form but do NOT issue a new patient ID number and code.
- Blood collected for serum and plasma repository must be collected even if already banked for NAFLD Adult Database 2.
- Blood for genetics repository:
  - If not already collected, have the patient sign the FLINT genetic consent and collect a sample and complete the FLINT CG form.
  - 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 Adult Database 2 was satisfactory, key the Adult Database 2 CG form (if not already keyed) and complete the FLINT CG form answering 'yes' to the question about prior blood draw for the Adult Database 2; the patient does not need to sign the FLINT genetic consent.
    - If the yield on the sample drawn when the patient screened for the Adult Database 2 was unsatisfactory, then have the patient sign the FLINT genetic consent form, draw the replacement sample, and complete the FLINT CG form (the Adult Database 2 CG form can remain in the data system).
- Interviews and questionnaires must be completed on the FLINT forms:
  - If available, data from the Adult Database 2 AD and LD forms may be transcribed to the corresponding FLINT forms, but the patient should be queried regarding any changes since the previous interviews; the date in item 4 on each FLINT form should be the date you review the information with the patient.
  - The FLINT BG form should be completed anew– it is different from the Adult Database 2 BG form.
  - The patient should complete the FLINT QF anew.
- The physical exam (PE) form must be completed anew.

2.6. Guidelines for transferring NAFLD Adult Database 2 and FLINT patients

- If the same biopsy is used for FLINT that was used for the Adult Database 2, the local pathologist should not review the slides again. A generic document, the Liver Biopsy Histology Worksheet (HW form) should be completed whenever a biopsy is evaluated for any study in NASH CRN. The Clinic Coordinator should transcribe the information from the HW form to the Liver Biopsy Histology Findings (HF) form for FLINT. The HF form will not need to be signed by the Study Pathologist since the signature on the HW form is sufficient as long as the HW form is attached to the FLINT HF form.
- The FLINT SD form needs to be completed; transcribe information from the Adult Database 2 SD. For items relating to slide sequence numbers, transcribe the slide numbers that were previously sent for the Adult Database 2 (i.e., only 10 unstained slides need to be sent from a single biopsy). If liver tissue preservation using RNA*later*® Solution was obtained, the FLINT LT form must be completed; transcribe information from the Adult Database 2 LT form. Where the FLINT form asks for the duplicate LT label to be pasted onto the LT form, write in the label information from the Adult Database 2 LT form and write in the margin 'see Adult Database 2 LT form'. The Adult Database 2 SD and LT forms can remain in the data system.
- Retain all Adult Database 2 forms completed for the patient in the patient's NASH CRN file.

Patient registered in FLINT, but found to be ineligible, now wants to register in the NAFLD Adult Database 2

- The patient should be closed out of FLINT by completing and keying the FLINT RZ form to document the reason(s) the patient was found to be ineligible.
- Have the patient sign the Adult Database 2 consent form.
- Complete and key the Adult Database 2 RG form but do NOT issue a new patient ID and code.
- Blood collected for serum and plasma can be used if the patient is ineligible for FLINT and opts to enroll in the Adult Database 2 study.
- Blood for genetics repository:
  - If blood was not already collected, have the patient sign the Adult Database 2 genetic consent, collect a sample, and complete the Adult Database 2 CG form.
  - If blood was already collected, do not send another sample unless the yield was unsatisfactory, but the CG form must still be completed.
- Interviews and questionnaires must be completed on the Adult Database 2 forms.

2.6. Guidelines for transferring NAFLD Adult Database 2 and FLINT patients

- If the same biopsy is used for the Adult Database 2 that was used for FLINT, the local pathologist should not review the slides again. A generic document, the Liver Biopsy Histology Worksheet (HW form) should be completed whenever a biopsy is evaluated for any study in NASH CRN. The Clinic Coordinator should transcribe the information from the HW form to the Liver Biopsy Histology Findings (HF) form for the Adult Database 2. The HF form will not need to be signed by the Study Pathologist since the signature on the form is sufficient as long as the HW form is attached to the Adult Database 2 HF form.
- If slides were previously sent for FLINT, the Adult Database 2 SD form must be completed referencing the slide numbers for the slides that were sent (i.e., only 10 unstained slides need to be sent from a single biopsy). If liver tissue preservation using RNA*later*® Solution was obtained, the Adult Database 2 LT form must be completed. Where the Adult Database 2 LT form asks for the duplicate LT label to be affixed to the LT form, write in the label information from the FLINT LT form and write in the margin 'see FLINT LT form'. The FLINT SD and LT forms can remain in the data system.
- Retain the FLINT forms in the patient's NASH CRN file.

These procedures are complicated. Please contact the DCC if you have questions or if you run into problems when trying to key forms or enroll/randomize a patient.

2.7. Procedures for patients who complete FLINT and return to NAFLD Adult Database 2

- Patients who complete participation in FLINT should resume participation in the NAFLD Adult Database 2 (if previously enrolled in the NAFLD Adult Database 2) or be invited to join the NAFLD Adult Database 2 (if not previously enrolled). A FLINT Closeout (CO) form should be completed at the F096 visit (or at the close of the F096 visit window) for all patients randomized in the FLINT.

- If the patient was previously enrolled in the NAFLD Adult Database 2, the patient resumes participation in the Adult Database 2 by completing the visit that is open on the patient's Adult Database 2 visit time windows guide.

- If the patient was not previously enrolled in the NAFLD Adult Database 2, the patient will receive a new visit schedule upon the keying of the FLINT Closeout (CO) form into the database. This new visit schedule will use the FLINT randomization date as the effective enrollment date into NAFLD Adult Database 2.

2.8. 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 Pat Belt); 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 Adult Database 2 staff
  - Each clinical center
- Certification for the NAFLD Adult Database 2 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 role and it is recommended that more than one staff member be certified for a role

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 Adult Database 2 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 Adult Database 2 procedures will constitute the initial round of NASH CRN certification activities. As other studies 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 Adult Database 2 must be certified for that participation
- Completion of the Clinical Center Certification (CC) form will be required
- IRB approval for the NAFLD Adult Database 2 protocol and consents will be required

Purpose of clinical center certification

- Provide information regarding how the clinical center will conduct different aspects of the protocol, and who will staff the study
- Guide a clinical center through the steps of getting ready for the NAFLD Adult Database 2– 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 role that requires certification (a person may be certified for more than one role)
- Obtain IRB approval of the most current NAFLD Adult Database 2 protocol and consent documents
- Receive written notice of approval (email) from the Data Coordinating Center that the site is certified
- Provide assurances that the study participants' protected health information will be kept confidential
- Provide assurances that the linkable information will not be transmitted to the DCC

3.3. Personnel certification

Staff functions requiring certification

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

Requirements

- Everyone
  - Read the NAFLD Adult Database 2 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
  - Study Physician must be an MD preferably a hepatologist
- Additional requirements for Pathologist
  - Be approved by David Kleiner and Cynthia Behling (only at parent clinics; satellite centers will use study pathologist of the parent clinic)
- 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 Adult Database 2 data system
- Staff can be certified for more than one role but will have only one PIN

4. Human subjects

4.1. Background

Consent to participation in the NAFLD Adult Database 2 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 Adult Database 2 consent process has three major stages:

- The patient is asked to consent to screening and enrollment into the NAFLD Adult Database 2
- The patient is asked to consent 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

Two prototype consent statements have been prepared for the NAFLD Adult Database 2:

- Consent for screening and enrollment in the NAFLD Adult Database 2
- Consent for the collection, storage, and use of blood samples for current and future genetic research

Clinics are expected to use these materials in their submissions to their institutional review boards (IRBs) for approval to participate in the NAFLD Adult Database 2. 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 Adult Database 2. Data Coordinating Center staff will review and compare the approved local consents to the prototype consents. 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 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 Adult Database 2 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 Adult Database 2 consents

It is assumed that patients referred to a clinical center for screening have heard about the NAFLD Adult Database 2, 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 involves two tasks:

(1) A NAFLD Adult Database 2 staff member must sit down with the patient and review the contents of the statement; explain the risks, benefits, and responsibilities of participation; review the alternatives to participation; and answer questions.

(2) A NAFLD Adult Database 2 certified study physician (i.e., a NAFLD Adult Database 2 certified adult hepatologist) 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 is one who has a broad role in the study.

Generally, the consent 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 Adult Database 2 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 Adult Database 2 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 Adult Database 2 consent is administered, except that it should not be signed until the patient has been determined to be eligible for the NAFLD Adult Database 2.

4.4. Time considerations for obtaining consent

- **The NAFLD Adult Database 2 Consent and HIPAA authorization** must be obtained at the start of the initial visit t0; 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 Adult Database 2 diagnostic tests. A check for signature of this consent statement occurs on the Registration (RG) form.

- **The NAFLD Adult Database 2 Consent for Collection, Storage, and Use of Blood Samples for Current and Future Genetic Research** must be obtained after eligibility for the NAFLD Adult Database 2 has been established. Signature of this consent is required prior to drawing blood for genetic research for the NAFLD Adult Database 2; a check for signature of this consent statement on the Genetic Consent and Blood Collection Documentation (CG) form. Signature of this consent statement is not required for NAFLD Adult Database 2 eligibility (i.e., the patient may choose not to participate in the genetic research component of the NAFLD Adult Database 2).

- A patient may be given the consent statements to review prior to the initiation of visit t0 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 Adult Database 2 staff member. The consents may be mailed to the patient prior to visit t0. Whatever timing is used by a clinic, the patient should be allowed enough time to reflect about the proposed NAFLD Adult Database 2 procedures, pose questions, and consult with other individuals that he/she considers relevant to their participation in the NAFLD Adult Database 2. 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 statements are important legal documents. These signed statements should be kept in the patient's NAFLD Adult Database 2 clinical center file together with his/her other NAFLD Adult Database 2 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 Adult Database 2. Consent statements will be examined during site visits.

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

- The NAFLD Adult Database 2 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 Adult Database 2.

- The NAFLD Adult Database 2 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 Adult Database 2.

4.6. Informing participants of changes to consent statement after enrollment

As new data become available during the conduct of the NAFLD Adult Database 2, 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. HIPAA considerations

NAFLD Adult Database 2 study clinical center 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 Adult Database 2 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. Linkable information should not be transmitted to the DCC nor any other outside institution.

People outside the clinical center who will receive NAFLD Adult Database 2 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 Adult Database 2 data for performance and safety
- The NIDDK Genetics Repository at Rutgers, the State University of New Jersey in Piscataway, 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 Precision for Medicine in Frederick, 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

4.7. HIPAA considerations

- The NASH CRN investigators, as well as outside researchers, to analyze and report NAFLD Adult Database 2 study data. Patient identity will not be disclosed in any reports or publications resulting from the study. While the NAFLD Adult Database 2 is ongoing, the use of the NAFLD Adult Database 2 study data must be approved by the NASH CRN Steering Committee and by the research ethics committee at your institution.

Patient agreement to join the NAFLD Adult Database 2 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 Adult Database 2. 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 Adult Database 2.

5. Study visits

5.1. Overview of visit schedule

Screening (must be completed within 90 days of starting)

- t0: Consent, baseline history, physical exam, liver biopsy (if available), questionnaires on alcohol use, beverage intake, blood draw for laboratory measures (hematalogy, chemistries, HbA1c, liver panel, lipids, and glucose and insulin), and additional laboratory measures for new patients (hepatitis B, hepatitis C, iron, HFE gene analysis, ceruloplasmin, alpha-1 antitrypsin, autoantibody studies, and TSH) if not available from archival records, blood draw specimen banking, and genetic research; and liver imaging studies as needed

Enrollment

- t0: Enrollment is an event, not a visit; enrollment occurs when the clinical center staff run the enrollment task on the NAFLD Adult Database 2 data system and the patient is found to be eligible

Follow-up

- t048: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

- t096: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

- t144: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

- t192: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

- t240: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

- t288: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

5.1. Overview of visit schedule

- t336: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

- t384: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

- t432: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

- t480: Followup medical history, physical examination, beverage intake, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

5.2. Visits, data forms, and procedures

Phase/Visit	Form abbr	Procedure
Screening		
t0	RG	Registration (document consent, sociodemographics, assign Ids)
	PL	Patient location (patient contact information)
	BG	Baseline history
	PE	Physical examination (detailed exam)
	SD	Liver biopsy materials documentation (if liver biopsy is reported on form BG; required for new patients)
	HF	Liver biopsy histology findings (reading at clinical center; if SD form says biopsy is adequate for scoring; required for new patients)
	LT	Liver tissue banking (if SD form says liver tissue was obtained for banking)
	IR	Liver imaging studies report (if imaging study is reported on BG form)
	AD	AUDIT (alcohol questionnaire)
	LD	Lifetime drinking history (Skinner; new patients only)
	BQ	Beverage questionnaire (self-administered by patient)
	LS	Lab tests done only during screening (etiologic tests)
	LR	Lab tests done during screening and followup (hematology, hepatic, clinical chemistry, HbA1c if needed)
	CG	Genetic consent and blood collection documentation
	BP	Blood processing for serum and plasma
	EN	NAFLD Adult Database 2 enrollment
Followup phase		
48 week followup visit		
t048	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)

Phase/Visit	Form abbr	Procedure
	BQ	Beverage questionnaire (self-administered by patient)
	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)
96 week followup visit		
t096		Same as 48 week followup visit
144 week followup visit		
t144		Same as 48 week followup visit
192 week followup visit		
t192		Same as 48 week followup visit
240 week followup visit		
t240		Same as 48 week followup visit
288 week followup visit		
t288		Same as 48 week followup visit
336 week followup visit		
t336		Same as 48 week followup visit
384 week followup visit		
t384		Same as 48 week followup visit
432 week followup visit		
t432		Same as 48 week followup visit
480 week followup visit		
t480		Same as 48 week followup visit

5.2. Visits, data forms, and procedures

Phase/Visit	Form abbr	Procedure
As needed		
	FI	Family member identification (patient's relative(s) enrolled in NAFLD Adult Database 2)
	CO	Database closeout (if transferring to a new study)

5.3. Guide for screening visit

Procedures

- Obtain signed consent for the NAFLD Adult Database 2
- 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 for new patients)
  - Fasting blood draw for serum and plasma banking (must be within 90 days of the biopsy, but cannot be collected in the 4 days immediately following the biopsy)
  - Liver biopsy (pathologist should grade slides from most recent biopsy and obtain 10 unstained slides for the 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
- Schedule standard of care biopsy if needed
- Laboratory tests (hematology, hepatic clinical chemistry, HbA1c, fasting lipid profile, fasting glucose and insulin) if needed
- Obtain patient location information
- Schedule additional screening visit if needed

Data collection forms

- Forms completed for all patients
  - RG - Registration
  - PE - Physical Examination
  - BG - Baseline History
  - BP - Blood Processing for Plasma and Serum
  - CG - Genetic Consent and Blood Collection Documentation
  - AD - Alcohol Use Disorders Identification Test (AUDIT)
  - BQ - Beverage Questionnaire – Screening and Follow-up
  - LR - Laboratory Results – Screening and Follow-up
  - LS - Laboratory Results – Tests Done Only During Screening
  - EN - Database 2 Enrollment
- Additional forms required under specific conditions
  - SD - Liver Biopsy Materials Documentation (if biopsy is reported on Form BG; required for new patients)
  - HF - Liver Biopsy Histology Findings (if liver biopsy is available and can be scored; required for new patients)

  - LT - Liver Tissue Banking (if liver tissue was obtained for banking)
  - LD - Lifetime Drinking History (Skinner) (for new patients only)

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 Adult Database 2 chart for patient and file the materials generated at registration
- 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
- 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
- Run Enrollment Task and generate enrollment materials (appointment schedule for followup visits)

5.4. Visit windows: enrollment and followup

- **Enrollment** must occur within 90 days of initiating screening
- **t048**: window runs from 24 weeks through 72 weeks (168-504 days), must be at least 24 weeks after enrollment; ideal date is 48 weeks (336 days) after enrollment date
- **t096**: window runs from 72 weeks through 120 weeks (505-840 days), must be at least 24 weeks after t048; ideal date is 96 weeks (672 days) after enrollment date
- **t144**: window runs from 120 weeks through 168 weeks (841-1176 days), must be at least 24 weeks after t096; ideal date is 144 weeks (1008 days) after enrollment date
- **t192**: window runs from 168 weeks through 216 weeks (1177-1512 days), must be at least 24 weeks after t144; ideal date is 192 weeks (1344 days) after enrollment date
- **t240**: window runs from 288 weeks through 264 weeks (1513-1848 days), must be at least 24 weeks after t192; ideal date is 240 weeks (1680 days) after enrollment date
- **t288**: window runs from 264 weeks through 312 weeks (1849-2184 days), must be at least 24 weeks after t240; ideal date is 288 weeks (2016 days) after enrollment date
- **t336**: window runs from 312 weeks through 360 weeks (2185-2520 days), must be at least 24 weeks after t288; ideal date is 336 weeks (2352 days) after enrollment date
- **t384**: window runs from 360 weeks through 408 weeks (2521-2856 days), must be at least 24 weeks after t336; ideal date is 384 weeks (2688 days) after enrollment date
- **t432**: window runs from 408 weeks through 456 weeks (2857-3192 days), must be at least 24 weeks after t384; ideal date is 432 weeks (3024 days) after enrollment date
- **t480**: window runs from 456 weeks through 504 weeks (3193-3528 days), must be at least 24 weeks after t432; ideal date is 480 weeks (3360 days) after enrollment date

5.5. 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 Adult Database 2 patient between scheduled NAFLD Adult Database 2 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 t048 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

- Screening for eligibility evaluation visit (visit t0)

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 (new patients only; continuing patients use the ID number that was previously assigned to him/her).
- 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 2 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 all Adult Database 2 patients
- 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
  - Menstrual history (female patients)
  - Medical history (answer items based on information from all sources available to you)
  - Medication use currently and in the past 3 months
- Flash Card # 11, Weight Pattern over Past 5 Years, is used with Form BG

When

- Visit t0

How

- Mix of interview data and data obtained by chart review
- The smoking interview should be an interview with the patient
- Other questions on the BG form can be answered by interview with the patient, 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 Baseline History (BG) and Follow-up Medical History (HI) forms

- 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 the BG form. 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 all Adult Database 2 patients
- Study Physician and Clinical Coordinator sign the form

What

- The form queries/reviews
  - Alcohol consumption since the last visit (AUDIT-C)
  - Tobacco cigarette smoking since the last visit
  - Medical history diagnoses and procedures since the last visit
  - Medication use since the last visit

When

- Visits t048, t096, t144, t192, t240, t288, t336, t384, t432, and t480

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 patient's partner
- Other questions on the HI form can be answered by interview with the patient, 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) form

Who

- All Adult Database 2 patients

What

- Anthropometry
  - Height
  - Weight
  - Waist circumference
  - Hip circumference
- Vital signs
  - Temperature
  - Blood pressure
  - Resting radial pulse
  - Respiratory rate
- System review
  - Acanthosis nigricans
  - Abdomen abnormalities
  - Focused liver signs
- Note: Tanner staging is not required

When

- Visits t0, t048, t096, t144, t192, t240, t288, t336, t384, t432, and t480

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 67020, 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 and weight measurement

Height

- 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

Weight

- 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)

6.6. Waist and hip circumference measurement

Waist

- 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

Hip

- 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

6.6. Waist and hip circumference measurement

- 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

6.7. Liver biopsy

- Details of liver biopsy procedures, tissue preservation using RNA*later*Solution®, slide preparation, and shipment of slides to the DCC are discussed in the SOP Part IV, Liver Biopsy and NAFLD Histology Scoring System document

6.8. Liver imaging studies (IR form)

Who/What

- Patients who have had a hepatic imaging examination (ultrasound, computerized tomography (CT), or magnetic resonance imaging (MRI) scan) within 6 months of screening (visit t0) or following enrollment (as part of standard of care)

Form

- Liver Imaging Studies Report (IR) form
- Report results of most recent scan of each type done 6 month prior to screening (visit t0) or in the period since the last study visit.

When

- Visit t0 if a liver imaging study is reported on the Baseline Medical History (BG) form
- Visits t048, t096, t0144, t192, t240, t288, t336, t384, t432, and t480 if a liver imaging study is reported on the Followup Medical History (HI) form

Comments

- Scan findings suggestive of NAFLD: fatty infiltration
- Scan findings suggestive of NASH -related cirrhosis: cirrhosis, ascites
- Scan findings suggestive of hepatic tumors: hepatic mass, hepatic cysts

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

What

- Alcohol Use Disorders Identification Test (AUDIT) (AD) form
- Skinner Lifetime Drinking History (LD) form
- Summary question on Enrollment (EN) form
- Questions on interval alcohol consumption on Follow-up Medical History (HI) form
- Flash Card #9, Drink Equivalents, can be used with the alcohol questionnaires
- Flash Card #10, 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 NASH-related cirrhosis
- Monitor alcohol use during follow-up

Who

- AD form: All Adult Database 2 patients
- LD form: Required for new patients only

How

- AD form is self-administered
- LD form is interviewer administered

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 (EN) 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

6.10. Laboratory measures (LS and LR forms)

Who

- LS form: Required for new patients and optional for continuing patients (but form must still be completed and keyed)
- LR form: All Adult Database 2 patients

What

- LS form covers assessments collected only at screening:
  - Screening etiologic tests
  - Iron assessments
  - HFE gene analysis
  - Ceruloplasmin measurement (required for patients 40 years or younger and, if available, for patients 40 years and older)
  - Alpha-1 antitrypsin assessment
  - Autoantibody studies
  - Immunoglobulin levels
  - Thyroid stimulating hormone

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

When

- LS form: Visit t0
- LR form: Visit t0 and annually thereafter (t048, t096, t144, t192, t240, t288, t336, t384, t432, and t480)
- Requirements for fasting – nothing by mouth except water for at least 12 hours before blood draw

Instructions for LS form

- Most of the tests on LS form 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
- Iron overload screening is required; hepatic iron index is recorded if available, but 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 18 through 40; and record, if available, for patients greater than 40 years old
- Alpha-1 antitrypsin assessment is required
- Autoantibody studies are required
- Immunoglobulin levels (IgA, IgG, IgM) are recorded if available
- Thyroid stimulating hormone is required

Instructions for LR Form

- The measures on LR form can also be obtained by chart review, both at screening and during follow-up; the time window for each type of assessment is specified on the form
- During follow-up, the time window for the assessment is "in the time window for the followup visit (check the patient's Visit time window guide)" – eg, t048 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 t048, you do not need to order another hematology at t048
- 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
- For baseline, the required time window is within 3 months of the screening visit date

6.11. Plasma and serum collection for Biosample Repository (BP form)

Purpose

- Collection of whole blood from NAFLD Adult Database 2 patients; when timed to coincide with a liver biopsy, blood should be collected ideally within ± 7 days and up to ± 90 days of the biopsy. Blood should not be collected in the 72 hour period after a biopsy
- Separation of plasma and serum at clinical center: Screening visit: ten 0.5 mL aliquots of plasma and thirty 0.5 mL aliquots of serum are to be obtained in 2.0 mL cryogenic vials. Follow-up visits: ten 0.5 mL aliquots of plasma and twenty 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 Precision for Medicine

Fasting Instructions

- Patient instructed to fast 12 hours (recommended) prior to blood draw; an 8-hour fast prior to blood draw is allowable

Forms / Materials

- BP - Blood Processing for Plasma and Serum
- Labels for heparin (green top) tube and serum separator SST tubes (red top)
- Labels for plasma and serum cryovials
- Barcode scanner
- SS - Specimen Shipment log and Excel Spreadsheet
- NIDDK Biosample Repository shipper

When

- Visit t0
- Annual followup visits (ie, t048, t096, t144, t192, t240, t288, t336, t384, t432, and t480)
- Batch shipments: Monthly or semi-monthly

By whom

- Phlebotomist
- Clinical Coordinator
- Person responsible for shipping to NIDDK Biosample Repository must have formal, documented training to package and ship hazardous goods, per DOT/IATA guidelines

6.11. Plasma and serum collection for Biosample Repository (BP form)

Equipment

Blood tubes/aliquot vials

- One 10 mL sodium heparin (green top) tube - *provided by clinical center*s
- Two to three 10 mL SST (red top) tubes - *provided by clinical centers*
- Up to forty 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 (10 mL heparin 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 10 mL heparin (green top) tube for plasma
- During visit t0 collect whole blood into three 10 mL SST (red top) tubes for serum.
- During follow-up visits, collect whole blood into two 10 mL SST tubes for serum.
- If sample appears to have hemolyzed, do not aliquot. Re-draw blood

Plasma

- Collect blood into heparin (green top; Becton-Dickinson) tube. Ensure that heparin 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 10 labeled 2.0 mL cryovials

- Freeze at -70° C immediately
- Processing of plasma should be completed within 30 minutes

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 20-30 labeled 2.0 mL cryovials
- Freeze at -70° C immediately
- Processing of serum should be completed within two hours

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 heparin plasma and the SST serum to the BP form
- Affix aliquot 00 cryovial labels to the BP form

Packaging Procedures

- Check that 1 absorbent pad is in the Saf T Pack Bio hazard plastic bag
- Insert frozen cryovial into small cardboard boxes with dividers. Place only one tube into each cardboard cell. Each cardboard box may hold 81 cryovials
- 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 envelope 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 the styrofoam cooler
- Place cardboard box in upright position in bottom of styrofoam cooler
- Surround the STP-111 inner brown cardboard box with abut 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

6.11. Plasma and serum collection for Biosample Repository (BP form)

- Place the "Empty Packaging" cover and shipping form, with Excel spreadsheet on the top of the cooler lid
- Place a completed Specimen Shipment Log (Form SS) on top of the cooler lid
- Close and seal outer cardboard box with tape

Labeling Shipper:

- Place a checkmark in the block on the outer cardboard box next to "BIOLOGICAL SUBSTANCE, CATEGORY B". Do not cover this marking with labels.
- Affix a label with your name and return address to the side of the box in the "Shipper:" block
- Affix the repository address label to the side of the box in the "Consignee:" block
- Affix the dry ice label below the repository address label. Enter the weight of dry ice on the label in kilograms
- Affix the "UN3373 BIOLOGICAL SUBSTANCE, CATEGORY B" label to the right of the dry ice label
- Use the preprinted Federal Express air bill to ship specimens to the 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 entry "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 adjacent to the labeled side. 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

  Do not ship frozen packages on Friday; the repository is closed on weekends

Shipping samples to the NIDDK Biosample Repository

- Specimens are to be batch-shipped monthly to Precision for Medicine on Monday, Tuesday, or Wednesday
- 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)
- Open the template Excel file used for shipments and scan each cryovial using the barcode scanner provided to your clinical center. The file should have the filename: NASHCRNsiteXXX_shipdate.xls. Replace the 'xxx' with your clinical center three digit site ID and replace 'shipdate' with the date of shipment.
- The Excel shipping file has column headings for barcode number, Site ID - Patient ID code, date collected, specimen type: plasma, sera, liver tissue, volume, units of measure, study number and visit code
- Record the Federal Express Airbill tracking number, at top of page 3, of the Specimen Shipping Log.

6.11. Plasma and serum collection for Biosample Repository (BP form)

- Complete Section A. *Center ID, shipment and study information* and section B. *Clinical Administrative information* of the Specimen Shipment Log (SS). Enclose a printed copy of the Specimen Shipment Log and the Excel spreadsheet with each shipment of specimens.
- Keep a notebook of all original completed Specimen Shipment Logs (Form SS) and Excel spreadsheet so that you have a record of all shipments to the NIDDK Biosample Repository
- Notify the NIDDK Biosample Repository of the shipment via email: niddk.mailbox@precisionformedicine.com and eduard.chani@precisionformedicine.com on the day the package is picked up by Federal Express. Include the tracking number in the subject line of the email, and attach the Excel shipment file.

6.12. Whole blood collection for Genetics Repository (CG form)

Purpose

- Collection of whole blood from NAFLD Adult Database 2 patients who consent for genetic research (new patients only unless original yield was low)
- Shipment of whole blood to the NIDDK Genetics Repository at Rutgers University for DNA banking

Forms

- NAFLD Adult Database 2 consent for genetic research
- Genetic Consent and Blood collection Documentation (CG) form
- NIDDK Genetics Blood Collection form

When

- Visit t0 (or any time during follow-up)
- 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 must have formal, documented training to package and ship hazardous goods, per DOT/IATA guidelines

Equipment

- One 10 mL NaEDTA vacutainer tube (purple top) - *provided by NIDDK Genetics Repository*
- Preprinted whole blood tube label and form CG label - *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
  - Two 18" strips of red waterproof tape
  - One press-lock plastic bag
  - One corrugated shipping carton with locking tabs
  - One pre-printed Fed Ex airbill with third party billing
  - One NIDDK Genetics Blood Collection form
  - Instructions for Blood Sample Collection form

6.12. Whole blood collection for Genetics Repository (CG form)

Blood collection procedures

- Affix MACO tube label onto the tube and avoid covering the barcode label
- Collect blood into one 10 mL NaEDTA (purple top) tubes
- Invert tube gently 6 times to mix blood with additives
- Keep blood at room temperature
- Check that patient ID information on preprinted tube label matches information recorded onto the NIDDK Genetics Blood Collection form

Packaging procedures

- Ship whole blood at ambient room temperature same day to the NIDDK Genetics Repository
- Package the whole blood tube in the body of the Safety Mailer (Model 472 Thermosafe Safety Mailer)
- Place absorbent material 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 Blood Collection 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.rutgers.edu
    peralta@biology.rutgers.edu
  - Fax: 1-732-445-1149
  - Telephone: 1-732-445-1498

6.12. Whole blood collection for Genetics Repository (CG form)

- 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.

6.13. Adverse event reporting (IE form)

Definitions

- **Adverse event** is defined as any unfavorable sign, symptom, state, condition, or laboratory finding in a NAFLD Adult Database 2 patient. Adverse events may result from appropriate application of the protocol in relation to the processes of enrolling, studying, or following NAFLD Adult Database 2 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 NAFLD Adult Database 2 protocol.

Reportable Adult Database 2 events

- Any serious and unexpected adverse event thought to be associated with a NAFLD Adult Database 2 procedure is reportable to the NAFLD Adult Database 2 Study.
- Any event threatening the integrity study of the NAFLD Adult Database 2 Study (eg, suspected fraud) or the well-being of a study participant is a reportable NAFLD Adult Database 2 Study event. We recognize that this category is not well-defined; however, it is included as a reminder that reportable unanticipated events can have a broader scope than anticipated events that happen to a patient.
- Deciding whether an event is reportable to the NAFLD Adult Database 2 Study (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 Interim Event Report (IE) 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.

6.13. Adverse event reporting (IE form)

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 Adult Database 2 Study. Regardless of what the Adult Database 2 Study requires, you must continue to meet your local IRB's requirements. If the local requirements are more stringent than the Adult Database 2 Study, you may report events locally that you do not report to Adult Database 2 Study.

- Since the NAFLD Adult Database 2 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 NAFLD Adult Database 2, it is likely that some will develop significant liver-related morbidity or mortality during the course of follow-up. While this information is important and should be documented on the Follow-up Medical History (HI) form, it would only be considered a reportable adverse event if it is related to the study in some way.

6.14. Procedures for missed or incomplete visits (MV form)

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 follow-up visit or for any follow-up 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

6.15. Procedures for patients lost to follow-up

Purpose

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

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 or evening hours
  - 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

6.16. Procedures for mortality closeout (DR form)

Purpose

- Record participant death

Forms

- Complete the Death Report (DR) form

By whom

- Study Physician and Clinical Coordinator

6.17. Medical management of patients

Since the NAFLD Adult Database 2 is an observational study, it will not be the role of study investigators to prescribe or prohibit use of medications as part of this research study. Nevertheless, to keep recommendations and care for participants in the study as uniform as possible, investigators should generally discuss with the participants what is laid out in the SOP Part V: Standard of Care for Adult Patients with Fatty Liver Disorders.

6.18. Beverage Questionnaire (BQ form)

Who/What

- Beverage Questionnaire (BQ)
- Patients age 18 years and older; self-administered
- The BQ form should be completed by parent for patients unable to complete the form

Purpose

- To determine patent's beverage intake
- To assess the frequency and quantity consumed for each beverage listed

When

- Screening Visit: t0
- Annual follow-up visits: t048, t096, t144, t192, t240, t288, t336, t384, t432, and t480

Procedures

- Provide the patient and/or parent or guardian with a pencil. Circle the number that best indicates how often each beverage was consumed in the past month, and how much of each beverage was consumed in the past month
- The BQ form is designed to be self-administered by the patient. If the patient is unable, the parent or guardian may complete the form with patient assistance
- Before giving the BQ form to the patient, the Clinical Coordinator must complete section A and affix the Beverage Questionnaire ID label in the designated area located at the right top corner of page 2
- The Clinical Coordinator should review the completed questionnaire for missing responses and resolve any problems/discrepancies before the patient leaves the clinical center.
- The Clinical Coordinator should reattach Page 1 to Page 2 and complete section C

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

Cleveland Clinic Foundation	CCF
Duke University	DUKE
Indiana University	IU
Saint Louis University	SLU
University of California, San Diego	UCSD
University of California, San Francisco	UCSF
Swedish Medical Center	SMC
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 (satellite centers should use site ID of parent clinic)

Cleveland Clinic Foundation	220
Duke University	222
Indiana University	221
Saint Louis University	223
University of California, San Diego	224
University of California, San Francisco	225
Swedish Medical Center	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

Cleveland Clinic Foundation	CCF	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
Swedish Medical Center	SMC	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

t0	Screening, baseline data collection, and enrollment
t048	48 weeks followup visit (approximately 1 year)
t096	96 weeks followup visit (approximately 2 years)
t144	144 weeks followup visit (approximately 3 years)
t192	192 weeks followup visit (approximately 4 years)
t240	240 weeks followup visit (approximately 5 years)
t288	288 weeks followup visit (approximately 6 years)
t336	336 weeks followup visit (approximately 7 years)
t384	384 weeks followup visit (approximately 8 years)
t432	432 weeks followup visit (approximately 9 years)
t480	480 weeks followup visit (approximately 10 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 t0 visit code would be completed and keyed as "t0 ").
- 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 2 Study 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 2 Patient ID: __ __ __ __

Form RG
Revision 1 (26 Oct 09) 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 2 6

- 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. Participants cannot be enrolled until all '?'s keyed on the forms have been resolved.

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

It is the standard of practice with NIH funded studies to certify study physicians who assume responsibility for the accuracy and integrity of the sponsored studies. Coordinators are certified separately based on their professional qualifications and privileges even though the functions fulfilled may overlap functions of the study physician.

On the NAFLD Database 2 data collection forms that require the Physician's signature, the signature is the assurance as the clinical center's principal investigator, that they are assuming responsibility for the accuracy of the data recorded on the study form. This does not require that the study physician completes the forms or performs the procedures, but does require assumption of responsibility signified by signing the NAFLD Database 2 forms. This is also the standard of practice required by the FDA for case-report forms completion.

7.11. Handling forms

Form duplication

- The individual forms and form sets specific to a particular visit are available on the NASH CRN website
- You can print master copies from the website 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 Adult Database 2 study should be kept in a single folder in a locked room or locked filing cabinet.
- Each patient who is enrolled in the NAFLD Adult Database 2 study 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 Adult Database 2 study 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 Adult Database 2 study, apply the rounding rule only at the last step, when required to record a quantity on the NAFLD Adult Database 2 study 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 Adult Database 2
- 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 forms for all participants including the date and signature of a witness
- Documents including NAFLD Adult Database 2 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. Representatives from the NIDDK or other clinical centers 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 forms and protocol

- Documents
  - SOPs
  - Protocol

- Enrollment and retention
  - Status
  - Recruitment and retention strategies
  - Problems
  - Losses to follow-up

- 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 follow-up
  - Data audit follow-up

Site visit follow-up

- 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 follow-up 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 recruitment and retention reports that will provide a count of participants enrolled at each clinical center

- On approximately a monthly 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 Adult Database 2 study 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.

8.3. Data quality surveillance

Forms audits

On a periodic 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 Adult Database 2 centers
- Discrepancy rates over time by clinical center are reported to the Steering Committee

NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

NAFLD Database 2
Standard Operating Procedures
Part IV:

Liver Biopsy
and
Histology Scoring System

06 April 2018

NASH CRN
NAFLD Database 2
Standard Operating Procedures - Part IV:
Liver Biopsy and Histology Scoring System

Contents

NAFLD Database 2 SOP Part IV: Liver Biopsy

1. Overview

1.1. Philosophy

Nonalcoholic fatty liver disease (NAFLD) is defined by hepatic macrovesicular steatosis $\geq$ 5% in the absence of known causes of liver disease and significant alcohol consumption. Nonalcoholic steatohepatitis (NASH) is a subset of NAFLD defined histologically by $\geq$ 5% steatosis, lobular inflammation, and distinctive pattern of zone 3 injury characterized by hepatocellular ballooning; Mallory's hyaline (Mallory-Denk bodies) 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. An adult trial and a pediatric treatment trial have been completed. A database of 1,635 patients is present from the first phase of the NASH CRN. This document specifies the procedures for liver biopsy and histology scoring for the NAFLD Database 2.

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 are 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 2 will obtain a piece of liver tissue for preserving in RNA*later*® Solution and 10 unstained slides for archiving from each biopsy evaluated for the NAFLD Database 2. However, because some of the biopsies evaluated for the NAFLD Database 2 will not provide these materials (e.g., 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 2 pathologist to be adequate for scoring for the slides to be used to satisfy NAFLD Database 2 liver histology criteria. A copy of the surgical pathology report must, however, be obtained for all slides that will be subsequently provided to the DCC. 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 done before screening and occurrence of liver biopsy during screening are queried on the Baseline History (BG) form. After enrollment in the NAFLD Database 2, biopsies should occur prospectively under the care of the NAFLD Database 2 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 2 visit. However, as a check to be sure that all biopsies on patients in Database 2 are procured, occurrence of a biopsy since the previous NAFLD Database 2 visit is queried on the Follow-up Medical History (HI) form.

The Liver Biopsy Materials Documentation (SD) form must be completed to document the outcome of all biopsies obtained for the NAFLD Database 2 (screening and follow-up) 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 prior to or during screening, then the local NAFLD Database 2 Study Pathologist must complete the Liver Biopsy Histology Worksheet (HW) form. After the HW form is completed by the Study Pathologist, the Clinical Coordinator will transcribe the data to the appropriate Liver Biopsy Histology Findings (HF) form.

Other forms that the NAFLD Database 2 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 seven forms (SD, LT, HW, HF, CR, TS, SS) are used to:

- Document what slides are available for scoring and sending to the DCC and whether liver tissue was obtained for banking (form SD)
- If liver tissue was obtained for banking for a biopsy done during screening or followup, document collection of tissue and procedures for banking (form LT)
- Document local scoring of a biopsy done for screening or followup (forms HW and HF)
- Document central scoring of biopsies (form CR)
- Document shipment of slides to the DCC (form TS)
- Document shipment of liver tissue in RNA*later*® Solution to the Biosample Repository (form SS)

The adult hepatologist and his/her pediatric counterpart, the Study Pathologist, and the Clinical Coordinator must work together to accomplish these tasks and each complete their sections of 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

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 in the NAFLD Database 2 has been obtained. In the case of (1), we will try to obtain 10 unstained slides for NAFLD Database 2 exclusive purposes, but limited biopsy materials may require that the NAFLD Database 2 borrow an 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 2 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 2 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 2 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 2 purposes. However, it is an unfortunate fact that the amount of tissue available in archived paraffin blocks may be very limited.

If a patient had a biopsy prior to screening (up to 90 days prior to the screening specimen collection), the Clinical Coordinator should request the original pathology materials: ideally, this includes the surgical pathology report, an H&E and Masson's trichrome-stained slide, and either 10 unstained slides or the paraffin block. The block is requested so that the 10 unstained slides for the DCC can be sectioned at the Investigator's institution. It is suggested that the Investigator format a request letter to the Director of Pathology at a non-Investigator site in order to briefly explain the necessity of obtaining these materials. The patient must also sign a Release Form in order for the Department of Pathology to release their materials. If there is no tissue remaining in the block for preparation of unstained slides, the Investigator should be prepared to request the outside institution's permission to utilize their original H&E and trichrome-stained slides for Central Reading. It should be made clear that the DCC will return them if so requested.

Upon receipt of the original pathology materials, the NAFLD Database 2 Clinical Coordinator should verify that all materials pertain to the NAFLD Database 2 patient they are said to, and should annotate the accompanying 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 with the biopsy slides.

The Study Pathologist must be asked to determine if the biopsy is adequate for scoring. If the biopsy is adequate for scoring for the NAFLD Database 2, the Study Pathologist should complete the NAFLD Database 2 Liver Biopsy Histology Worksheet (HW) form using the institution's H&E stained slide and Masson's trichrome stained slide, as described later in this document.

After this determination is made, the Clinical Coordinator will:

- 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 two unstained slides are available for sending to the DCC, the institution's stained slides should be sent to the DCC

- 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 2 is borrowing the stained slides from the institution or if the NAFLD Database 2 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 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 2.

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 2, 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 2; other NASH CRN studies may have more rigorous requirements.

The NAFLD Database 2 should request that the slides be provided outright, with no arrangements to return the slides at the end of the NAFLD Database 2. 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 2. 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 2 should be returned upon determination that the patient will not enroll; these slides should not be sent to the DCC.

2.3. Baseline and followup biopsies performed after consent for screening – biopsy procedures

---

Each clinical center investigator will notify his/her NAFLD Database 2 pathologist (e.g., via local institutional requisition sheet) when a biopsy is performed on a NAFLD Database 2 patient so that when the block is initially cut for the local institution's requirements, the additional 10 unstained slides for the NAFLD Database 2 can be cut at the same time, minimizing the chance of tissue loss with refacing the block.

In order to ensure adequate material for thorough histologic review, investigators should obtain needle core biopsies of at least 2.0 cm length using a 16 gauge or greater needle. Laparoscopic biopsies should be done as a needle biopsy, rather than a wedge biopsy, and should be taken from the right lobe. If the biopsy is done at the time of a cholecystectomy, the biopsy should be done as soon as possible into the procedure to avoid the inflammation that can occur in surgical liver biopsies.

If more than 2.0 cm of liver tissue is available, place a 1-2 millimeter segment of liver tissue into a labeled polypropylene cryovial prefilled with approximately 1 mL of RNA*later*® Solution. Tissue is to be placed in RNA*later*® Solution within one minute (preferably) but no more than five minutes after biopsy.

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 2 Study Pathologist for the local evaluation (i.e., for completion of Form HW).

For both baseline and follow-up 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 chance 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:	$119.75 per gross (144 slides/gross); $1,024.44 per case of 10 gross
Tele:	1-800-766-7000

Coverslips should not be placed on the unstained slides. Slides should be labeled with the patient specific preprinted NASH CRN labels for stained slides. The 10 (or as many as 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

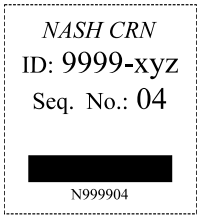
The requirements for the labeling scheme for slides are:

- 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

2.5. Labeling stained and unstained slides at the clinical center

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

(1)



(2)

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

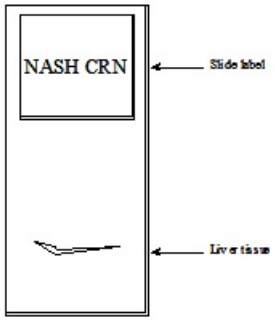
The unique identifier for a slide will consist of the patient ID number and a sequential number. The sequence numbers on the labels for unstained slides run from 01 to 60 (60 is the maximum number of unstained slides that we think we will ever get for any one patient over the lifetime of all NASH CRN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

2.5. Labeling stained and unstained slides at the clinical center

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

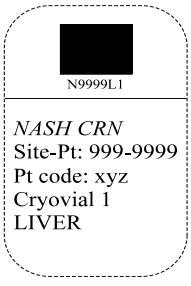
The extra piece of liver tissue (minimum 1-2 mm or greater) will be placed in RNA*later*® Solution as follows:

Labeling procedures

- Apply a pre-printed white, "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 to ensure that the inside of the vial remains sterile
  - Position 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:



- The vial used for banking extra liver tissue should be a 2.0 mL polypropylene cryogenic vial (13.5 mm wide x 48.3 mm tall), that is self-standing with externally threaded vials and silicone washers. This vial is designed for ultra low temperatures (-196 ° C, liquid nitrogen vapor) (supplier: CIC, catalog number 5012-0020; phone 877-738-8247)

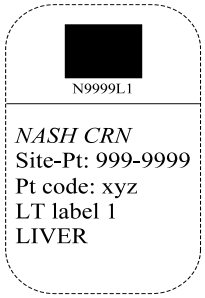
2.6. Liver tissue for banking at Biosample Repository

- Preferably within one minute, and no more than five minutes after biopsy, place the liver tissue into the vial, pre-filled with approximately 1 mL of RNA*later*® Solution. If the sample is not placed in RNA*later*® Solution within 5 minutes, discard the sample. Refrigerate the cryovial at 4° C overnight to allow thorough penetration of the liver tissue and then transfer to -70° C freezer for storage.

- RNA*later*® Solution may be ordered online at http://www.ambion.com/catalog/CatNum.php?AM7020

- The catalog number for 100mL of Ambion RNA*later*® Solution is Cat# AM7020.

- Complete the Liver Tissue Banking (LT) form; apply the corresponding "LT form" label to the LT form



- Complete the Specimen Shipment Log (SS) form. In the NASH CRN Excel shipment file under column J, enter "R".

- Batch ship cryovials monthly to the NIDDK Central Biosample Repository at Precision for Medicine on Monday, Tuesday, or Wednesday; after refrigerating overnight at 4° C, store temporarily in -70° C freezer at the clinical center until the next batch shipment

- Make sure you use the "Cryovial" and "LT form" labels from the same set (i.e., with the same sequence number)

3. Development of the history 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.

3.2. Methods and validation

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, a feature scoring system for NAFLD and NASH was devised 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 has used this system to evaluate liver biopsies for features of NAFLD for the NAFLD Database, and the PIVENS and TONIC trials. Modifications will be made for Database 2, but not to the final score.

4. Local pathology Evaluation at the clinical center (See Forms HW and HF)

4.1. Introduction

The local site NAFLD Database 2 Study Pathologist will evaluate baseline biopsies only, for eligibility determination only. In the case of the NAFLD Database 2, the evaluation determines what category the patient is in (histologically confirmed NAFLD or histologically confirmed cryptogenic cirrhosis).

The local site NAFLD Database 2 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 Worksheet (HW) form. The information recorded on the HW form will be transcribed to the study-specific HF form by the Clinical Coordinator. Copies of the HW and HF forms are 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 2 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. Length of biopsy

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

4.2.2. Steatois 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.

4.2.3. Steatosis location

Steatosis location has 4 choices for characterization:

Zone 3: distribution means not panacinar and not strictly zone 1

Zone 1: definite periportal location with sparing of zone 3

Azonal: macrosteatosis present in an irregularly distributed fashion

Panacinar: implies steatosis that appears to involve the whole acinus equally, rather than the more random azonal pattern

4.2.4. 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

4.2.5. Portal chronic inflammation

0: None

1: Mild

2: More than mild

"More than mild" portal inflammation is scored if more than one portal area shows a mononuclear infiltrate that is either focally dense (like a lymphoid aggregate) or a diffusely increased infiltrate of moderate density.

4.2.6. Lobular inflammation (0-3; 30x)

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

4.2.7. Portal chronic inflammation (0-2)

0: None

1: Mild

2: More than mild

4.2.8. Hepatocellular ballooning (0-2)

0: None

1: Few

2: Many

4.2.9. 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: Not NAFLD

1: NAFLD but not NASH

2: Suspicious/borderline/indeterminate: Zone 3 pattern (1A)

3: Suspicious/borderline/indeterminate: Zone 1, periportal patten (1B)

4: 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).

4.2.10. 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

Diagnoses 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 HF form.

4.2.11. 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.

4.2.12. 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. NAFLD Activity score (0-8)

The NAFLD 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.

4.4. Unscheduled liver biopsy

Unscheduled biopsies (i.e., biopsies done after screening) will be evaluated locally for standard of care and also centrally by the Pathology Committee. Forms HW and HF will not be completed for unscheduled liver biopsies, but the biopsy slides should be obtained and the SD form should be completed. Form CR will be completed upon central review by the Pathology Committee. The CR form will use the visit code of the visit window that was open at the time of the biopsy.

5. Central pathology evaluation (See Form CR)

5.1. Procedures

Each pathology review session will include attendance by at least three 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 2 days. Two 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 2 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:

- Length of biopsy
- Steatosis grade
- Steatosis location
- Fibrosis stage
- Lobular inflammation
- Portal, chronic, 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

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. Steatosis

5.3.2a Types of Macrovesicular steatosis

0: Predominantly large droplet macrovesicular steatosis

1: Mixed large and small droplet macrovesicular steatosis

2: Predominantly small droplet macrovesicular steatosis

5.3.2b Microvesicular steatosis, continguous 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.

5.3.2. Ballooning

5.3.3a: Severe ballooning present

0: No

1: Yes

A score of "severe ballooning present" should be made if large, classical balloon cells are seen from low magnification in multiple areas throughout the biopsy. The biopsy should already have a score of "2" for ballooning to qualify for severe ballooning.

5.3.3b: Classical balloon cells present

0: No

1: Yes

Classical balloon cells are ones that are easily recognized at low to medium magnification, stand out from the surrounding parenchyma and have cytoplasm that is clumped. They may have Mallory-Denk bodies. A positive score requires only 1 classical balloon cell.

5.3.3. Fibrosis

5.3.4a: Perisinusoidal fibrosis grade

0: No perisinusoidal fibrosis present

1: Perisinusoidal fibrosis present that requires a Masson stain to identify

2: Perisinusoidal fibrosis present that is visible on the H&E stain

Note that stage 1A fibrosis would automatically get a score of 1 and stage 1B fibrosis would automatically get a score of 2 on this scale.

5.3.4b: Predominant location of fibrosis (scored only if fibrosis stage is 1A, 1B, 1C, 2 or 3)

0: Fibrosis is more prominent around or between portal areas

1: No portal or central predominance to the fibrosis

2: Fibrosis is more prominent around or between central veins

Note that stage 1A and 1B fibrosis would automatically get a score of 2 and stage 1C fibrosis would automatically get a score of 0 on this scale.

5.3.4. Microgranulomas seen

0: No

1: Yes

A positive score is recorded if only one microgranuloma is seen.

5.3.5. Large lipogranulomas seen

0: No

1: Yes

A positive score is recorded if only one large lipogranuloma is seen. These are typically found in portal areas or adjacent to central veins.

5.3.6. Acidophil bodies

0: Rare/absent

1: Many

"Many" acidophil bodies are scored when more than one acidophil body is identified during examination of the biopsy.

5.3.7. Pigmented macrophages (Kupffer cells)

0: Rare/absent

1: Many

"Many" pigmented macrophages has a very low threshold. If a cluster of pigmented macrophages are seen in a single microgranuloma or lipogranuloma, this would qualify as "Many".

5.3.8. Megamitochondria

0: Rare/absent

1: Many

"Many" Megamitochondria is scored if several hepatocytes are identified with megamitochondria.

5.3.9. Mallory-Denk bodies

0: Rare/absent

1: Many

"Many" Mallory-Denk bodies are recorded when several hepatocytes are identified with unequivocal Mallory-Denk bodies.

5.3.10. Glyogen nuclei

0: Rare/absent

1: Present in patches

A "Patch" of Glyogen nuclei is a group of 5 or more hepatocytes in close proximity that have glycogen nuclei.

5.3.11. Glycogenosis of hepatocytes

0: Not present

1: Focal, involving less than 50% of the hepatocytes

2: Diffuse, involving more than 50% of the hepatocytes

5.3.12. 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

"Blue blush" staining of hepatocytes is not scored as it is not granular.

5.3.13. Iron: hepatocellular distribution

0: Periportal

1: Periportal and midzonal

2: Panacinar

3: Zone 3 or nonzonal

5.3.14. Nonhepatocellular iron grade (0-2)

0: None (skip nonhepatocellular iron distribution)

1: Mild (generally only a few scattered positive cells)

2: More than mild

5.3.15. 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

5.3.16. Steatohepatitis diagnosis

99: Not NAFLD

0: Not NASH

1a: Suspicious/borderline/indeterminale: Zone 3 pattern

1b: Suspicious/borderline/indeterminale: Zone 1, periportal pattern

2: Yes, definite

5.3.17. 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 (#021227854). 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 inventory 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 institutional slides to the clinical center

- Log the slides out of the DCC slide inventory
- 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

NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

NAFLD Database
Standard Operating Procedures

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

13 October 09

Standards of Care for Adult Patients with Fatty Liver Disorders

Contents

Standards of Care for Adult Patients with Fatty Liver Disorders

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. The document was revised for the continuation of the NASH CRN in 2009 and approved by the steering committee by vote at the Steering Committee meeting in Baltimore, October 8-9, 2009. 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.

2. Specific recommendations

2.1.1.1. 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. The importance of portion control will be discussed, especially in reference to eating at restaurants. Avoidance of calorie dense fast food and sugar sweetened beverages will be stressed.
c. Patients with known or newly discovered type 2 diabetes will receive specific recommendations as promulgated by the ADA (Appendix 2).
d. Recommendations regarding the use of specific nutritional supplements are addressed below.
e. Dietary guidelines may not apply to all persons or situations.

2.1.1.2. 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.

2.1.1.3. 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.

2.1.1.4. 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.

2.1.1.5. 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.

2.1.1.6. 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 (hemoglobin A_{1c} will be continued on their current treatment regimens.

iii. Patients with sub optimally controlled diabetes (hemoglobin $A_{1C} \geq 7\%$) will receive a recommendation for follow-up with their primary physician for improved glycemic control.

b. Hypertriglyceridemia
   i. 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
   i. 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
   i. 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)
   i. 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
    i. 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.

2.1.1.7. Possibly helpful concomitant medication use

a. Vitamin E
    i. Recommendations regarding the use of vitamin E will be individualized and should not exceed 800 IU all natural vitamin E daily.
b. 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.
c. 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.
d. Fibrates
    i. Fibrates used to treat hypertriglyceridemia may be continued with dose escalations as clinically indicated.
e. Statins
    i. Statins used to treat hypercholestereolemia may be continued with dose escalations as clinically indicated.
f. 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) will be at the discretion of the patient's physicians based on accumulating data regarding potential benefits and risks.

2.1.1.8. Possibly harmful concomitant medication use

a. Acetaminophen
    i. Acetaminophen should be restricted to < 3 grams in any given day in patients

without cirrhosis and < 2 grams in any given day in patients with cirrhosis.
   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
   i. 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)
   i. 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.

2.1.1.9. Possibly helpful concomitant dietary supplement use

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

2.1.1.10. Posibily 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 use these standards of care in the patients followed at all clinical centers. There will be no standardized lead-in period during which these standards will be applied before patients are enrolled in NASH CRN studies. 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 continue to use 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 annually 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

4.1.1.1. Physician summary of guidelines

a. Physicians at the study sites will need ongoing reminders of the specifics of the standards of care

b. A 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/cdbase/DB2/SOP/NASHCRNPocketStandardsofCare2.pdf)

4.1.1.2. Patient brochures

a. Brochures that should be available for NASH CRN patients
   i. Healthy eating
   ii. Healthy weight loss
      1. BMI formula
      2. Goals
   iii. General NASH CRN brochure to cover most other recommendations
      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

4.1.1.3. 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.

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.