NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

Treatment of Nonalcoholic Fatty Liver Disease in Children (TONIC)

Standard Operating Procedures

Part I: Clinical Center Operations

25 May 2007

Contents

Contents

Contents

TONIC SOP Part I: Clinical Center Operations

1. Design overview

1.1. Design synopsis

Title
- Treatment of Nonalcoholic Fatty Liver Disease in Children (TONIC)

Sponsor
- NIDDK and NICHD

Objective
- To determine whether 96 weeks of treatment with either metformin or vitamin E leads to sustained reduction in serum ALT in nondiabetic children with NAFLD compared to treatment with placebo

Type of study
- Phase III randomized clinical trial
- Multicenter, masked, placebo-controlled

Treatment groups
- Group 1: Metformin, 500 mg PO b.i.d. and vitamin E-placebo b.i.d.
- Group 2: Vitamin E, 400 IU natural form PO b.i.d. and metformin-placebo b.i.d.
- Group 3: Metformin-placebo b.i.d. and vitamin E-placebo b.i.d.

Population
- Children with NAFLD aged 8-15 years at first screening visit

Study duration – per patient
- Up to 4 months (120 days) of screening prior to randomization, including at least 3 months of drug washout for those using antiNAFLD or antidiabetic medications prior to randomization
- 96-week treatment
- 24-week post-treatment follow-up

Study duration – per calendar time
- Recruitment phase: August 2005 - September 2007
- Follow-up phase: August 2005 - October 2009

Sample size justification

- Total of 180 patients in 3 groups of equal size (60 per group)
- Primary comparisons
  - Metformin vs placebo
  - Vitamin E vs placebo
- Error protection
  - Type I = 0.025 (0.05, Bonferroni protected)
  - Type II = 0.10 (90% power)
- Effect size for sustained reduction in ALT after 96 weeks of treatment
  - Expected percent with sustained reduction in the placebo group: 20%
  - Expected percent with sustained reduction in the metformin or vitamin E groups: 50%
- Statistical test and sample size software
  - Chi-squared test for two proportions
  - Dupont and Plummer PS software
- Source of data for response rates
  - Open-label pilot studies of metformin and vitamin E at the UCSD NASH CRN clinic
  - Consensus of NASH CRN clinicians regarding expected response in the placebo group

Inclusion criteria

- Age 8 through 17 at first screening visit
- Histologic evidence of NAFLD – biopsy cannot be older than 6 months (183 days) as of randomization
- ALT level > 60 U/L on two separate occasions at least 30 days apart but no more than 6 months (183 days) apart. One of these measures must be obtained at the TONIC clinical center during screening for TONIC. The other measure may be historic (ie., obtained prior to initiation of screening in TONIC)
- Consent
- Randomized within 4 months (120 days) of starting screening

Exclusion criteria

- History of significant alcohol intake (AUDIT questionnaire) or inability to quantify alcohol consumption
- Diabetes mellitus
  - Fasting serum glucose of 126 mg/dL or greater

  OR
  - 2-hour serum glucose of 200 mg/dL or greater (from oral glucose tolerance test, OGTT)

  OR
  - History of diabetes mellitus
- ALT > 400 U/L on most measurement closest in time to randomization
- Clinical or histologic evidence of cirrhosis
- Evidence of other chronic liver disease
- Serum creatinine of 1.5 mg/dL or greater for males and 1.4 mg/dL or greater for females

- Use of drugs historically associated with NAFLD (systemic glucocorticoids, tetracyclines, anabolic steroids, valproic acid, salicylates, tamoxifen, other known hepatotoxins) for more than 2 weeks in the 2 years prior to randomization
- Use of antidiabetic drugs (insulin, biguanides, glucosidase inhibitors, sulfonylureas, meglitinides, metformin, thiazolidinediones) in the 3 months prior to randomization
- Use of antiNAFLD drugs (metformin, vitamin E, thiazolidinediones, UDCA, SAM-e, betaine, milk thistle, probiotics) in the 3 months prior to randomization
- Use of any over-the-counter or herbal remedy for hyperlipidemia in the 3 months prior to randomization
- Metabolic acidosis
- Renal dysfunction
- Coagulopathy
- History of bariatric or hepatobiliary surgery
- History of total parenteral nutrition during the past 3 years prior to screening
- Inability to swallow study medication
- Vitamin E supplementation of greater than 100 I/U per day
- Disease considered by study physician to be significant
- Females of childbearing potential: positive pregnancy test during screening or at randomization or unwillingness to use an effective form of birth control during the trial
- Females of childbearing potential: breast feeding
- Any other condition, which in the opinion of the investigator would impede compliance or hinder completion of study

Outcome measures

- Primary: Sustained reduction in ALT to either 50% of baseline value or < 40 U/L
- Secondary:
  - Sustained reduction in serum AST
  - Sustained reduction in serum GGT
  - Change in histologic feature scores determined by standardized scoring of liver biopsies at baseline and after 96 weeks of treatment
  - Change in liver fibrosis, inflammation, or steatosis
  - Change in body mass index
  - Change in insulin resistance indices
  - Change in serum vitamin E levels
  - Change in serum cytokine and fibrosis marker levels
  - Change in serum lipid profile
  - Change in QOL scores

Randomization

- Centrally administered randomization stratified by clinical center and blocked by calendar time

Visit schedule

- Baseline/screening: at least 1 visit separated by at least 1 calendar day from randomization; screening period can last no more than 4 months (120 days)
- Randomization (final pre-treatment interview, dispensing of study drugs)
- Follow-up visits
  - 4 weeks after randomization
  - 12 weeks after randomization and every 12 weeks thereafter up to 96 weeks
  - 120 weeks after randomization (24 weeks after treatment ends)
- Liver biopsy at 96 week visit

Statistical analysis

- All analyses will be on an "intention-to-treat" basis

Safety monitoring

- NIDDK appointed Data and Safety Monitoring Board will monitor the data for safety and efficacy for outcomes such as metabolic acidosis, hepatotoxicity, hypoglycemia, pregnancy, new onset diabetes, and any other outcomes or events identified as safety-related

Number of clinics

- 8

1.2. Data collection schedule

Assessment/Procedure	Screening visits S1	Screening visits S2	RZ	Follow-up visits Weeks from randomization 4	12	24	36	48	60	72	84	96	120
Consent	X	.	X	.	.	.	.	.	.	.	.	.	.
Baseline (B) or interim (I) medical history	B	.	.	I	I	I	I	I	I	I	I	I	I
Review for adverse effects	X	.	.	X	X	X	X	X	X	X	X	X	X
Review of concomitant medications	X	.	.	X	X	X	X	X	X	X	X	X	X
AUDIT (A) or interim (I) alcohol quest	A	.	.	I	I	I	I	I	I	I	I	I	I
Study drug dispensing	.	.	X	.	X	X	X	X	X	X	X	.	.
Review of study drug adherence	.	.	.	X	X	X	X	X	X	X	X	X	.
Detailed (D) or focused (F) physical exam	D	.	.	F	F	F	F	D	F	F	F	D	D
DEXA scan for body fat	.	X	.	.	.	.	.	.	.	.	.	X	.
MRI for body fat (optional)	.	X	.	.	.	.	.	.	.	.	.	X	.
Liver biopsy (H for ≤6 months)	H	.	.	.	.	.	.	.	.	.	.	X	.
Block Brief nutrition questionnaire	.	X	.	.	.	.	.	X	.	.	.	X	X
Modifiable activity questionnaire	.	X	.	.	.	.	.	X	.	.	.	X	X
Pediatric quality of life	.	X	.	.	.	.	.	X	.	.	.	X	X
Liver symptom questionnaire	.	X	.	.	.	.	.	X	.	.	.	X	X
Labs													
Metabolic panel	X	.	.	X	X	X	X	X	X	X	X	X	X
Hematology	X	.	.	.	.	X	.	X	.	X	.	X	X
Fasting lipid profile	X	.	.	.	.	X	.	X	.	X	.	X	X
Fasting serum glucose	X	.	.	.	.	X	.	.	.	X	.	.	.
Hepatic panel	.	X	.	X	X	X	X	X	X	X	X	X	X
Vitamin B12	.	X	.	.	.	X	.	X	.	X	.	X	X
GGT, prothrombin time, HbA1c	.	X	.	.	.	.	.	X	.	.	.	X	X
OGTT with insulin and C-peptide	.	X	.	.	.	.	.	X	.	.	.	X	X
Free fatty acid, leptin, C-reactive protein	.	X	.	.	.	.	.	X	.	.	.	X	X
Urine pregnancy test (females)	X	X	X	X	X	X	X	X	X	X	X	X	X
Banking													
Fasting serum, plasma banking	.	X	.	.	.	X	.	X	.	X	.	X	.
Serum vitamin E (on banked specimen)	.	X	.	.	.	.	.	X	.	.	.	X	.
DNA for banking	.	X	.	.	.	.	.	.	.	.	.	.	.
Closeout form	.	.	.	.	.	.	.	.	.	.	.	.	X

Note: **Detailed (D) physical** includes measurement of height, weight, waist, hips; vital signs (temperature, heart rate, blood pressure); triceps skin fold thickness; mid-upper arm circumference; examination for scleral icterus and pedal edema and auscultation of the heart and lungs; general physical findings (hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting, fetor); Tanner staging. **Focused (F) physical** includes measurement of height, weight; vital signs (temperature, heart rate, blood pressure); examination for scleral icterus and pedal edema and auscultation of heart and lungs.
OGTT: Blood samples will obtained only at baseline and at 2 hours after administration of glucose solution.
Lipid profile: total cholesterol, triglyceride, LDL, HDL.
Hematology: Hemoglobin, hematocrit, WBC, platelet count.
Metabolic panel: sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein.
Hepatic panel: total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase.
Fasting visits: All visits except RZ.

1.3. Whole blood (mL) to be drawn at screening and follow-up visits

	Study visit (wk)												
Procedure	**s1**	**s2**	**4**	**12**	**24**	**36**	**48**	**60**	**72**	**84**	**96**	**120**	**Total**
OGTT w/insulin	.	10	.	.	.	.	10	.	.	.	10	10	40
Fasting lipid	5	.	.	.	5	.	5	.	5	.	5	5	30
Hematology	5	.	.	.	5	.	5	.	5	.	5	5	30
Metabolic panel	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 and others	.	5	.	.	.	.	5	.	.	.	5	5	20
Plasma: banking	.	3	.	.	.	.	3	.	.	.	3	.	9
Serum: fibrosis	.	10	.	.	.	.	10	.	.	.	10	.	30
Serum: banking	.	25	.	.	20	.	25	.	20	.	25	.	115
Serum: Vit E	.	5	.	.	.	.	5	.	.	.	5	.	15
DNA	.	20	.	.	.	.	.	.	.	.	.	.	20
Total	15	83	10	10	40	10	78	10	40	10	78	35	**419**

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

OGTT w/insulin: also includes c-peptide and fasting glucose (baseline and 2-hour samples only are collected)
Hematology: hemoglobin, hematocrit, WBC, platelet count
Metabolic panel: sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein
Hepatic panel: total bilirubin, AST, ALT, alkaline phosphatase
HbA1c and others: HbA1c, GGT, prothrombin time (INR)

Note: Fasting serum glucose (visits s1, f024, f072), **Vitamin B12** (visits s2, f024, f048, f072, f096, f120), and **free fatty acid, leptin, and C-reactive protein** (visits s2, f048, f096, f120) are also collected, but these measures can be obtained from the blood drawn at these visits for one of the other tests specified in the table (ie, these measures do not add to the volume of blood needed to be drawn at the visit)

1.4. Treatment groups

A patient whose parent/guardian has signed an informed consent statement and who meets the eligibility criteria will be randomly assigned to one of three groups for 96 weeks of treatment:

Group 1: Metformin (500 mg b.i.d.) and vitamin E-placebo (b.i..d.)
Group 2: Vitamin E (400 IU, natural form, b.i.d.) and metformin-placebo (b.i.d.)
Group 3: Metformin-placebo (b.i.d.) and vitamin E-placebo (b.i.d.)

The randomization plan will be prepared and administered centrally by the Data Coordinating Center (DCC) but will not require real time interaction with a DCC staff member. Requests for randomizations will be made by the clinics using a web-based application. An assignment will be issued only if the database shows that the patient is eligible, the parent/guardian has signed the consent statement, and the patient has had all required baseline data keyed to the database.

2. Eligibility and enrollment

2.1. Inclusion and exclusion criteria

Inclusion criteria

- Age 8 through 15 years at first screening visit
- Histological evidence of NAFLD based on a liver biopsy obtained in the 6 months (183 days) prior to randomization: a minimum of 5% of hepatocytes with macrovesicular fat, without other etiologies for the presence of fat being identified
- Serum alanine aminotransferase elevation (ALT > 60 U/L) on two separate occasions at least 30 days apart but no more than 6 months (183 days) apart. One of these measures must be obtained at the TONIC clinical center during screening for TONIC. The other measure may be historic (i.e., obtained prior to initiation of screening in TONIC)
- Consent
- Randomized within 4 months (120 days) of first screening visit

Exclusion criteria

Patients who satisfy any of the following exclusion criteria will be ineligible for enrollment in the trial:

- History of significant alcohol intake per AUDIT questionnaire or inability to quantify alcohol consumption
- Diabetes mellitus
  - Fasting serum glucose of 126 mg/dL or greater at visit s1 or at oral glucose tolerance test (OGTT; 2 g/kg glucose load, maximum 75 g) at visit s2
    OR
  - 2-hour serum glucose of 200 mg/dL or greater at OGTT at visit s2
    OR
  - History of diabetes mellitus
- ALT > 400 U/L on measurement occasion closest in time to randomization
- Clinical or histologic evidence of cirrhosis
- Evidence of other chronic liver disease
  - Alpha-1 antitrypsin deficiency
  - Bile duct anomalies
  - Hemochromatosis
  - Hepatitis - autoimmune or viral
  - Wilson's disease
- Serum creatinine of 1.5 mg/dL or greater for males and 1.4 mg/dL or greater for females
- Use of drugs historically associated with NAFLD (systemic glucocorticoids, tetracyclines, anabolic steroids, valproic acid, salicylates, tamoxifen, other known hepatotoxins) for more than 2 weeks in the 2 years prior to randomization
- Use of antidiabetic drugs (insulin, biguanides, sulfonylureas, metformin, thiazolidinediones) in the 3 months prior to randomization
- Use of antiNAFLD drugs or herbal remedies (metformin, vitamin E, thiazolidinediones, UDCA, SAM-e, betaine, milk thistle, probiotics) in the 3 months prior to randomization
- Use of any over-the-counter medication or herbal remedy for improving hyperlipidemia in the

3 months prior to randomization
- Metabolic acidosis
- Renal dysfunction
- Coagulopathy
- History of bariatric or hepatobiliary surgery
- History of total parental nutrition during the past 3 years prior to screening
- Inability to swallow study medication
- Vitamin E supplementation greater than 100 I/U per day
- Current, untreated disease and/or considered by study physician to be significant (cardiac, renal, pulmonary, psychiatric, neoplastic, chronic inflammatory disease besides liver, coagulopathy)
- Females of childbearing potential: positive pregnancy test during screening or at randomization or unwillingness to use an effective form of birth control during the trial
- Females of childbearing potential: breast feeding
- Any other condition, which in the opinion of the investigator would impede compliance or hinder completion of study

2.2. Run-in period

Patients must not have used any prescription or over-the-counter medication or herbal remedy taken with an intent to improve or treat NAFLD or diabetes in the 3 months prior to randomization. AntiNAFLD agents include: metformin, vitamin E, thiazolidinediones, UDCA, SAM-e, betaine, milk thistle, and probiotics. Antidiabetic agents include but are not limited to: insulin, biguanides, sulfonylureas, metformin, and thiazolidinediones. These agents are not to be used during screening nor for the duration of the trial (except in the form of assigned study treatment or treatment for new onset diabetes).

Any over-the-counter medication or herbal remedy that is being taken with an intent to improve hyperlipidemia will not be allowed for at least 3 months prior to randomization and will be discouraged after randomization. Patients will be allowed to continue on prescription anti-hyperlipidemic agents. Patients will be interviewed in a detailed fashion at screening and at every clinic visit to document the absence of such use.

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

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

Viral hepatitis (070)

Malignant neoplasm of liver and intrahepatic bile ducts (155)

Malignant neoplasm of gallbladder and extrahepatic bile ducts (156)

Acquired hypothyroidism (244)

Diabetes mellitus (250)

Ovarian dysfunction (256)
- Polycystic ovaries (256.4)

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

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

Gout (274)

Disorders of mineral metabolism (275)
- Disorders of iron metabolism (275.0)
  - Hemochromatosis, iron overload
- Disorders of copper metabolism (275.1)
  - Wilson's disease

Disorders of fluid, electrolyte, and acid-base balance (276)
- Acidosis (276.2)
- Fluid overload, retention (276.6)

Other metabolic disorders (277)
- Cystic fibrosis (277.0)
- Amyloid degeneration of liver (277.3)
- Disorders of bilirubin excretion, Gilbert's syndrome (277.4)
- Alpha-1 antitrypsin deficiency (277.6)
- Dysmetabolic syndrome X (277.7)

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

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

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

Schizophrenic disorders (295)

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

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

Personality disorders (301)

Epilepsy (345)

Muscular dystrophies and other myopathies (359)

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

Ischemic heart disease
- Acute myocardial infarction (410)

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

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

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

Atherosclerosis (440)

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

Portal vein thrombosis, portal obstruction (452)

Budd-Chiari syndrome, hepatic vein thrombosis (453)

Esophageal varices with bleeding (456.0)

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

Chronic liver disease and cirrhosis (571)
Chronic hepatitis (571.4)
Cirrhosis of liver without mention of alcohol (571.5)
Biliary cirrhosis (571.6)
Other chronic nonalcoholic liver disease (571.8)

Unspecified chronic liver disease without mention of alcohol (571.9)

Liver abscess and sequelae of chronic liver disease (572)
Abscess of liver (572.0)
Portal pyemia (572.1)
Hepatic coma (572.2)
Hepatic encephalopathy
Hepatocerebral intoxication
Portal-systemic encephalopathy
Asterixis: An abnormal tremor consisting of involuntary jerking movements, especially in the hands, frequently occurring with impending hepatic coma.
Portal hypertension (572.3)
Hepatorenal syndrome (572.4)
Other sequelae of chronic liver disease (572.8)
Hepatopulmonary syndrome

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

Cholelithiasis (574)

Other disorders of gallbladder (575)

Other disorders of biliary tract (576)

Diseases of pancreas (577)

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

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

Hydronephrosis (591)

Calculus of kidney and ureter (592)

Other disorders of kidney and ureter (593)

Other conditions in the mother classifiable elsewhere, but complicating pregnancy (648)

Abnormal glucose tolerance in pregnancy, **gestational diabetes** (648.8)

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

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

Diffuse diseases of connective tissue
Systemic lupus erythematosus (710.0)
Rheumatoid arthritis (714.0)

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

Other disorders of soft tissues (729)
Myalgia and myositis, unspecified (729.1)

Other congenital anomalies of digestive system (751)
Anomalies of gallbladder, bile duct, and liver (751.6)
Congenital cystic disease of liver (751.62)

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

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

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

Other symptoms involving abdomen (789)
Abdominal pain (789.0)
Hepatomegaly (789.1)
Splenomegaly (789.2)
Ascites (789.5) Lymph fluid that has leaked into the peritoneal cavity, is one of the principal clinical manifestations cirrhosis and portal hypertension.

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

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

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

2.4. 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. Guidelines for when to rescreen patients deemed ineligible at the time of initial screening are:

- An ineligible liver biopsy – the participant may be rescreened after 12 months at the discretion of the investigator
- Ineligibility determined on measurements of albumin, INR, conjugated bilirubin, ALT, fasting glucose, and creatinine – the participant may be rescreened after 6 months at the discretion of the investigator
- Pregnancy – the participant may be rescreened 3 months following end of pregnancy and breast feeding at the discretion of the investigator
- Unwilling to participate – the participant may be rescreened after 3 months at the discretion of the investigator

2.5. Rescreening for TONIC after temporary ineligibility resolves

- Affirm that the patient and parent/guardian continue to consent/assent to TONIC participation.
- Delete all forms from the patient's original screening from the data system except the RG, BC, and CG forms; update any RG items in sections B, C, or D that have changed since the previous screening and update section G but do not edit items in section A, E, or F.
- Complete the Rescreen in TONIC (RC) form and key it (this form must be keyed before additional screening forms may be keyed for the patient); a new set of screening phase tube and questionnaire labels will be available for printing upon keying the RC form.
- Blood for serum and plasma repository
  - Blood must be collected for the serum and plasma repository even if banked during the previous screening, regardless of the time between previous and current screening; complete and key a new BP form; use a new set of serum and plasma labels for the cryovials.
- Blood for genetics repository
  - If not already collected, have the patient and parent/guardian sign the TONIC genetic consent/assent and collect a sample and complete the TONIC BC and CG forms.
  - If the yield on the sample drawn when the patient was previously screened for TONIC was satisfactory, leave the previously completed BC and CG forms in the data system; new forms do not need to be completed for this cycle of screening.
  - If the yield on the sample drawn when the patient previously screened for TONIC was unsatisfactory, draw the replacement sample, delete the original BC form from the data system, and complete and key the BC form anew; leave the previously completed CG form in the data system.
- If a new revision of the LR form has been issued since the previous screening, complete the current version of the LR form; lab results reported on the previously completed LR form may be transcribed to the new form if they are within the time windows specified on the form. If the same version of the LR form completed previously is still in use, you may update the LR form – change the date in item 4 to the current date, update results as needed, and update the review date. If a test is now out of the time window for the test, it must be repeated. Key the LR form.
- If a new revision of the LU form has been issued since the previous screening, complete the current version of the LU form; lab results reported on the previously completed LU form may be transcribed to the new form if they are within the time windows specified on the form (note that ALT must be obtained anew). If the same version of the LU form completed previously is still in use, you may update the LU form – change the date in item 4 to the current date, update results as needed (ALT must be obtained anew), and update the review date. If a test is now out of the time window for the test, it must be repeated. Key the LU form.

2.5. Rescreening for TONIC after temporary ineligibility resolves

- If a new revision of the LS form has been issued since the previous screening, complete the current version of the LS form; lab results reported on the previously completed LS form may be transcribed to the new form if they are within the time windows specified on the form. If the same version of the LS form completed previously is still in use, you may update the LS form – change the date in item 4 to the current date, update results as needed, and update the review date. If a test is now out of the time window for the test, it must be repeated. Key the LS form.
- All interviews and patient questionnaires (drinking history, AUDIT, baseline history, liver symptoms, quality of life, physical activity, and food questionnaire) must be completed and keyed anew.
- The physical exam (PE) form must be completed and keyed anew.
- If the liver biopsy used during the previous screening is again being used to satisfy eligibility (keep in mind that the biopsy for TONIC must have been done within 6 months of the date of randomization), additional slides do not need to be sent to the DCC, but the pathologist must rescore the biopsy and a new HF form must be completed. If a new revision of the SD form has been issued since the previous screening, complete the current version of the SD form; you may reference the slide numbers of materials already sent to the DCC. If the same version of the SD form completed previously is still in use, you may update the SD form – change the date in item 4 to the current date and update results as needed (remember to update the review date). Key the SD form.
- If the patient is again found to be ineligible for TONIC, the patient may be rescreened at a later date; delete the RC form from the previous rescreening, complete a new RC form, and continue as described above

2.6. Transferring patients between NAFLD Database and TONIC

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

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

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

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

- Blood for genetics repository
  - If not already collected, have the patient sign the TONIC genetic consent and collect a sample and complete the TONIC BC and CG forms
  - If blood was already collected, do not send another sample unless the yield was unsatisfactory
  - If the yield on the sample drawn when the patient screened for the Database was satisfactory, leave the Database BC and CG forms in the data system and complete the TONIC BC form answering 'yes' to the question about prior blood draw for the Database; the patient does not need to sign the TONIC genetic consent nor does the TONIC CG form need to be completed
  - If the yield on the sample drawn when the patient screened for the Database was unsatisfactory, have the patient sign the TONIC genetics consent form, draw the replacement sample, and complete the TONIC BC and CG forms; the Database BC and CG forms should remain in the data system
- Lab results reported on the Database LR and LS forms may be used on the TONIC LR, LU, and LS forms if they were obtained within the time windows specified on the forms
- All interviews and patient questionnaires (drinking history, AUDIT, baseline history, liver symptoms, quality of life, physical activity, and food questionnaire) must be completed anew for TONIC
- The physical exam (PE) form must be completed anew for TONIC
- If the biopsy used for TONIC is the same one that was used for the NAFLD Database (keep in mind that the biopsy for TONIC must meet date and medication requirements not imposed in the NAFLD Database), the local pathologist must review the slides again and complete the TONIC HF form. The TONIC SD form must be completed; transcribe information from the Database SD (or SE or SF) form. For items relating to slide sequence numbers, transcribe the slide numbers for the slides that were previously sent for the Database (i.e., only 10 unstained slides need to be sent from a single biopsy). There will be more than 1 form in the data system pointing to the same numbered slides (Database SD and TONIC SD forms), but this is ok since the patient enrolled in the Database.
- If the patient is eventually randomized in TONIC, have the patient complete TONIC visits and forms; you do not need to complete the MV form for the missed Database visits, but you do need to complete the Database Closeout (CO) form to suspend the patient's participation in the NAFLD Database. The CO form can be completed prior to or after randomization in TONIC, but our advice is to complete it upon randomization in TONIC. The patient remains enrolled in the NALFD Database while participating in TONIC, but the patient is not subject to completion of NAFLD Database visits.
- Retain all Database forms completed for the patient in the patient's NASH CRN file
- Retain the patient's Database visit windows schedule since it will be needed once TONIC is completed

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

- The patient should be closed out of the NAFLD Database by completing and keying the Database ED form to document the reason(s) why the patient didn't enroll in the Database. Answer as many of the questions in sections B, C, D, E and F of form ED as you can, coding an item as 'm' if you do not know the answer. If the answer to ED item 23 is 'No' (2), then for item 24, pick the response that best matches the situraiton for your patient. If you check 'No' (2) to item 24, then you must check 'No' (2) for item 25 and check 'Other reason' in item 26c and write in 'opted to go directly into TONIC' in the specify line. If you check 'Yes' (1) for ED item 23 or 'Yes' (1) for ED item 24 or 'Task not run' (3) for ED item 24, go directly to item 26 and indicate the reason(s) for ineligibility.
- Have the patient sign the TONIC consent form
- Complete and key the TONIC RG form but do NOT issue a new patient ID number and code
- Blood for serum and plasma repository
  - If more than 8 weeks have elapsed since the previous blood draw or if serum and plasma were not obtained for the Database, you must do the blood draws for serum and plasma for TONIC
  - If less than 8 weeks have elapsed since the previous blood draw for serum and plasma and the serum and plasma have been kept frozen or were sent to the repository at Fisher BioServices:
    - The blood draw for serum and plasma does not need to be repeated
    - Transcribe information from the Database BP form to the TONIC BP form; the date in item 4 of the TONIC BP form should be the current date, but enter the actual date of blood draw (ie, the date in item 9 on the Database BP form) in item 9 of the TONIC BP form; when you are asked to apply duplicate labels to the TONIC BP form, write in the label information and in the margin write "see Database BP form"; key the TONIC BP form. If the Database BP form has been keyed, it can remain in the data system.
- Blood for genetics repository
  - If not already collected, have the patient sign the TONIC genetic consent and collect a sample and complete the TONIC BC and CG forms
  - If blood was already collected, do not send another sample unless the yield was unsatisfactory
  - If the yield on the sample drawn when the patient screened for the Database was satisfactory, key the Database BC and CG forms (if not already keyed) and complete the TONIC BC form answering yes to the question about prior blood draw for the Database; the patient does not need to sign the TONIC genetic consent nor does the TONIC CG form need to be completed

  - If the yield on the sample drawn when the patient screened for the Database was unsatisfactory, then have the patient sign the TONIC genetic consent form, draw the replacement sample, and complete the TONIC BC and CG forms based (the Database BC and CG forms can remain in the data system)
- Interviews and questionnaires must be completed on the TONIC forms
  - Data from the Database AD form may be transcribed to the corresponding TONIC form, but the patient should be queried regarding any changes since the previous interviews; the date in item 4 on the TONIC form should be the date you review the information with the patient
  - The TONIC BG form should be completed anew – it is different from the Database BG form, and the TONIC BG form data help establish that the biopsy is a medication free biopsy (medication use is not an issue with Database biopsies)
  - The patient should complete the TONIC LP, quality of life, and MA forms anew
  - The patient should complete the Brief Block Food Questionnaire anew and the BD form should be completed anew
- The physical exam (PE) form must be completed anew
- If the same biopsy is used for TONIC that was used for the Database (keep in mind that the biopsy for TONIC must meet date requirements not imposed in the NAFLD Database), the local pathologist must review the slides again and complete the TONIC HF form. The TONIC SD form needs to be completed; transcribe information from the Database SD (or SE or SF form). For items relating to slide sequence numbers, transcribe the slide numbers that were previously sent for the Database (ie, only 10 unstained slides need to be sent from a single biopsy). The Database SD/SE/SF forms can remain in the data system.
- Retain all Database forms completed for the patient in the patient's NASH CRN file

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

- The patient should be closed out of TONIC by completing and keying the TONIC EC form to document the reason(s) the patient was found ineligible
- Have the patient sign the Database consent form
- Complete and key the Database RG form but do NOT issue a new patient ID number and code
- Blood for serum and plasma repository
  - If serum and plasma were not obtained in TONIC, do the blood draws for serum and plasma in the Database
  - If more than 8 weeks have elapsed since the blood draw for serum and plasma, do the blood draws for serum and plasma for the Database anew
  - If 8 weeks or less have elapsed since the previous blood draw for serum and plasma and the serum and plasma have been kept frozen or were sent to the repository at Fisher BioServices:
    - The blood draw for serum and plasma does not need to be repeated

    - ○ Transcribe the data from the TONIC BP form to the Database BP form; the date in item 4 of the Database BP form should be the current date, but enter the actual date of blood draw (ie, the date from item 9 on the TONIC BP) in item 9 of the Database BP form; when you are asked to apply duplicate labels to the Database BP form, write in the label information and write "see TONIC BP form" in the margin. Key the Database BP form; if the TONIC BP form has already been keyed, it may remain in the data system.
- Blood for genetics repository
  - If blood was not already collected, have the patient sign the Database genetic consent and collect a sample and complete the Database BC and CG forms
  - If blood was already collected, do not send another sample unless the yield was unsatisfactory
  - If the yield on the sample drawn when the patient screened for TONIC was satisfactory, make sure that the TONIC BC and CG forms have been keyed. Complete the Database BC form: answer "No" to item 9 on the Database BC form and write "collected when screening for TONIC" in the specify line. Key the Database BC form. The patient does not need to sign the Database genetics consent and the Database CG form does not need to be completed.
  - If the yield on the sample drawn when the patient screened for TONIC was unsatisfactory, then have the patient sign the Database genetic consent form, draw the replacement sample, and complete the Database BC and CG forms based (the TONIC BC and CG forms can remain in the data system)
- Interviews and questionnaires must be completed on the Database forms
  - Data from the TONIC BG and AD forms may be transcribed to the corresponding Database forms, but the patient should be queried regarding any changes since the previous interviews (**NOTE**: care should be taken in transcribing the BG form since the BG forms are similar, but are not the same); the date in item 4 on each Database form should be the date that the information is reviewed with the patient
  - Patient should complete the Database LP, MA, and quality of life forms anew even if they were completed for TONIC
  - The Database physical examination (PE) form must be completed anew if more than 8 weeks have elapsed since the TONIC PE form was completed; if fewer than 8 weeks have elapsed, repeat the measurement data anew (height, weight, circumferences, skin fold etc) and review the Examination findings (section C) for any findings that are likely to have changed; if the Examination findings are unlikely to have changed, transcribe those data to the Database PE form; otherwise, complete the Examination findings anew; date the PE form with the current date (ie, the date when the measurements were redone)
  - If the patient completed the Brief Block Food Questionnaire in TONIC within the past 8 weeks, he/she does not need to complete it again for the Database. The data from the TONIC BD form should be transcribed to the Database BD form. Put the current date in item 4 of the Database BD form but enter the date when the Brief Block food questionnaire was completed in item 8 of the BD form. The date in item 8 should match the date completed on the questionnaire booklet (bubble

areas) and on the label on the booklet. Where you are asked to affix a label to the BD form, transcribe the information from the label affixed to the TONIC BD form and write in the margin "see TONIC BD form". The TONIC BD form can remain in the data system.

- If the same biopsy is used for the Database that was used for TONIC, the local pathologist must review the slides again and complete the Database HF/HE form. If slides were previously sent for TONIC, the Database SD/SE form must be completed referencing the slide numbers for the slides that were sent (ie, only 10 unstained slides need to be sent from a single biopsy). The TONIC SD form can remain in the data system.
- Retain the TONIC forms in the patient's NASH CRN file

2.7. Co-enrollment in NAFLD Database

- When a NAFLD Database patient enrolls in TONIC, the visit schedule and requirements of TONIC take precedence over the requirements for the NAFLD Database – NAFLD Database requirements are suspended for the duration of the participant's time in TONIC

- NAFLD Database patients may enroll in the TONIC trial after being enrolled in the NAFLD Database for three months or less, depending on the discretion of the principal investigator

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

2.8. Roll over into NAFLD Database after completion of TONIC (form CO)

- Patients who complete participation in TONIC should resume participation in the NAFLD Database (if previously enrolled in the Database) or be invited to join the NAFLD Database (if not previously enrolled).

- The Closeout (CO) form should be completed at the f120 visit (or at the close of the f120 visit window) for all patients randomized in TONIC.

- For patients aged 18 or over, ask the participant if he/she consents to re-entering or enrolling in the NAFLD Database.

- For patients under the age of 18, ask the participant's guardian if he/she consents to their child re-entering or enrolling in the NAFLD Database (follow your institutional IRB guidelines for having the child sign the assent form).

- Participants willing to re-enter or join the NAFLD Database should sign the most recent version of the NAFLD Database assent and/or consent documents approved by your IRB (follow your institutional IRB guidelines for re-consenting participants previously enrolled in the NAFLD Database).

- Each participant who has signed the NAFLD Database consent documents should be scheduled for a NAFLD Database follow-up visit approximately 6 months after the date of their TONIC f120 visit.

- For patients previously enrolled in the NAFLD Database, consult the patient's NAFLD Database visit schedule (time windows guide) generated at their enrollment and schedule the NAFLD Database visit that is open 6 months from the date of their TONIC f120 visit (this visit will be f144 or f192).

- For patients who were not previously enrolled in the NAFLD Database, a new NAFLD Database visit schedule (time windows guide) will be automatically generated when the TONIC Closeout form (CO) is keyed into the online database. The new visit schedule will use the TONIC randomization date as the effective date of enrollment into the NAFLD Database. Schedule the participant approximately 6 months from their TONIC f120 visit for their f144 NAFLD Database follow-up visit.

- For TONIC participants who decline to participate in the NAFLD Database; inform them that the study results and their treatment assignment will be available to them sometime after the close of the TONIC trial.

2.9. Randomization and eligibility checking

Randomization steps

- Complete collection of baseline data and key baseline data forms
- Run electronic check on eligibility (i.e., run the Enroll task, but opt out of randomization and resolve any ineligibility conditions)
- Run the Enroll task and confirm that you want to randomize the patient "now"; this task will officially randomize the patient in TONIC and the randomization assignment and materials needed in followup will be generated (ie, labels, visit time window guide); this task will categorize each patient into one of three treatment groups:
  - Metformin and vitamin E-placebo
  - Vitamin E and metformin-placebo
  - Vitamin E-placebo and metformin-placebo

Overriding eligibility criteria

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

Randomization

- The randomization date is the date on which the clinical center runs the Enroll task and confirms that the patient is to be randomized "now" and the treatment group is assigned
- The randomization date is the "time zero" for reckoning the time windows specified on the patient's TONIC visit time window guide

3. Certification

3.1. Certification overview

What is certification?

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

Why do we require it?

- Primary purpose is to help assure consistent conduct of the study over time, within and across clinics. The conduct of procedures should be similar across patients and in serial testing of the same patient over the duration of follow-up.
- 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 TONIC trial.
- 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.

3.2. Clinical center certification

General comments

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

Purpose of clinical center certification

- Provide information regarding how the clinical center will conduct different aspects of the protocol, who will staff the study
- Guide a clinical center through the steps of getting ready for TONIC – provide a checklist of what needs to be in place before patient activities begin

Requirements for certification of a site

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

3.3. Personnel certification

Staff functions requiring certification

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

Requirements

- Everyone
  - Read the TONIC 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 Pathologist
  - Be approved by David Kleiner and Elizabeth Brunt
- Additional requirements for Data Entry Technician
  - Complete the Data Entry Certification/Decertification Request (DC) form
  - Complete the data system tutorial (personnel previously certified as Data Entry Technician in another NASH CRN study do not need to complete the data system tutorial a second time)

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 TONIC data system
- Staff can be certified for more than one function and may be certified for more than one study (NAFLD Database, PIVENS, TONIC), but will have only one PIN

4. Human subjects

4.1. Background

Consent to participation in the TONIC trial must be completed before screening for TONIC may begin. The patient's parent/guardian must consent to procedures offered to and performed on the patient for screening, as well as to the followup visits which the patient will face in the future. The patient should assent to participation in an age appropriate manner (e.g., written or oral) as deemed appropriate by the site's IRB.

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 TONIC consent process has three major stages:

- The patient/guardian is asked to assent/consent to screening and randomization into TONIC
- The patient/guardian is asked to assent/consent to the collection, storage, and use of blood samples for genetic research
- The patient/guardian is asked to sign the HIPAA authorization to disclose protected health information

4.2. Institutional review board process

Five prototype consent/assent statements have been prepared for the TONIC trial:

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

Clinics are expected to use these materials in their submissions to their institutional review boards (IRBs) for approval to participate in TONIC. Each clinical center must send copies of the consent and assent statements to be used at the clinical center, stamped with their IRB's seal, to the Data Coordinating Center prior to initiating patient activities in TONIC. 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 statement has been approved by an institution's IRB, it cannot be changed without the IRB's approval.

The study protocol, consent and assent statements, 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 TONIC until the site has IRB approval for TONIC and the DCC has certified the site for initiation of TONIC 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

Patients referred to a clinical center for screening may have heard about TONIC, 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 TONIC consents involves two tasks:

(1) A TONIC staff member must sit down with the patient and parent/guardian 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 TONIC study physician (i.e., a TONIC certified hepatologist) must sign the consent/assent 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/assent statements should be offered to the parent/patient to read through at least a day before signature is requested. The consent/assent will then be reviewed with the parent/patient by the staff member designated to obtain consent; the consenter may opt to read the statement to the parent/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 and parent/guardian should sign the assent/consent statement in the presence of the TONIC staff member after all questions have been answered and when the parent/patient has asserted orally that he/she is ready to sign the consent/assent. After the patient and parent/guardian have signed and dated the consent/assent, the parent/patient should meet with a TONIC study physician for the physician to sign the statements; ordinarily this meeting should take place on the same day that the parent/patient signed the statement. The physician should ask the parent and patient to confirm their voluntary consent/assent and query the parent/patient about any questions or concerns about participation. All signatures on the consent/assent statements must be in a non-erasable ink pen. If the physician cannot meet with the parent/patient on the same day that the parent/patient signs the consent/assent 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 TONIC consent is administered, except that it should not be signed until the patient has been determined to be eligible for the TONIC trial. Patients who have already consented to collection and banking of blood for genetic research as part of the NAFLD Database do not need to sign this consent again as part of the TONIC trial (unless the sample obtained for the NAFLD Database was inadequate and a new sample must now be obtained).

4.4. Time considerations for obtaining consent

- The **TONIC Consent and HIPAA authorization** must be obtained at the start of the initial (s1) visit; documents from the referring physician (if any) or from the NAFLD Database 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 TONIC diagnostic tests. A check for signature of this consent statement occurs on the Registration (RG) form.

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

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

4.5. Consent and assent handling

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

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

- The TONIC consent statement is an “all or none” form. The parent/guardian either accepts it in its entirety and signs it, or does not. The parent/guardian must consent to the evaluation procedures, the follow-up evaluations, and the banking of the child’s serum and plasma. If the parent/guardian refuses any part, the child may not enroll in TONIC.

- The TONIC Consent for Genetic Research has been made a separate statement so that the parent/guardian can opt out of genetic research and still have the child participate in the TONIC trial. Adolescents should provide assent to use of their DNA for genetic research.

4.6. Informing participants of changes to consent/assent statements after randomization

As new data become available during the conduct of TONIC, the consent/assent 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/assent statements from the DCC

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

Procedures for reviewing changes to consent/assent statements with participants

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

4.7. Consenting roll over patients from NAFLD Database

If the patient previously enrolled in the NAFLD Database

- Consent as for a new TONIC patient

If the patient previously consented to DNA banking as part of the NAFLD Database

- Patient does not need to sign new consent for genetic banking as part of TONIC

4.8. HIPAA considerations

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

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

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

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

Patient agreement to join the TONIC trial 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 TONIC. The only exception is refusal to provide blood for genetic research; patients may refuse to provide blood for genetic research and still enroll in TONIC.

5. Study visits

5.1. Overview of visit schedule

The patient-related activities of the TONIC trial can be divided into 4 phases:

- Screening for eligibility for enrollment (2 visits over a maximum of 4 months (120 days))
- Randomization to treatment (1 visit)
- Treatment phase (9 visits over 96 weeks)
- Post-treatment washout phase (1 visit at 120 weeks)

5.2. Visits, data forms, and procedures

Phase/ Visit	Form abbr	Procedure
Screening		
s1	RG	Registration (document consent, sociodemographics, assign IDs)
	BG	Baseline history
	PE	Physical exam (detailed)
	SD	Liver biopsy materials documentation
	HF	Liver biopsy histology (reading at clinical center)
	CR	Central histology review
	AD	AUDIT (alcohol questionnaire)
	LR	Lab tests done during at visit s1 and during follow-up
	LS	Lab tests done only during screening (etiologic tests)
	PL	Patient location (patient contact information)
s2	BD	Documentation of completion of the Brief Block food questionnaire
	LP	Symptoms of liver disease (children)
	MA	Modifiable activity questionnaire
	PQ/PR/PW/PY	Pediatric quality of life (parent and child reports)
	DX	DEXA scan for body fat
	MR	MRI report (if available)
	LU	Lab tests required at visit s2
	CG	Genetic consent documentation
	BC	Blood collection for DNA
	BP	Blood processing for serum and plasma
Randomization		
Rz	EC	Eligibility checklist
	RD	Study drug dispensing and return
	PL	Patient location (update as needed)
4 week follow-up visit		
f004	HI	Follow-up medical history
	PF	Physical exam (focused)
	LR	Lab tests done at visit s1 and during follow-up
	RD	Study drug dispensing and return
	PL	Patient location (update as needed)

Phase/ Visit	Form abbr	Procedure
12 week follow-up visit		
f012	HI	Follow-up medical history
	PF	Physical exam (focused)
	LR	Lab tests done at visit s1 and during follow-up
	RD	Study drug dispensing and return
	PL	Patient location (update as needed)
24 week follow-up visit		
f024	HI	Follow-up medical history
	PF	Physical exam (focused)
	LR	Lab tests done at visit s1 and during follow-up
	BP	Blood processing for plasma and serum
	RD	Study drug dispensing and return
	PL	Patient location (update as needed)
36 week follow-up visit		
f036	HI	Follow-up medical history
	PF	Physical exam (focused)
	LR	Lab tests done at visit s1 and during follow-up
	RD	Study drug dispensing and return
	PL	Patient location (update as needed)
48 week follow-up visit		
f048	HI	Follow-up medical history
	PE	Physical exam (detailed)
	BD	Documentation of completion of the Brief Block food questionnaire
	LP	Symptoms of liver disease (children)
	MA	Modifiable activity questionnaire
	PQ/PR/PW/PY	Pediatric quality of life
	LR	Lab tests done at visit s1 and during follow-up
	BP	Blood processing for plasma and serum
	RD	Study drug dispensing and return
	PL	Patient location (update as needed)
60 week follow-up visit		
f060	HI	Follow-up medical history
	PF	Physical exam (focused)
	LR	Laboratory results
	RD	Study drug dispensing and return
	PL	Patient location (update as needed)

Phase/ Visit	Form abbr	Procedure
72 week follow-up visit		
f072	HI	Follow-up medical history
	PF	Physical exam (focused)
	LR	Lab tests done at visit s1 and during follow-up
	BP	Blood processing for plasma and serum
	RD	Study drug dispensing and return
	PL	Patient location (update as needed)
84 week follow-up visit		
f084	HI	Follow-up medical history
	PF	Physical exam (focused)
	LR	Laboratory tests done at visit s1 and during follow-up
	RD	Study drug dispensing and return
	PL	Patient location (update as needed)
96 week follow-up visit		
f096	HI	Follow-up medical history
	PE	Physical exam (detailed)
	SD	Liver biopsy materials documentation
	CR	Central histology review
	DX	DEXA scan for body fat
	MR	MRI report (if available)
	BD	Documentation of completion of the Brief Block food questionnaire
	LP	Symptoms of liver disease (children)
	MA	Modifiable activity questionnaire
	PQ/PR/PW/PY	Pediatric quality of life (parent and child reports)
	LR	Laboratory tests done at visit s1 and during follow-up
	BP	Blood processing for plasm and serum
	RD	Study drug return
	PL	Patient location (update as needed)
120 week follow-up visit		
f120	HI	Follow-up medical history
	PE	Physical examination
	BD	Documentation of completion of Brief Block food questionnaire
	LP	Symptoms of liver disease (children)
	MA	Modifiable activity questionnaire
	PQ/PR/PW/PY	Pediatric quality of life (parent and child reports)
	LR	Lab tests done during screening and follow-up
	PL	Patient location (update as needed)
	CO	Closeout form

5.2. Visits, data forms, and procedures

Phase/ Visit	Form abbr	Procedure

5.3. Guide for visit s1

Procedures

- Obtain signed consent/assent
- Obtain permission to abstract data from patient's medical records
- Initiate data collection for screening and baseline values
  - Interview for baseline history (responses may be modified or expanded upon chart review)
  - Physical exam and anthropometry
  - Have patient demonstrate ability to swallow study medication as needed
  - Liver biopsy (biopsy must have been done within 6 months of randomization; pathologist should grade locally stained slides and obtain 10 unstained slides if possible or arrange for biopsy if appropriate)
  - Alcohol use questionnaire
  - Laboratory testing
- Obtain patient location information
- If patient appears eligible at the close of visit s1
  - Schedule patient for visit s2
  - Schedule patient for any needed etiologic tests
  - Schedule patient for biopsy if appropriate

Data collection forms

- Forms completed for all patients
  - RG - Registration
  - BG - Baseline History
  - PE - Physical Examination
  - SD - Liver Biopsy Materials Documentation
  - HF - Liver Biopsy Histology Findings
  - AD - AUDIT
  - LS - Laboratory Results – Tests Done Only During Screening
  - LR - Laboratory Results – Tests Done at Visit S1 and During Followup
- 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
- Set up TONIC chart for patient and file the materials generated at registration that will be used at visit s2
- Key completed data forms
- Package biopsy slides for sending to the DCC

5.4. Guide for visit s2

Before the patient arrives for the visit

- Abstract data from patient's medical records as needed
- Gather the most recent prior liver biopsy slides as available
- Apply labels to forms as appropriate
- Gather specimen collection and mailing materials (labels, tubes, shippers)
- If advisable, alert phlebotomy lab staff of need to obtain plasma and serum samples (up to 46 samples per patient)
- Confirm eligibility with respect to whatever data have been keyed

Procedures

- Have patient complete diet, activity, and quality of life questionnaires
- Have parent complete parent versions of quality of life questionnaires
- Obtain consent for DNA banking (if available)
- Collect blood for genetic specimen banking (2 tubes) and biosample banking (5 tubes)
- Confirm eligibility (hand/eyeball review of unkeyed data)
- Explain to the patient that you will electronically confirm eligibility after keying the data collected at this visit but that since you believe the patient to be eligible, you would like to schedule the patient for randomization

Data collection forms (form abbreviation)

- Forms completed for all patients
  – DX - DEXA Scan for body fat
  – MR - MRI Report (if available)
  – BD - Food Questionnaire Documentation
  – Brief Block Food Questionnaire (use the printed booklet provided by the DCC)
  – LP - Symptoms of Liver Disease (children)
  – MA - Modiable Activity Questionnaire
  – PQ/PR/PW/PY - Pediatric quality of life (parent and child reports)
  – LU - Lab Tests Done at Visit S2
  – BP - Blood Processing for Serum and Plasma
  – CG - Genetic Consent Documentation (this form documents both consent and refusal)

- Additional forms for patients who consent to blood draw for DNA extraction
  – BC - Blood Collection for DNA

Forms for clinical center use only

- Check for updates to Patient Location (PL)

After the patient leaves the clinical center

- Key data collection forms
- Run Enroll task and re-check eligibility
- Package whole blood tubes for DNA banking for mailing and ship to Genetics Repository
- Process blood to serum and plasma and aliquot for banking; store in local freezer until batch sufficient for mailing accumulates
- Hold food questionnaires for batch mailing to the DCC

5.5. Randomization visit

Procedures

- Patient must have demonstrated ability to swallow medication
- Randomization visit to be conducted as a visit separate from s2
- Patient will be randomized to study drug assignment
- Request for randomization will be made by clinical center using a web based application
- A randomization assignment will be issued only if the database shows that the patient is eligible, that the parent/guardian has signed the consent statement, and that all required baseline data have been keyed to the database
- Patient is given the assigned study drugs, with numbers unique to the patient, instructed about starting the drugs and monitoring for adverse effects, and begins taking study drugs
- Patients of childbearing potential must complete the randomization visit in person (because of the pregnancy test); it is preferable that all patients complete the visit in person, but if the patient is not of childbearing potential, then the visit may be done over the telephone; in that case, the clinical coordinator must affirm over the phone on the day of randomization that the patient feels well and still consents to randomization

Data Collection Forms

- EC - Eligibility Checklist
- RD - Study Drug Dispensing and Return

Comment

- The date of randomization visit is the date for reckoning all follow-up visits

5.6. Visit windows: randomization and follow-up

- **Randomization** must occur within 4 months (120 days) of date of biopsy
- **f004**: window runs from (2 weeks+1 day) through 8 weeks, must be at least 2 weeks after randomization date; ideal date is 4 weeks (28 days) after randomization date
- **f012**: window runs from (8 weeks+1 day) through 18 weeks, must be at least 4 weeks after f004; ideal date is 12 weeks (84 days) after randomization date
- **f024**: window runs from (18 weeks+1 day) through 30 weeks, must be at least 6 weeks after f012; ideal date is 24 weeks (168 days) after randomization
- **f036**: window runs from (30 weeks+1 day) through 42 weeks, must be at least 6 weeks after f024; ideal date is 36 weeks (252 days) after randomization
- **f048**: window runs from (42 weeks+1 day) through 54 weeks, must be at least 6 weeks after f036; ideal date is 48 weeks (336 days) after randomization
- **f060**: window runs from (54 weeks+1 day) through 66 weeks, must be at least 6 weeks after f048; ideal date is 60 weeks (420 days) after randomization
- **f072**: window runs from (66 weeks+1 day) through 78 weeks, must be at least 6 weeks after f060; ideal date is 72 weeks (504 days) after randomization
- **f084**: window runs from (78 weeks+1 day) through 90 weeks, must be at least 6 weeks after f072; ideal date is 84 weeks (588 days) after randomization
- **f096**: window runs from (90 weeks+1 day) through 108 weeks, must be at least 6 weeks after f084; ideal date is 96 weeks (672 days) after randomization
- **f120**: window runs from (108 weeks+1 day) through 132 weeks, must be at least 12 weeks after f096; ideal date is 120 weeks (840 days) after randomization

5.7. 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 performed for a TONIC patient at a time other than the baseline and f096 visit, complete the SD (Liver Biopsy Materials Documentation) form; use the visit code for the followup visit that is open (check the patient's visit time windows guide); if a visit window is not open, use visit code "n"

6. Study procedures

6.1. Assignment of study identifiers

What

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

Materials

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

When

- Eligibility evaluation visit (visit s1)

By whom

- Clinical Coordinator

Procedures

- Complete the TONIC Registration (RG) form; if the patient remains eligible at the close of the form, assign the ID number and code by peeling a label of the label sheet and affixing it to the specified item on form RG
- The patient will be known by these IDs for the duration of the NASH CRN, including participation in any other NASH CRN studies
- Key the Registration (RG) form into TONIC data system; this must be the first form keyed
- The Registration (RG) form should be keyed for each patient screened for TONIC, including patients already enrolled in the NAFLD Database

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 (form BG)

What

- The form queries:
  - Family history of liver disease
  - Information on initial diagnosis of NASH
  - 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 2 years
  - Willingness to use birth control methods

When

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

How

- Mix of interview data and data obtained by chart review
- The smoking interview should be an interview with the patient
- Other questions on the BG form can be answered by interview with the patient, or use all sources to get the most accurate information that you can

Definitions of hepatic events queried on Forms BG and HI

- The TONIC trial 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. Any occurrence of these should be documented on the BG or 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. Follow-up history (form HI)

What

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

When

- All follow-up visits: f004, f012, f024, f036, f048, f060, f072, f084, f096, f120.

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 HI form can be answered by interview with the patient, or use all sources to get the most accurate information that you can

Definitions of hepatic events queried on Forms BG and HI

- The TONIC trial 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. Any occurrence of these should be documented on the BG or HI form. The following guidelines may be useful in defining these events:

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  - **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.

6.4. Physical examination (forms PE and PF)

What

- Anthropometry
  - Height
  - Weight
  - Waist circumference
  - Hip circumference
  - Triceps skinfold
  - Mid-upper arm circumference
- Vital signs
  - Temperature
  - Blood pressure
  - Resting radial pulse
  - Respiratory rate
- System review (limited review for focused physical examination (PF) form)
  - Skin, including grading of acanthosis nigricans
  - Head, eyes, ears, nose, throat
  - Neck
  - Lymphatic
  - Chest and lungs
  - Heart
  - Abdomen
  - Liver and spleen
  - Extremities
  - Genitourinary/pelvis (may be skipped)
  - Nervous system (may be skipped)
  - Tanner stagging (for those who have not reached full sexual maturity and are less than 18 years old)
- Demonstration that patient can swallow TONIC study medication (visit s1/PE form only)
  - Prior to randomization, the Study Physician/Clinical Coordinator must state that they believe that patient will be able to swallow the TONIC study medication
  - The DCC has provided each clinical center with a bottle of placebo metformin capsules (which exceed the size of the vitamin E/placebo vitamin E softgels)
  - Clinic staff may use their judgment about requiring the patient to demonstrate his/her ability to swallow the study medication
  - The PE form includes a reminder about this eligibility criterion

When

- Detailed physical (form PE) at visit s1 and at f048, f096, f120
- Focused physical (form PF) at f004, f012, f024, f036, f060, f072, f084
- All visits are fasting visits

How

- Ideally, use a stadiometer for height measurement.
- Ideally, use the Gulick II tape measure for waist and hip measurement; this device may be obtained from www.fitnessmart.com (608-735-4718, model 67019, listed at $36); it is manufactured by Country Technology Inc: 608-735-4718.
- Ideally, use Harpenden skin fold calipers in the performance of triceps skin fold measurements; this device may be obtained from www.bodytrends.com (800-549-1667), listed at $419.99). Other less expensive calipers are also available.

6.5. Height measurement

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

6.6. Weight measurement

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

6.7. Waist circumference measurement

- Waist circumference may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
- If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
- Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
- Ideally, waist circumference is measured in the morning after voiding and before breakfast; this should be possible in the TONIC since most follow-up 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 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 breathe naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
- Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
- Record the measurement to the nearest tenth (one decimal place)
- Remove the tape, retract the tape, and repeat the procedure
- If the tape cannot be made horizontal across the waist markings, default to the right hip and note this in the margin of the form

6.8. Hip circumference measurement

- Hip circumference may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
- If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
- Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
- Ideally, hip circumference is measured in the morning after voiding and before breakfast; this should be possible in the TONIC since most follow-up 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 stand with feet together
- Pull an appropriate amount of tape out of the housing
- Ask the patient to adjust his/her clothing to allow measuring the hips over the patient's underwear
- Wrap the tape once around the hips over the underwear: the measure should be taken at fullest part of the hips (maximum extension of the bullocks)
- 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 correctly, the patient should be asked to keep his/her arms at the sides and breathe naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
- Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
- Record the measurement to the nearest tenth (one decimal place)
- Remove the tape and repeat the procedure

6.9. Triceps skinfold measurement

- Tricep skin fold measurement should be recorded in millimeters
- Two measurements are to be taken and recorded; repeat measurements until you have two within 10mm of each other
- Ideally a Harpenden skin fold caliper will be used; the accuracy of measurement is dependent upon accuracy of the equipment used, the correct selection and location of the skinfold sites, the proper technique in taking the measurements, and the experience of the tester
- To measure tricep skinfold, measure a vertical fold on the posterior midline of the upper arm, over the triceps muscle, halfway between the acrosion process, and olecranon process. The elbow should be extended and the arm relaxed
- Measurement should be taken on dry skin. Moist skin is harder to grasp and can influence the measurement
- Instruct the rest subject to keep muscles relaxed during test
- Take all measurements on the right side of the body. An exception might be where a deformity or missing limb would necessitate using the left side
- Mark the skinfold site using a pen. Use a tape measure to accurately find the mid points
- The skinfold should be firmly grasped by the thumb and index finger, using the pads at the tip of the thumb and finger. Gently pull skinfold away from the body
- The calipers should be placed perpendicular to the fold on the site marked, dial up at approximately 1 cm below the finger and thumb. While maintaining the grasp of the skinfold. Try to visualize the location of the true double fold of skin thickness, and place calipers there
- A minimum of two measurements should be taken at each site. If repeated tests vary by more than 10 mm, repeat the measurement. If consecutive measurements become increasingly smaller, the fat is being compressed; test the site a little later

6.10. Mid-upper arm circumference measurement

- Mid-upper arm circumference may be recorded in inches or centimeters
- Two measurements are to be taken and recorded; repeat measurements until you have two with 1.5 inches (3.8 cm) of each other
- Measure right arm, with elbow extended and arm relaxed, midway between the acrosion process and the olecranon process

6.11. Baseline and follow-up liver biopsy

- A baseline liver biopsy should be obtained prior to randomization for patients who have been found to be eligible for TONIC with respect to all other criteria and
  - who have not had a liver biopsy within 6 months of randomization, or
  - whose previous liver biopsy is of inadequate quantity

- A followup liver biopsy should be obtained at the f096 visit for all patients enrolled in TONIC

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

6.12. Alcohol use questionnaires (form AD and others)

What / Who

- AUDIT (AD) form
- Summary question on Eligibility Checklist (EC) form
- Questions on interval alcohol consumption on Followup Medical History (HI) form
- Flash Card #11, Drink Equivalents, can be used with the alcohol questionnaires

Purpose

- At screening, obtain a detailed history of the patient's alcohol consumption patterns
- Monitor alcohol use during followup

Who

- All TONIC patients

How

- AD form is self-administered for patients ages 13-17; interviewer administered for patients ages 8-12

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 the AD form, interviews, chart review, discussions with those who know the patient etc; the clinic staff use their best judgement to answer the eligibility question on the Eligibility Checklist (EC) form
- The clinical coordinator should complete section A on page 1 of Form AD and apply labels to subsequent pages before asking the patient to complete the form

6.13. Liver symptom questionnaire (form LP)

What

- LP - Symptoms of Liver Disease (children)

Purpose

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

When

- Visit s2
- At follow-up visits f048, f096, and f120

How

- Self-administered for patients ages 13-17; interviewer administered patients ages 8-12
- Clinical coordinator must be available to answer questions and to review form for completeness

6.14. Diet questionnaire (form BD)

Who/What

- Brief Block Food Questionnaire
- Food Questionnaire Documentation (BD) form
- Portion size illustration (part of the Block Food Questionnaire booklet)
- Parents may complete the food questionnaire for their child

Purpose

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

When

- Visit s2
- Follow-up visits f048, f096, and f120

Procedure

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

- Complete the Food Questionnaire Documentation (BD) form
- Put the completed booklet in a box (or other collection site) at the clinical center to hold for batch mailing to the DCC

Mailing completed questionnaires to the DCC

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

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

Comments

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

6.15. Modifiable activity questionnaire (form MA)

Purpose

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

What / Who

- MA - Modifiable Activity Questionnaire

When

- Visit s2
- Follow-up visits f048, f096, and f120

How

- Self-administered for patients ages 13-17; interviewer administered for patients ages 8-12
- Clinical coordinator should train patient in form completion
- Clinical coordinator should complete pages 2 and 4 and affix labels to pages as needed

6.16. Pediatric quality of life questionnaires (forms PQ, PR, PW, and PY)

Purpose

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

What / Who

- All TONIC patients (form PW [age 8-12] and form PY [age 13-17])
- All parents (form PQ [child age 13-17] and form PR [child age 8-12])

When

- Visit s2
- Follow-up visits f048, f096, and f120

Procedure

- Clinical Coordinator should complete Part A and apply labels to subsequent pages as needed before giving the forms to the patient and parent to complete
- All forms are self administered
- Clinical Coordinator should instruct patient/parent in completion of forms
- Clinical Coordinator should check returned forms for completeness before the patient/parent leaves the clinical center

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6.17. Laboratory measures (forms LS, LR, LU)

Who

- All TONIC patients

What

- Form LS covers assessments collected only at screening:
  - Screening etiologic tests
  - Autoantibody studies
  - Ceruloplasmin measurement
  - Alpha-1 antitrypsin assessment
  - Iron assessments
- Form LR covers assessments collected at visit s1 and during follow-up
  - Initial screening ALT (visit s1; one of two ALT measures during screening; may be historic)
  - Hematology (visits s1, f024, f048, f072, f096, f120)
  - Metabolic panel (visits s1, f004, f012, f024, f036, f048, f060, f072, f084, f096, f120)
  - Fasting lipid profile (visits s1, f024, f048, f072, f096, f120)
  - Fasting serum glucose (visits s1, f024, f072)
  - Hepatic panel (visits f004, f012, f024, f036, f048, f060, f072, f084, f096, f120)
  - Vitamin B12 (visits f024, f048, f072, f096, f120)
  - Prothrombin time, GGT, and HbA1c (f048, f096, f120)
  - OGTT (visits f048, f096, f120)
  - Free fatty acids, leptin, C-reactive protein (visits f048, f096, f120)
  - Urine pregnancy test (all study visits if of childbearing potential)
- Form LU covers assessments collected at visit s2
  - Hepatic panel
  - Vitamin B12
  - Free fatty acids, leptin, C-reactive protein
  - Prothrombin time, GGT, and HbA1c
  - OGTT
  - Urine pregnancy test

When

- Form LS: Visit s1
- Form LR: Visit s1 and all follow-up visits
- Form LU: Visit s2
- Given the extent of information recorded on form LS, you may still be completing it at visit s2 or drawing blood for tests at visit s2; use visit code s1 anyway
- Requirements for fasting – nothing by mouth except water for at least 12 hours before blood draw

6.17. Laboratory measures (forms LS, LR, LU)

Instructions for Form LS

- Most of the tests on Form LS are intended to be obtained by chart review; time windows for the allowed age of tests are specified on the form
- Serological assessment to exclude viral causes of chronic liver disease is required for all patients
- Iron overload screening is required for all patients; hepatic iron index is recorded if available, but is not required
- Ceruloplasmin is required for patients age 40 or younger
- Alpha-1 antitrypsin assessment is required for all patients
- Autoantibody studies are required for all patients

Instructions for form LR

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

Instructions for form LU

- Many measures on form LU may be obtained by chart review; the time window for each type of assessment is specified on the form; the exception is the hepatic panel which must be obtained at the TONIC clinical center and on or after the date when TONIC screening started
- Note that there must be two ALT measures done within 6 months of randomization and both must be > 60 U/L for the patient to be eligible and the one done closest to randomization may not be greater than 400 U/L
- If the chart review tests are out of the time window or the test conditions can't be ascertained or differ from what is required, the measures have to be done at the study visit and can be charged to the study

6.18. DEXA scan for body fat (form DX)

Who/What

- All patients (complete form DX indicating why the scan was not done if patient's size exceeds the capacity of the scanner)

Purpose

- Record scan/scanner information
- Record trunk % fat and total % fat

When

- Visit s2
- Visit f096

Procedure

- Order a DEXA scan for body fat
- If your scanner reports both tissue % fat and region % fat, record region % fat in the trunk % fat item on the DX form
- If your scanner reports both tissue % fat and region % fat, record region % fat in the total % fat item on the DX form

6.19. MRI report (form MR)

Who

- All patients, if available

Purpose

- Record upper abdominal MRI findings

When

- Visit s2
- Visit f096

6.20. Plasma and serum collection for Biosample Repository

Purpose

- Collection of whole blood from all TONIC patients
- Separation of plasma and serum at clinical center: up to three 0.5 mL aliquots of plasma and up to forty 0.5 mL aliquots of serum are to be obtained in 2.0 mL cryogenic vials
- Store plasma and serum aliquots at -70° C prior to batch shipping to the NIDDK Biosample Repository at Fisher Bioservices

Forms / Materials

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

When

- Visit s2
- Follow-up visits f024, f048, f072 and f096 (amount of whole blood collected varies by visit, see BP form)
- Batch shipments: Monthly or semi-monthly

By whom

- Phlebotomist
- Clinical Coordinator

Equipment

Blood tubes/aliquot vials

- One 4.5 mL CTAD (blue top) tube - *provided by the DCC*
- Four 10 mL SST (red top) tubes - *provided by clinical centers*
- 45 or more 2.0 mL cryogenic vials - *provided by clinical centers*
  - vials should be able to withstand -196 degrees C
  - vials should be self standing (flat bottom, not curved), externally threaded, 13.5 mm wide x 48.3 mm tall, with silicone washers
  - one model/vendor that may be used is:
    Model # 5012-0020
    Cyrogenic Storage Systems and Supplies
    243 Lawyers Road, NW
    Vienna, VA 22180
    703-319-8247
    877-738-8247
    703-938-9351 (fax)

Labels

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

Equipment

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

Blood processing procedures

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

Plasma

- Collect blood into CTAD (blue top; Becton-Dickinson) tube
- Fill vacutainer tube with 3 ml whole blood
- Mix gently by inversion 5 times
- Within 30 minutes, centrifuge at 1800 x g for 15 minutes at 4° C, preferably with a swing out rotor
- Immediately after centrifugation, insert a 5 mL pipette below surface of plasma
- Remove the clear plasma while avoiding blood cells
- Transfer plasma into aliquots of 0.5 mL each into 5-6 labeled 2.0 mL cryovials
- Freeze at -70° C immediately

Serum

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

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

6.20. Plasma and serum collection for Biosample Repository

Blood Processing for Plasma and Serum (BP) form

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

6.21. Specimen collection for Genetics Repository

Purpose

- Collection of whole blood from TONIC patients who consent for genetic research
- Shipment of whole blood to the NIDDK Genetics Repository at Rutgers University for DNA banking
- Do not repeat genetic research consent or blood draw for patients who have had blood drawn for genetic research as part of the NAFLD Database

Forms

- TONIC consent for genetic research
- Genetic Consent Documentation (CG) form
- Blood Collection for DNA (BC) form
- NIDDK Genetics Initiative Phlebotomy (GP) form

When

- Visit s2 (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

Equipment

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

Blood collection procedures

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

- Phlebotomist to sign and date the section: To Be Completed by Phlebotomist on the NIDDK Genetics Initiative Phlebotomy form

Applying labels to vials

- Attach the label to the vial when the vial is at room temperature
- Leave the cap on the vial when labeling; the inside of the vial is sterile
- Apply the label to the vial so that the long edge of the label is parallel to the floor when the vial is held in an upright position. The label should not trail off the bottom of the vial or over the cap
- While holding the vial in an upright position, affix the colored portion of the label to the vial first
- Wrap the clear tail around the perimeter of the vial. The end of the clear tail should overlap the colored portion of the label
- Press firmly on the entire label. Verify that all edges of the label adhere to the vial
- When possible, allow newly labeled vials to set at room temperature for several hours prior to subjecting them to colder temperatures. (24-48 hours is optimal)

Packaging procedures

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

Shipping procedures

- Use the preprinted Federal Express shipping label, marked for *Priority Overnight Delivery*, to ship whole blood to the NIDDK Genetics Repository
- Affix the UN3373 BIOLOGICAL SUBSTANCE, CATEGORY "B" label to the outside cardboard box
- 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: Web portal: http://rucdr.rutgers.edu/shippingblood
  - email: witt@biology.rutgers.edu
    peralta@biology.rutgers.edu
  - Fax: 1-732-445-1149
  - Telephone: 1-732-445-1498
- Ship whole blood to:
  Rutgers University/Cell Repository/NIDDK
  604 Allison Rd., Room C120A
  Nelson Laboratory
  Piscataway, New Jersey 08854-8000

Genetics Repository Web Portal System
(Rutgers University Cell and DNA Repository - RUCDR)

- Establishing a Username and Password

  http://rucdr.rutgers.edu/scripts/up.exe?AIMACTION=vnewaccountconiddk&enforce_color=ON&skey=10925637151082500795

- Go to the URL listed above and then just follow the directions on the top of the page. You can sign up for multiple NIDDK sites (if you are associated with more than one) at once. (Phlebotomists performing off-site draws will send a notice from http://rucdr.rutgers.edu/shippingblood.)

Logging in to the System

- The URL for the RUCDR Web Portal is http://rucdr.rutgers.edu. Click on the square for NIDDK to get to your login screen. Enter your newly created username and password. If you ever forget your username or password there is an option on this screen to "Retrieve Lost Password". You will need to remember what email address you used to create your account to use this function!

Announcement Board

- When you enter the web portal you will see announcements from the RUCDR. The dates of future holiday closings will be listed here.

Navigating the Web Portal

- Click the tabs on the top of the screen to access the different parts of the web portal. The functions accessible from each tab are listed below.

Request Functions

- From the "Request Functions" tab you can do two things: "Submit Request" or "Look Up Status of Request".

1. Submit Request

- To get to these options, pick a function from the drop-down menu: Shipping Blood, Request Mailers, or Question.

- Next, pick a site number from the drop-down menu.

- Fill out the section of the form corresponding with the function you chose. Even if your function choice was not "Question" you can add information to any request in the textbox under the heading "Special Notes/Special Instructions/Questions".

- *Good thing to know! If you choose "Shipping Bloods" you can only enter one FedEx tracking number per submission, but if you have more than one sample in the box you can list all the NIDDK ID numbers separated by commas. As always, do not overpack the mailers and enclose a separate piece of paperwork for each sample.*

- In Section 2: Attachments (a light grey area towards the bottom of the page) you can add a file.

2. Look Up Status of Request

- You can search your recent requests to see their status in multiple ways. These are self-explanatory. If you just hit the search button without selecting any search criteria all the requests you have made will be shown.

- There are 4 different status assignments a request can have:
  - Open
  - Assigned
  - Pending
  - Closed

- **Open:** This status signifies that a request has been submitted, but is not yet assigned.
- **Assigned:** This status signifies that an open request is assigned to a particular staff person.
- **Pending:** This status signifies that a request has been assigned and a staff person is working on it, but hasn't yet completed the job.
- **Closed:** When a request is completed the status is set to closed.

Self Help Resources

- This tab is a holding area for useful documents.

1. **FAQ –** If you have a question, hopefully it is already answered here.
2. **Download Center –** These instructions are here! Also, any paperwork enclosed with mailer kits is here in case you need to print off extras.
3. **View Announcements –** In case you missed the announcement page when you first logged in to the web portal you can read it again.
4. **Support Resources** – Links that may be of interest to visit.

Account Management

- From this tab you can "Modify Your Profile" or "Change Password".

Important Information Regarding Blood Shipments

- When a package is received, a mailer request is filled or a question is answered, you will receive an email from us and the status will be changed to "closed".

*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.

6.22. Study drug dispensing and return (form RD)

Forms

- RD - Study Drug and Return form

Drug supply

- Metformin and metformin-placebo: 500 mg capsule, 100 capsules/bottle, taken orally twice a day (bid) with meal. Distributed to patients in a white HDPE plastic bottle
- Vitamin E and vitamin E-placebo: 400 IU/softgel, 100 softgels/bottle, taken orally twice a day (bid) with meal. Distributed to patients in a white HDPE plastic bottle

Distribution of study drug

- Study drug to be dispensed at: Visits Rz, f012, f024, f036, f048, f060, f072, and f084

Return of study drug

- Study drug bottles should be returned at: Visits f012, f024, f036, f048, f060, f072, f084 and f096

By whom

- TONIC clinical coordinator or pharmacist

Ordering procedures at clinical center

- Inventory current drug supplies
- Study drug supplies are shipped to arrive within 2 working days of receipt of order

Handling and disposal

- All unopened bottles of metformin/placebo, or vitamin E/placebo returned by patients should be disposed of per protocol, do not dispense returned drug to patients
- Unused portions of open bottles in the possession of patients should be considered contaminated and handled accordingly
- Returned capsules and softgels should be counted by the pharmacist or clinical coordinator and the number of capsules/softgels and the number of TM series and TE series bottles returned, should be recorded on the RD form

Storage and stability

- Store at room temperature (77 degrees F) in a cool dry place

6.23. Metformin dosing and titration

- Study drugs will be shipped to each clinical center's pharmacy in well marked active or placebo containers. The research pharmacy staff will then provide the investigator with masked study drug based on the DCC randomization schedule. Patients will be dispensed one bottle labeled ***"metformin or placebo"*** and one bottle labeled ***"vitamin E or placebo"***.

- Each day in the morning and evening, one pill from the "metformin or placebo" bottle and one pill from the "vitamin E or placebo" bottle will be taken with food. One of the pills will be a tablet which will be either 500 mg metformin or its placebo. The second pill will be 400 IU soft gel capsule of vitamin E or its placebo.

- The dosage of metformin administered in this study is 500 mg twice daily. However, its administration will take place in two phases: a titration phase and a treatment phase. The use of metformin at the dose of 500 mg twice daily is associated with gastrointestinal side effects at the onset of treatment. These side effects are reduced if the medication is taken with food and the dose titrated from once daily to twice daily over two weeks.

- During the first 2 weeks following randomization, the metformin dose will be 500 mg once daily (q.d.) with the evening meal or bedtime snack. After the first 2 weeks, the metformin dose will then be raised to the full 500 mg twice daily (b.i.d.).

- The dose of metformin may be reduced if intolerable gastrointestinal symptoms develop, based on the investigator's discretion, as follows:

  - If the patient has intolerable symptoms while on 500 mg once daily (q.d.) dosing during the first 2 weeks after randomization, a 2 week hiatus followed by a 2 week re-attempt at 500 mg once daily (q.d.) dosing will occur. The rationale for the re-attempt is to rule out an alternative reason such as intercurrent illness.

  - If the patient has intolerable symptoms while on 500 mg twice daily (b.i.d.) dosing after successful completion of 2-week titration phase, the patient dosing will be reduced to 500 mg once daily (q.d.) dosing.

- At the end of 2-week titration phase, dose will be 500 mg twice daily, AM dose with morning meal and PM dose with the evening meal or bedtime snack.

- If a participant develops side effects thought to be due to study drug and require cessation of study drug, the drug will be stopped for two weeks. If the side effects disappear, a second attempt will be made to reintroduce the study drug after two weeks. If the symptoms reappear, the study physician will discuss termination of study drug with the members of the Executive Committee.

6.23. Metformin dosing and titration

- In case of gastrointestinal side effects, investigators can initially attempt dose reduction instead of stopping therapy. Metformin dose can be cut back during the treatment phase to once daily dosage.

6.24. Adverse event reporting

Definitions

- **Adverse event** is defined as any untoward medical occurrence that may present itself during treatment with a pharmaceutical product or clinical procedure and which may or may not have a causal relationship with the treatment. Adverse events include any unanticipated problems involving risks to participants, or breaches of protocol which might entail risk to participants. The term "unanticipated problem" includes both new risks and increased rates of anticipated problems.
- **Serious adverse event** is defined as an adverse event occurring at any time during the study that results in death, life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Other events may also be considered a serious adverse event if, based on medical judgement, the event jeopardized the patient to the point of requiring medical or surgical intervention to prevent the occurrence of any of the conditions for a serious adverse event listed above.
- **Unexpected adverse event** is defined as any adverse event with specificity or severity that is not consistent with the risk information in the TONIC protocol, current study drug investigator brochure, or current study drug package insert.
- **Associated with the use of the drug** means that there is a reasonable possibility that the adverse experience may have been caused by the study drug.

FDA and "Common Rule" reporting requirements

- The FDA requires notification of occurrence of any serious, unexpected adverse event thought associated with study treatment; notification must be within 15 days of discovery of the event (7 days if the event was fatal or life threatening)
- The "Common Rule" (45 CFR Part 56, Subpart C) requirements are assumed to be fulfilled by two types of reporting in TONIC:
  - Each participating clinical center will send a copy of each adverse event report submitted to the FDA to their IRB (unless their IRB has indicated that the IRB does not wish to receive these reports)
  - Each clinical center must report locally occurring events to their local IRB as required by their local IRB; this may result in an event that happened at your own site being reported to your local IRB but not reported on a TONIC AN form

Patient adverse event reporting in TONIC

- Report unexpected serious adverse events thought to be associated or possibly associated with study treatment or participation on the AN form upon discovery at the clinical center
- Report all other adverse events on another TONIC form(s) promptly; depending on the nature of the event, the form could be the Death Report (DR) form, the Followup Medical History (HI), the followup lab results (LR) form, or the Interim Event (IE) form; the clinic will choose which form(s) to use
- Deciding what category an event is in (eg, what type of event it is and therefore how it gets reported in TONIC) is 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

Other type of adverse event that may occur in a study

- Any event threatening the integrity or well-being of the TONIC trial (eg, suspected fraud or breach of confidentiality of participants) is a reportable event to the study and to your IRB. We recognize that this category is not well-defined; however, it is included as a reminder that reportable events can have a broader scope than events that happen to a patient.
- This type of event is likely best formally reported in a letter to the DCC/NIDDK project officer, with a telephone call in advance of the letter; consultation with your IRB about what to do also may be appropriate

CTCAE v3.0

- The NASH CRN uses the Common Terminology Criteria for Adverse Events, (CTCAE v3.0) to specify and grade adverse events reported on the AN form
- 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 reported on the AN form
- Events which are given severity grade 1 (mild AE) or 2 (moderate AE) should not be reported on the AN form (because they are not considered serious adverse events); use some other TONIC form to report their occurrence (see discussion above)

Clinical center responsibilities regarding a patient adverse event that occurs at your clinical center

- Figure out what type of event it is and complete the appropriate form (see discussion above)
- Deciding what type of event has occurred is the responsibility of the TONIC Principal Investigator of the center; the study chairs, the NIDDK project officer, and staff at the Data Coordinating Center are available for consultation
- If you report the event on the AN form (ie, you decide it is serious, unexpected and related or possibly related to TONIC treatment or participation), then do the following upon discovery of the event:
  - Complete the AN form and key it
  - Prepare a narrative description of the event and your subsequent course of action – describe what happened, the actions taken in response to the event, and the relationship of the event to the TONIC drugs or procedures
  - Prepare correspondence to your IRB reporting the event
  - Send a paper copy of the AN form, the narrative (in paper or electronic form), and the report to your IRB (in paper or electronic form) to the DCC
  - Be sure to refer to the patient by his/her NASH CRN patient ID number and code; do not use the patient's name
  - Confirm receipt of the report at the DCC by telephone or email

  - Provide a followup report when the event is resolved or if there is a significant change in the patient's condition or in the physician's judgment about the event since the previous AN form was within one month to report the details of the disposition of the event
- If you report the event on forms other than the AN form (ie, it is unrelated to study participation or treatment or it is not serious although related to study participation or treatment), then be sure to key the forms promptly; no other action is required by the clinic with regard to reporting for TONIC (if the event meets your local IRB's requirements for reporting to it, then proceed as required by your local requirements)
  - The DCC will review the electronic database periodically to cull this information and report it to the study investigators and the DSMB as appropriate
- The clinical center investigator may also be responsible for completing an FDA MedWatch 3500 form

Data Coordinating Center responsibilities regarding patient adverse events

- The DCC will send a copy of each AN form received, the narrative, and the local report to the IRB to:
  - to each clinical center, with instructions for each center to forward the report to their IRB
  - the NIDDK project officer
  - the Data and Safety Monitoring Board (DSMB)
  - the FDA
- The Data Coordinating Center will maintain a list of all events reported on AN forms for review at Steering Committee meetings and DSMB meetings
- The DCC will prepare tallies of events reported on other forms as appropriate and as requested by the DSMB and or Steering Committee

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

- When you receive a report from the DCC regarding occurrence at another NASH CRN clinical center of a serious adverse event thought to be associated or possibly associated with TONIC treatment or participation, you should forward that report to your IRB unless your IRB has indicated to you that it does not want to see reports of these events.

Local reporting requirements

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

6.25. Adverse event reporting management

- Potential adverse effects of metformin and safeguards to minimize risks are:
  - Gastrointestinal side effects: as many as 30% of patients receiving metformin report diarrhea, nausea, metallic taste, abdominal bloating, flatulence, or anorexia; these should be minimized by recommending that metformin be taken with food and titrating the dose over two weeks when metformin is initiated
  - Anemia: about 6-9% of patients receiving metformin may develop reduced vitamin B12 levels; vitamin B12 levels will be measured at baseline and every 24 weeks thereafter during treatment (at f024, f048, f072, f096); patients with low B12 levels at baseline or with diminishing B12 levels during follow-up will be treated according to standard clinical practice
  - Lactic acidosis: Reported incidence is about 0.03 cases/1000 person-years; however the fatality rate when it occurs is 50%. Patients with renal dysfunction or with serum creatinine >1.5 mg/dL (males) / >1.4 mg/dL (females) or with cirrhosis will not be enrolled. Patients and families will be educated about the need to avoid intravenous contrast while taking study medication. If intravenous contrast is needed electively or emergently, the study drug will be stopped for 3 days starting with the day of dye administration and serum creatinine will be measured on day 3 (i.e., 48 hours after dye administration). Study drug will then be restarted on the 4th day (72 hours after dye administration) only if the serum creatinine from day 3 is <1.5 mg/dL (males) / <1.4 mg/dL (females). During follow-up, serum creatinine is measured at every visit to monitor for renal dysfunction

- Potential adverse effects of vitamin E and safeguards to minimize risks are:
  - Toxicity of vitamin E is very low
  - Development of new onset diabetes: It is assumed that the risk of development of new onset diabetes in the vitamin E group will be similar to the risk in the placebo group; measures to identify patients who have new onset diabetes include frequent assessment of symptoms consistent with uncontrolled hyperglycemia, measurement of fasting serum glucose at f024, f048, f072, f096, f120. Patients who have fasting serum glucose of 126 mg/dL or greater will have the measurement repeated within 6 weeks. If diabetes is diagnosed, the patient will be managed as described in the protocol and the primary care physician will be notified.

- Potential adverse effects of placebo and safeguards to minimize risks are:
  - Development of new onset diabetes: It is assumed that the risk of development of new onset diabetes in the vitamin E group will be similar to the risk in the placebo group; measures to identify patients who have new onset diabetes include frequent assessment of symptoms consistent with uncontrolled hyperglycemia, measurement of fasting serum glucose at f024, f048, f072, f096, f120. Patients who have fasting serum glucose of 126 mg/dL or greater will have the measurement repeated within 6 weeks. If diabetes is diagnosed, the patient will

be managed as described in the protocol and the primary care physician will be notified.

- Other potential adverse events are those related to blood draws for the study (such as hematoma, cellulitis, phlebitis, and arterial puncture) or to liver biopsy procedures; if such an event occurs, appropriate medical care should be provided immediately in the clinic.

- If a suspected adverse event is reported by telephone at the time of the event or 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.

- It is possible that some TONIC patients will develop significant liver-related morbidity or liver-related 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 may also be considered a reportable adverse event according to the local institutional guidelines.

6.26. Procedures for unmasking treatment assignment

- Treatment assignments will remain masked until all data collection for the TONIC trial has been completed (i.e., until every patient has completed the f120 visit) except as described below

- Unmasking of study drugs will occur under the following conditions:
  - **Severe allergic reaction (Stevens-Johnson Syndrome)**: Study drugs will be stopped indefinitely. The patient, primary care provider (PCP), and the investigator will be unmasked.
  - **Pregnancy during the study**: Study drugs will be stopped indefinitely. The patient, PCP, and investigator will be unmasked and notified of the assigned treatment's risks of teratogenicity.
  - **Development of hepatotoxicity**: Study drug will be discontinued according to the guidelines outlined in the TONIC protocol. The patient, PCP, and the investigator will be unmasked.
  - **New onset diabetes**: The patient will be referred to their PCP and managed according to the stepped care approach defined in the TONIC protocol. The patient and PCP may be unmasked, but the investigator will continue to be masked.

- In unforeseen situations where the clinical center principal investigator considers unmasking is in the best interest of the participant's health and well being, unmasking may be done after notifying the Executive Committee.

- The Data and Safety Monitoring Board will review all instances of unmasking that occur.

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

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

6.28. 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/evening
  - Send letter via regular or certified registered mail to determine if patient is still at listed address
- Check current telephone directory for listings both for the patient and the patient's contacts specified on the PL form, eg., next of kin, health care professionals
- Check post office for forwarding address; ask patient's contacts for forwarding address
- Check obituaries
- Check state vital records

6.29. Procedures for mortality closeout

Purpose

- Record participant death

Forms

- Complete the Death Report (DR) form

By whom

- Study Physician and Clinical Coordinator

6.30. Medical management of patients (standard of care)

To keep recommendations and care for participants in the study as uniform as possible, investigators should generally discuss with participants what is laid out in the Standards of Care Documents for Pediatric Patients with Fatty Liver Disorders. These can be found in TONIC SOP Part V.

6.31. Transferring patients from TONIC to the NAFLD Database (form CO)

Purpose

- To close out a patient's participation in TONIC and document the patient's assent and guardian's consent to join or re-enter the the NAFLD Database

Form

- Closeout (CO) form

When

- The Closeout form should be completed at the f120 visit (or at the close of the f120 visit window) for all patients randomized in TONIC.

By whom:

- Clinical coordinator

Instructions

- Ask the patient and their guardian if he/she consents to re-entering or enrolling in the NAFLD Database

- Guardians of patients willing to re-enter or join the NAFLD Database should sign the most recent version of the NAFLD Database consent documents approved by your IRB (follow your institutional IRB guidelines for re-consenting participants previously enrolled in the NAFLD Database).

- Each patient whose guardian has signed the consent documents should be scheduled for a NAFLD Database follow-up visit approximately 6 months after the date of their TONIC f120 visit. For patients previously enrolled in the NAFLD Database, consult the patient's NAFLD Database visit schedule (time windows guide) generated at their enrollment and schedule the NAFLD Database visit that is open 6 months from the date of their TONIC f120 visit (this visit will be f144 or f192).

- For patients who were not previously enrolled in the NAFLD Database, a new NAFLD Database visit schedule (time windows guide) will be automatically generated when the TONIC Closeout form (CO) is keyed into the online database. The new visit schedule will use the TONIC randomization date as the effective date of enrollment into the NAFLD Database. Schedule the participant approximately 6 months from their TONIC f120 visit for their f144 NAFLD Database follow-up visit.

6.31. Transferring patients from TONIC to the NAFLD Database (from CO)

- For TONIC participants who decline to participate in the NAFLD Database; inform them that the study results and their treatment assignment will be available to them sometime after the close of the TONIC trial.

7. Forms management

7.1. Clinical center ID codes

Alphabetic IDs

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

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

Numeric site IDs

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

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

7.2. Patient identifiers

What

- Patient ID number
- Patient code

Patient ID number

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

Ranges of patient IDs assigned to clinics

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

Patient code

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

7.3. Visit ID code

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

s1	Screening
s2	Screening and baseline data collection
rz	Randomization
f004	4 weeks follow-up visit (approximately 1 month)
f012	12 weeks follow-up visit (approximately 3 months)
f024	24 weeks follow-up visit (approximately 6 months)
f036	36 weeks follow-up visit (approximately 9 months)
f048	48 weeks follow-up visit (approximately 12 months)
f060	60 weeks follow-up visit (approximately 15 months)
f072	72 weeks follow-up visit (approximately 18 months)
f084	84 weeks follow-up visit (approximately 21 months)
f096	96 weeks follow-up visit (approximately 24 months)
f120	120 weeks follow-up visit (approximately 30 months)
n	Unscheduled visit

7.4. General guidelines for forms completion

Ink

- Forms should be completed in blue or black ink that is dark enough to photocopy legibly; use pencil only for Block questionnaires.

Changing responses on forms

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

Multipage forms

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

Miscellaneous

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

Calculations

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

7.5. Instruction box

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

7.6. Form skips, stops, ineligibility symbols

Skip pattern

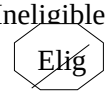
- Form navigation (skip pattern) instructions are indicated in **boldface**. Skips are designated by an arrow from that response to a box with the number of the next item to be completed.

Stop sign

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

Ineligibility sign

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



Other

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

7.7. Headers and footers

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

TONIC Patient ID: __ __ __ __

Form RG TONIC
Revision 0 (17 Feb 04) RG - Registration 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. 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: TONIC 3

- The form and revision number will be printed on the form in item 6; if a form is only used for one specific visit, the visit code will also be printed on the form

- 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 – e.g., 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 permanently missing height would be completed as m _ _._ and a temporarily missing height would be completed as ? _ _._).

- 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.

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, this area may also include the PIN and signature of other staff

7.11. Handling forms

Form duplication

- The forms 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 – 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 randomized in TONIC should be kept in a single folder in a locked room or locked filing cabinet.
- Each patient who is randomized in TONIC 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 TONIC 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 TONIC, apply the rounding rule only at the last step, when required to record a quantity on the TONIC 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:

- Liver biopsy pathology reports
- DEXA (Dual Energy X-ray Absorptiometry) scan reports
- MRI scan reports
- Laboratory test result reports
- Medical records for archival information
- Institutional drug accountability logs.
- 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 TONIC
- Tour facilities
- Discuss with clinical center personnel any problems that have occurred or that are expected to occur in conducting the study

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

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

Participants

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

Reviewed during site visit

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

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

- Enrollment and retention
  - Status
  - Recruitment and retention strategies
  - Problems
  - Losses to 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
  - Study drug storage and dispensing
  - 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 enrollment reports that will provide a count of participants enrolled at each clinical center

- 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 TONIC 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, after receipt of data transmittals from the clinical centers, DCC will generate a database report of:
  - number of participants enrolled
  - missed visits
  - incomplete visits (missing or pending forms)
  - missed specimen collection or shipment
  - edits (see below)

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

Forms audits

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

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

NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

TONIC Trial

Standard Operating Procedures

Part IV:

Liver Biopsy
and
NAFLD/NASH Histology Scoring System

24 May 2005

TONIC SOP IV: Biopsy and Histology Scoring

Contents

TONIC SOP IV: Biopsy and Histology Scoring

1. Overview

1.1. Philosophy

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

The procedures specified by the NASH CRN for their studies are based on two concepts. First, a primary goal of the NASH CRN is to collect a database of specimens along with clinical and laboratory data, so that this information remains available for research after the NASH CRN has disbanded. The other concept behind these procedures is the necessity of a system of evaluation of NASH that can be utilized in evaluating efficacy in treatment trials. Because there are no serum markers or other diagnostic modalities that can accurately stage or grade the liver disease in these patients, a liver biopsy remains the standard against which all other methods will be compared. Every effort should be made to build a slide archive that is 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, study consent statements 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 should not be enrolled in the NASH CRN.

Histologically confirmed NAFLD is an inclusion criterion for the TONIC trial. The baseline biopsy may have been done prior to screening (within specified time limits) or it may be done as standard of care as part of the screening procedure. TONIC patients will also have a followup biopsy after 96 weeks of treatment in the trial. Unscheduled biopsies also may occur after screening. Unlike PIVENS and the NAFLD Database, TONIC is not banking liver tissue samples. Ideally, the TONIC trial will obtain 10 unstained slides for archiving from each of these biopsies. However, because some of the biopsies evaluated for TONIC may not provide these materials (eg, 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.

1.1. Philosophy

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

1.2. Tasks and forms related to liver biopsy

The TONIC eligibility criteria include histologic evidence of NAFLD according to the NASH CRN protocol for histology scoring. The biopsy that is used to satisfy eligibility may be a historical biopsy (done no more than 6 months prior to randomization) or it may be done prospectively under the care of the TONIC investigator as a screening procedure. Each randomized patient will have another biopsy after 96 weeks of treatment. In addition, a patient may have an interim biopsy as needed for standard of care. As a check to be sure that all biopsies that occur are caught, occurrence of a biopsy since the previous TONIC visit is queried on the Followup Medical History (HI) form.

Information about the biopsy procedure and materials is captured on the Liver Biopsy Materials Documentation (SD) form. The SD form also documents the outcome of the biopsy with regard to availability of stained and unstained slides for scoring and archiving. If the biopsy is a screening biopsy (ie, done/evaluated to determine eligibility for TONIC), then the local TONIC Study Pathologist must complete the Liver Biopsy Histology Findings (HF) form. The steatosis score must be at least 1 ($\geq$ 5%) for the patient to be eligible. Other forms that the TONIC trial 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 four forms (SD, HF, CR, TS) are used to:

- Document what slides, if any, are available for scoring and sending to the DCC and whether liver tissue was obtained for banking (form SD)
- If the biopsy is the screening biopsy, remind the clinical center that the screening biopsy cannot be older than 6 months at the time of randomization (form SD)
- Document local scoring of baseline biopsy (form HF)
- Document scoring of baseline and followup biopsy by the NASH CRN Pathology Committee (form CR)
- Document shipment of slides to the DCC (form TS)

1.2. Tasks and forms related to liver biopsy

The TONIC hepatologist, the Study Pathologist, and the Clinical Coordinator must work together to accomplish these tasks and complete the required forms. Considerable cooperation and close communication will be required to complete these tasks successfully.

2. Obtaining liver biopsy materials for scoring for TONIC

2.1. Overview

Baseline (screening) 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 for TONIC has been obtained. In the case of (1), we will try to obtain 10 unstained slides for the exclusive use by TONIC, but limited biopsy materials may require that the institution's biopsy slides be borrowed. In the case of (2), sufficient tissue should be collected to provide for the institutional pathology slides AND 10 unstained slides for TONIC exclusive use. Followup liver biopsies should all be in category (2).

Baseline biopsies will be scored by the local TONIC Study Pathologist (to determine eligibility) and also centrally (after randomization), by the Pathology Committee. Biopsies obtained 96 weeks after randomization will be scored centrally only, by the Pathology Committee. Unscheduled biopsies 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 TONIC 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 TONIC purposes. However, it is an unfortunate fact that the amount of tissue available in archived paraffin blocks may be very limited.

Upon learning that a patient had a biopsy in the 6 months prior to screening (and not so close to being 6 months old that screening cannot be completed prior to closure of the 6 month window at randomization), the Clinical Coordinator should obtain the original pathology materials: the surgical pathology report, the institution's H&E and Masson's trichrome slides, and the paraffin block so that 10 unstained slides may be obtained (see below for how to prepare and label those slides).

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

- Determine the number and type of the institution's stained slides available for the local review of the biopsy and determine if any stained slides need to be sent to the DCC; if fewer than 2

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

- 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 TONIC is borrowing the stained slides from the institution or if TONIC 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

The Study Pathologist should complete the TONIC Liver Biopsy Histology Findings (HF) form using the institution's H&E stained slide and Masson's trichrome stained slide, as described later in this document. If the steatesis score is 0 (zero), the patient is ineligible for TONIC.

If there is no H&E stained slide or if there is no Masson's trichrome stained slide, the biopsy is insufficient for evaluation for entry into TONIC. However, the patient may be eligible for the NAFLD Database under criteria that do not require histologic evidence of disease (e.g., the NAFLD Database includes patients with suspected disease as well as those with histologically confirmed disease).

If only the H&E and Masson's trichrome slides are available, these should be reviewed locally. If the patient is found to be eligible, these slides will need to be sent to the DCC for central review by the Pathology Committee. Both of these slides must be available for central review for the patient to be found eligible for TONIC.

The TONIC trial should request that the slides be provided outright, with no arrangements to return the slides at the end of the trial. 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 trial. 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 are not randomized in TONIC should be returned upon determination that the patient will not be randomized.

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

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

In order to insure adequate material for histologic review, investigators should obtain needle core biopsies of at least 1.5 cm length. Each clinical center will follow their centers guidelines for pediatric liver biopsies. Suggested guidelines are: Patient will present fasted with no liquids for at least 12 hours. Intravenous sedation will be done on prepped and draped patient. Under sterile field, a needle biopsy device will be positioned by ultrasound guidance via the right axillary approach. One core will be biopsied from the right lobe of the liver to be placed in formalin for later preparation of slides for light microscopy and stains including H & E and Masson's trichrome. Following the procedure, subjects will be placed and on their right side and monitored for 4 hours to include vital check every 30 minutes and hematocrit values. All abnormalities will be followed to resolution and per good clinical practices guidelines.

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 TONIC Study Pathologist for the local evaluation (i.e., for completion of form HF).

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

2.4. Preparation of slides

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

SuperFrost Plus slides, Precleaned

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

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

2.5. Labeling stained and unstained slides at the clinical center

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

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

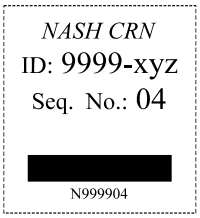
The requirements for the labeling scheme for slides are:

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

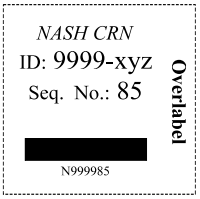
The solution to these requirements is to use Brady labels in the format shown below: (1) labels for unstained slides and (2) labels for stained slides which are borrowed.

2.5. Labeling stained and unstained slides at the clinical center

(1)



(2)



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

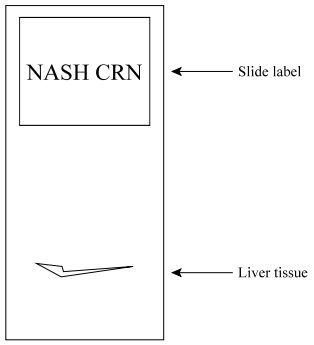
The unique identifier for a slide will consist of the patient ID number and a sequential number. The sequence numbers on the labels for unstained slides run from 01 to 60 (60 is the maximum number of unstained slides that we think we will ever get for any one patient over the life time of all NASH CRN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

The slide labels include the following information:

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

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).



3. Development of the histology scoring system

3.1. Background

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

3.2. Methods and validation

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

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

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

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

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

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

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

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

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

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

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

4.1. Introduction

The local TONIC Study Pathologist will evaluate baseline biopsies only, for eligibility determination. TONIC patients must have histologically confirmed steatosis.

The local TONIC Study Pathologist should make his/her evaluation using the locally stained H&E slide (all features except fibrosis) and Masson's trichrome slide (fibrosis only) and should complete the Liver Biopsy Histology Findings (HF) form. A copy of the HF form is included at the end of this document for your information; please obtain blank forms for completion for a patient from the study website (www.nashcrn.com).

4.2. Guidelines for features scored in the local evaluation

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

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

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

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

4.2.2. Steatosis location

Steatosis location has 4 choices for characterization:

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

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

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

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

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

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

4.2.5. Portal chronic inflammation (0-1)

0: None to minimal
1: Greater than minimal

4.2.6. Hepatocellular ballooning (0-2)

0: None
1: Few
2: Many

4.2.7. Steatohepatitis diagnosis

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

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

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

4.2.8. Exclusion of other liver disease and other features

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

Diagnosis of any chronic liver disease (aside from NAFLD) and diagnosis of cirrhosis are exclusionary; these diagnoses are marked with Caution symbols on the HF form.

4.2.9. NASH Activity Score (NAS)

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

4.2.10. Comments

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

4.3. Unscheduled liver biopsy

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

5. Central pathology evaluation (for Form CR)

5.1. Procedures

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

Following central review, slides will be returned to storage at the DCC. Slides that are only loaned to the TONIC trial 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 viewed will be recorded on the CR form for each case.

5.3. Guidelines for features scored in the central evaluation

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

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

Steatohepatitis diagnosis and amount of portal inflammation are scored both centrally and locally, but the scoring of these features for the central evaluation differs from the scoring for the local evaluation.

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

5.3.1. Length of biopsy

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

5.3.2. Microvesicular steatosis, contiguous patches

0: Absent
1: Present

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

5.3.3. Microgranulomas seen (yes/no)

5.3.4. Large lipogranulomas seen (yes/no)

5.3.5. Portal chronic inflammation

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

5.3.6. Acidophil bodies

0: Rare/absent
1: Many

5.3.7. Pigmented macrophages (Kupffer cells)

0: Rare/absent
1: Many

5.3.8. Megamitochondria

0: Rare/absent
1: Many

5.3.9. Mallory's hyaline

0: Rare/absent
1: Many

5.3.10. Glyogen nuclei

0: Rare/absent
1: Many

5.3.11. Iron: hepatocellular grade (0-4)

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

5.3.12. Iron: hepatocellular distribution

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

5.3.13. Nonhepatocellular iron grade (0-2)

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

5.3.14. Nonhepatocellular iron distribution

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

5.3.15. Steatohepatitis diagnosis

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

5.3.14. Iron: sinusoidal lining cell iron distribution

5.3.16. Comments

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

6. Shipping slides to the Data Coordinating Center

6.1. Packing and shipping slides

The steps in shipping slides to the Data Coordinating Center are:

- Complete the Histology Slide Transmittal Log (Form TS), specifying the sequence numbers for the stained and unstained slides for each patient included in the shipment; specify the biopsy date for the slides 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
  - Wrap the box in bubble wrap and tape securely
  - Place the bubble wrapped box inside a padded jiffy bag. Place the jiffy box and pathology reports and Form TS in a Federal Express box; stuff the Federal Express box as needed with newspaper or other packing material so that the bubble wrapped box does not move around
  - Ship second day arrival to:

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

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

6.2. Receipt of slides at the Data Coordinating Center

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

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

6.3. Returning slides to the clinical center

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

7. Appendices

Central Histology Review

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

A. Clinic, patient and visit identification

__ __ __ __ 1. Center ID

__ __ __ __ 2. Patient ID

__ __ __ 3. Patient code

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

__ __ __ __ 5. Visit code

c r 1 6. Form and revision

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

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

B. Slide sequence number

9. Sequence number for

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

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

__ __ . . . c. Iron stained slide

__ __ . . . d. Other slide

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

C. Administrative information

__ __ __ 10. CC Initials

_____ 11. CC Signature

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

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

_____ 14. Followup with clinic (*Specify*):

_____ *Patient ID* **D. Histology**

_____ 15. Biopsy length (mm)

H & E stain

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

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

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

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

17. Inflammation

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

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

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

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

18. Liver cell injury

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

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

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

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

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

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

Masson's trichrome stain

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

22. Iron stain

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

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

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

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

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

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

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

26. Features suggestive of steatohepatitis etiology for cryptogenic cirrhosis:

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

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

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

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

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

27. Other comments: _____

TONIC

HF - Liver Biopsy Histology Findings

Purpose: Record results of local histologic evaluation of slides from liver biopsy.

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

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

Instructions: The Study Pathologist should complete this form using the institution's H & E slide and if available, the institution's Masson's trichrome slide. Upon completion of this form, the Study Pathologist should give the original HF form to the Clinical Coordinator. If fewer than 2 unstained slides are available for the biopsy, the institution's H & E and Masson's trichrome slides must be sent to the DCC for central pathology review. If 2 or more unstained slides are available for the biopsy, only the unstained slides need to be sent to the DCC. The Study Pathologist should forward the stained slides (if needed) and up to 10 unstained slides to the Clinical Coordinator for forwarding to the Data Coordinating Center. If Elig is checked for any item, the patient is not eligible for TONIC and the form should not be keyed. If △C is check for any item, use caution. If the Study Physician agrees with the diagnosis, the patient is ineligible for TONIC and the form should not be keyed.

A. Center, patient and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date of reading:

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

5. Visit code: s 1 ____ ____

6. Form & revision: h f 1

7. Study: TONIC 3

B. Biopsy information

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

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

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

a. H & E: (1)

b. Masson's trichrome: (1)

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

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

a. Grade:

< 5% (0) Elig

5-33% (1)

34-66% (2)

> 66% (3)

b. Location:

Zone 3 (0)

Zone 1 (1)

Azonal (2)

Panacinar (3)

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

0: None (0)

1a: Zone 3, perisinusoidal (requires trichome) (1)

1b: Zone 3, perisinusoidal (easily seen on H&E) (2)

1c: Portal/periportal only (3)

2: Zone 3 and periportal, any combination (4)

3: Bridging (5)

4: Cirrhosis (6)

12. Inflammation

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

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

b. Amount of portal, chronic inflammation:

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

13. Hepatocellular ballooning:

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

14. Is steotohepatitis present:

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

D. Exclusion of other liver disease

15. Is there evidence of primary biliary cirrhosis:

Yes (* 1) C No (2)

** Caution: Primary biliary cirrhosis is exclusionary*

16. Is there evidence of Wilson's disease:

Yes (* 1) C No (2)

** Caution: Wilson's disease is exclusionary*

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

a. Bile duct loss/infiltration/sclerosis: (* 1) C

b. Florid duct lesions: (1)

c. Cholate stasis: (1)

d. Copper deposition: (1)

e. Other *(specify):* (1)

_____

f. None: (1)

** Caution: Bile duct anomalies*

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

a. Vascular lesions of ALD/B-C/OVD: (1)

b. Inflammation suggestive of AIH, HCV: (* 1) C

** Caution: Autoimmune liver disease and HCV are exclusionary*

c. Pigment suggestive of HH: (* 1) C

** Caution: Hemochromatosis is exclusionary*

d. Globules suggestive of A1AT: (* 1) C

** Caution: Alpha-1 antitrypsin deficiency is exclusionary*

e. Hepatocellular changes suggestive of HBV: (* 1) C

** Caution: HBV is exclusionary*

f. Granulomas suggestive of sarcoid, PBC, infection: (* 1) C

** Caution: Primary biliary cirrhosis is exclusionary*

g. Other *(specify):* (1)

_____

h. None: (1)

19. Is there evidence of cirrhosis:

Yes (1) No (2)

E. Other features

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

a. Mallory's hyaline (r/o cholate stasis): (1)

b. Perisinusoidal fibrosis away from septa: (1)

c. Hepatocyte ballooning: (1)

d. Megamitochondria: (1)

e. Other *(specify):* (1)

_____

f. None: (1)

F. Other comments

21. Other comments:

_____

_____

_____

_____

_____

G. Administrative information

22. Study Pathologist PIN: ____ ____ ____

23. Study Pathologist signature:

_____

24. Clinical Coordinator PIN: ____ ____ ____

25. Clinical Coordinator signature:

_____

26. Date form reviewed:

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

TONIC

SD - Liver Biopsy Materials Documentation

Purpose: To document that the liver biopsy required for screening was obtained under the time and medication restrictions required by the protocol. The number and type of slides available for archival at the Data Coordinating Center is noted for both screening and followup biopsies. If slides cannot be archived at the Data Coordinating Center, the source of the slides and the time by which the slides must be returned to the clinical center are recorded.

When: Visits s1, f096, and as needed for biopsies at interim times.

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

Instructions: This form provides information about the tissue and slides from the biopsy and alerts the DCC to expect receipt of slides from the biopsy at the Data Coordinating Center. It also provides a record of the source of the slides, the number and type of stained slides available for review at the clinical center, the need (if any) to borrow those stained slides or provision of those stained slides to the NASH CRN without requiring return of the slides, and the number of unstained slides to be provided to the NASH CRN. A copy of the original surgical pathology report for the biopsy must be obtained; the patient's name should be blacked out, the report should be annotated with the patient's NASH CRN ID number and code, and the annotated report should be stapled to the back of this form. The surgical pathology report documents the date of biopsy. The slides should be labeled using the labels provided by the DCC. For unstained slides use permanent labels with sequence numbers 01-60, the borrowed slides should be labeled with removable overlabels with sequence numbers 81- 90.

A. Center, patient and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date form initiated:

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

5. Visit code ____ ____ ____ ____

6. Form & revision: s d 1

7. Study: TONIC_3

B. Surgical pathology report

8. Is a copy of the report annotated with the patient's NASH CRN ID number and code and with name blacked out attached to this form:

(Yes 1) (No * 2) STOP

** Obtain a copy of the report, annotate it, and attach it. You can use one of the pathology labels to annotate the report.*

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

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

C. Requirements for screening biopsy

10. Is this visit s1:

(Yes 1) (No 2) → **12.**

11. Is the date in item 9 within 6 months (183 days) of the anticipated date of randomization:

(Yes 1) (No * 2) Elig

** Biopsy date must be within 6 months of randomization.*

D. Biopsy specimens and stained slides at the clinical center

12. What stained slides from the biopsy are available at the clinical center *(check all that apply)*

a. H & E stain: (1)

b. Masson's trichrome stain: (1)

c. Iron stain: (1)

d. Other *(specify):* (1)

_____

e. Other *(specify):* (1)

_____

E. Unstained slides to be sent to the DCC

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

(Yes 1) (No 2)

16. ___

14. How many unstained slides will be sent to the DCC: ___ ___

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

a. Slide sequence number: ___ ___ 01-60

b. Slide sequence number: ___ ___ 01-60

c. Slide sequence number: ___ ___ 01-60

d. Slide sequence number: ___ ___ 01-60

e. Slide sequence number: ___ ___ 01-60

f. Slide sequence number: ___ ___ 01-60

g. Slide sequence number: ___ ___ 01-60

h. Slide sequence number: ___ ___ 01-60

i. Slide sequence number: ___ ___ 01-60

j. Slide sequence number: ___ ___ 01-60

F. Stained slides to be sent to the DCC

(The institution's stained slides must be sent to the DCC only if fewer than 2 unstained slides will be sent to the DCC)

16. Are any stained slides to be sent to the DCC:

(Yes 1) (No 2)

23. ___

17. How many stained slides to be sent to the DCC: ___

18. Sequence number of slides to be sent to DCC

a. Slide sequence number: ___ ___ 81-90

b. Slide sequence number: ___ ___ 81-90

c. Slide sequence number: ___ ___ 81-90

d. Slide sequence number: ___ ___ 81-90

19. Are any stained slides to be returned to the clinic:

(Yes 1) (No 2)

22. ___

20. List sequence numbers of those slides to be returned

a. Slide sequence number: ___ ___ 81-90

b. Slide sequence number: ___ ___ 81-90

c. Slide sequence number: ___ ___ 81-90

d. Slide sequence number: ___ ___ 81-90

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

Immediately after central review (1)

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

22. Which pathology department did these slides come from:

NASH CRN clinical center's pathology department (1)

23. ___

Other, *(specify):* (2)

_____
name

_____
address

_____
address

_____
address

_____
phone

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

G. Administrative information

23. Clinical Coordinator PIN: ____ ____ ____

24. Clinical Coordinator signature:

_____

25. Date form reviewed:

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

TONIC

TS - Histology Slide Transmittal Log

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

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

A. Center, date, shipping and study identification

1. Center ID: ___ ___ ___ ___

2. Date form completed:

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

3. Study: TONIC 3

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

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

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

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

_____

6. Shipment tracking number:

_____

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

_____
please print

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

_____

_____

_____

B. Slide shipment information

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

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

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

26. Other comments regarding contents of shipment received:

_____

_____

_____

27. Person receiving shipment (please print):

_____

28. Date shipment received and reviewed:

____ ____ - ____ ____ ____ - ____ ____
day month year

NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

Treatment of Nonalcoholic Fatty Liver Disease in Children (TONIC)

Standard Operating Procedures

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

12 September 2005

Contents

TONIC SOP 6 Standard of Care for Pediatric 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 Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) to the care of pediatric patients with fatty liver disorders. As directed by the NASH CRN Steering Committee, these standards were developed so that pediatric 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 NASH CRN Steering Committee and approved in principal at its meeting on September 22, 2002 in Atlanta, GA. Committee members are aware that application of the standards described here could be viewed as an intervention in itself. However, the committee also felt that these standards are essential to fulfill our obligation to provide the best care to the participating pediatric patients.

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

2. Specific recommendations

2.1 Evaluation of patients with suspected NASH

a. Obtain the following:
   i. AST, ALT, GGT, fasting lipid profile, insulin and glucose
   ii. Alpha-1-antitrypsin pi type, ceruloplasimin, hepatitis panel
   iii. Imaging study (US, CT, MRI) to evaluate fat in the liver

b. Liver biopsy can be considered in patients with suspected NAFLD to confirm the diagnosis and stage the degree of injury. Imaging and degree of abnormality in serum aminotransferases may suggest but not reflect the diagnosis or degree of injury

2.2 Dietary intake

a. A "heart healthy", food-guide pyramid-based diet will be recommended to patients. Specific recommendations will include:
   i. Less than 30% of calories from fat
   ii. "Five a day" (five servings of fruits and vegetables per day)
   iii. If subject is overweight, then modest total calorie restriction, calculated from expected needs based on height and age, with weight loss goal of 1-4 pounds per month.

b. Materials from the USDA, the NCES hot food facts and Fitness Foundation, and the Weight Control Information Network (WIN) will be provided to support these recommendations. General healthy eating principles, including three meals a day, minimal juice and soda, and avoidance of fast food, will be emphasized.

c. Patients with known or newly discovered type 2 diabetes will receive specific recommendations as promulgated by the ADA.

d. Recommendations regarding the use of specific nutritional supplements are addressed below.

e. Dietary recommendations may not apply to all persons or situations.

2.3 Weight loss

a. While weight loss recommendations will be individualized, very overweight subjects (BMI $\geq$ 95%) will be given a goal of losing and sustaining the loss of 10% of body weight or down to BMI< 85%. This weight loss should be achieved at a rate of 2 - 4 pounds per month per expert committee recommendations.

b. Food guide pyramid and other age-appropriate materials will be used as instructional material.

c. Patients will be instructed not to fast as a means of achieving weight loss.

d. Alternative diet plans will not be recommended.

e. Family education regarding healthy eating, fast food, etc. will take place. Educational materials on parenting skills and obesity will be developed.

2.4 Alcohol consumption

a. Pediatric patients will be reminded that alcohol consumption is prohibited.

2.5 Exercise

a. Patients will be instructed to exercise for a minimum of 60 minutes or more 5 or more times per week. This will be defined as continuous physical exertion sufficient to raise the heart rate of 130 and "break a sweat." Patients/families will be advised to limit TV-watching and video/ computer game time to less than 2 hours per day.

2.6 Preventive medicine

a. **Vaccination for viral hepatitis**. Hepatitis B vaccine is standard of care for children. The AAP Redbook on Infectious Disease recommends that hepatitis A vaccine be considered in patients with chronic liver disease. Therefore disparities in site-specific practices would have no impact on the studies of the NASH CRN.

b. **Hepatocellular carcinoma screening.** Although recent data suggest that cirrhosis caused by NASH is associated with a similar risk of developing hepatocellular carcinoma as other major causes of cirrhosis, there is lack of consensus in the field regarding an optimal cost-effective screening strategy, particularly in pediatrics. Screening methods will not be standardized across sites, but will be in accordance with local standards.

2.7 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 a pediatric endocrinologist for appropriate management.

ii. Patients with controlled diabetes (HgB $A_{1C} < 7\%$) will be continued on their current treatment regimens.

iii. Patients with suboptimally controlled diabetes (HgB $A_{1C} \geq 7\%$) will receive a recommendation for follow-up with their pediatric endocrinologist for improved glycemic control.

b. **Hypertriglyceridemia**

Patients with fasting triglycerides > 150 will be referred to dietitian and those with triglycerides > 250 should be further evaluated.

c. **Hypercholesterolemia**

Patients with fasting total cholesterol levels > 200 mg/dL will be referred to a dietitian for step 1 diet (NCEP) and those with cholesterol > 220 should be further evaluated.

d. **Hypertension**

Patients with repeated systolic blood pressure or diastolic blood pressure > 95% for age and height (NHLBI) will be referred for further evaluation.

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

f. **Hyperandrogenism and polycystic ovary syndrome (PCOS)**
Girls with hirsutism (facial and/or chest hair) and menstrual irregularities not associated with prepubescence will be referred to the appropriate specialist.

g. **Occupational exposure to hepatotoxins, recreational drugs, etc**.
A history of ongoing exposure to volatile hydrocarbons or recreational drugs will be sought. All subjects with positive histories will be cautioned regarding dangers of use and instructed to avoid/stop usage.

h. Evaluation of pediatric NASH patients with age < 5 years or developmental delay will include urine organic acids to evaluate for underlying metabolic disease.

2.8 Possibly helpful concomitant medication use

a. Ursodeoxycholic acid (UDCA, Actigall, Urso, Ursodiol)
   i. UDCA will generally be stopped unless new data is published to indicate a significant benefit for patients with NASH.
   ii. UDCA washout may be required before therapy studies.
   iii. UDCA will be continued in patients who have shown a significant improvement associated with treatment in liver histology or surrogate markers for NAFL such as ALT or imaging studies.

b. Metformin
   i. Patients receiving metformin as a treatment for diabetes will remain on the drug.
   ii. Patients receiving metformin as a treatment of other disorders of insulin resistance (e.g., PCOS) will remain on the drug.

c. Fibrates: There is little experience in children. Use will be decided on an individual case basis in children with hypertriglyceridemia.

d. Statins: There is little experience in children. Use will be decided on an individual case basis in children with hypercholesterolemia.

e. Thiazolidinediones (TZDs; pioglitazone, rosiglitazone)
   i. Patients receiving a TZD as a treatment for diabetes will remain on the drug.

2.9 Possibly harmful concomitant medication use

a. Acetaminophen
   i. Acetaminophen should be restricted to < 45 mg/kg/d in any given day.
   ii. Repeated use of > 20 mg/kg daily for more than 3 consecutive days should be discouraged.
   iii. Families should be warned that many OTC preparations contain acetaminophen. Labels should be read carefully.
b. Anticonvulsants: Children with seizure disorder will continue on previous anticonvulsants. Neurologists treating children with NASH with valproate will be asked to change to a different anticonvulsant if possible.
c. Estrogens (OCP, HRT)
   i. Estrogen use as oral contraception will be permitted.
d. Iron supplements
   i. Patients with iron deficiency will be allowed to continue the use of iron supplements until iron stores are sufficient. 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%.
   ii. 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.
e. Accutane
   i. Accutane may cause elevations in liver enzymes as well as lipids. In the context of NASH, this drug should be discontinued.
f. Prednisone
   i. Use should be minimized.

2.10 Possibly helpful concomitant dietary supplement use

a. Multivitamins. A daily multivitamin with iron content < 20 mg daily will be allowed.
b. Betaine. Betaine should not be used outside of a trial.
c. S-adenosylmethionine. SAM should not be used outside of a trial.
d. Herbals will not be allowed.
e. Creatine and other bodybuilding supplements will be forbidden.

2.11 Possibly harmful concomitant dietary supplement use

a. Vitamin A supplements in excess of that contained in a daily multiple vitamin (5000 IU) should not be used.
b. Herbal supplements will not be allowed.

3. Implementation

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

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

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

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

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

4.1 Physician summary of guidelines

a. Physicians at the study sites will need ongoing reminders of the specifics of the standards of care.
b. Perhaps a pocket card and a small poster for patient care areas are needed.

4.2 Patient brochures

a. What brochures are needed:
    i. Healthy eating
    ii. Healthy weight loss
        (1) BMI formula and curves
        (2) Goals
    iii. Handout on parenting skills (See Section 5.1)
    iv. General NASH brochure to cover most other recommendations
        (1) Acetaminophen use
            a. Allowable amounts
            b. List of medications
        (2) Supplemental iron use
        (3) Vitamins
            a. Allowable vitamin A
            b. MVI daily
        (4) Warning about herbal remedies
        (5) Symptoms to report
            a. Sleep apnea
            b. Irregular menstruation, facial hair
b. Brochure development
    i. Content: Standards of Care Committee (See Section 5.2)
    ii. Design: Need a professional aesthetically pleasing design
    iii. Printing: Local center to arrange for printing, distribution, cost recovery
c. Updates to the brochures
    i. Content to be reviewed annually and discussed at Steering Committee meetings
    ii. Revised content and design to be prepared within 4 weeks of review at Steering Committee. Revisions to be distributed to the Steering Committee members for final approval.

4.3 Referring physician information

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

4.4 Website

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

5. Parent guidelines and patient brochure

Printable versions of the Parent Guidelines and Patient Brochure are available on the NASH CRN website: http://www.jhucct.com/nash/closed/docs/sop/database/sop.htm. If you print the guidelines or brochure from the SOP, the headers and footers will cause the documents to not fit the page correctly; therefore, you must go to the website to print the documents.

5.1 Parent Guidelines: Helping your overweight child

Your family will have to make real changes to help your child lose weight. Use the following guidelines to plan small steps towards change.

- **Offer healthy choices.** Let your child choose between an apple and popcorn, not between an apple and a cookie. Offer choices of activities (say bike riding vs. basketball) without TV as an option.

- **Do not keep tempting foods around.** If your child does not have access to regular soda, chips or candies, then he'll have to make other choices. Provide snacks at snack time rather than keeping the pantry open.

- **Parents decide what food is offered and when; the child decides whether to eat.**

- **Help your child get 5 servings of fruit and vegetables/day.** Not only does this encourage weight loss, it protects from a number of diseases.

- **Limit portion sizes.** We often feel satisfied when the plate is empty – if serving sizes are smaller, your child may feel full with less food. Parents can control serving size by preparing the plate in the kitchen rather than allowing children to serve themselves.

- **Avoid fried foods.** Fat has twice as many calories as protein or carbohydrates. Minimize fast food, which is almost always high fat. Read labels. Choose low fat foods at restaurants (salads and no fries).

- **Never use food as a reward.** Use activity or time with parents as a reward.

- **Don't require child to "clean" his plate.**

- **Have regular family mealtimes, offering 3 meals and perhaps one snack each day.**

- **Encourage 30 minutes to 1 hour of physical activity each day.** This should be activity vigorous enough to "break a sweat." Ideally, spend some time exercising with your child.

5. Parent Guidelines: Helping your overweight child

- **Limit TV viewing (and computer/video games) to 1 to 2 hours/day**, as recommended by the American Academy of Pediatrics. Studies have shown a strong association between prolonged TV watching and obesity. This is probably partly due to inactivity, partly due to "munching while watching" and partly due to high fat food advertising.

- **Find reasons to praise healthy behavior.** Remember that while behavior can be good or bad, children are always good.

- **Model healthy eating behavior.** If your child sees you eating healthy and exercising, he is more likely to imitate your behavior. Don't have your child on a different "diet" than the rest of family.

Be consistent – you will probably have to guide your child over and over before you make real, permanent lifestyle changes. Good luck!

Adapted from Obesity Evaluation and Treatment, Expert Committee Recommendations. Barlow et al: Pediatrics 102(3), 1998.

(Parent Guidelines for Overweight Child)

5.2 Patient brochure

A dietitian can help you figure out the best way for *you* to eat and suggest changes that fit with your schedule and tastes.

Physical Activity:

Limit television and computer games to one hour a day or less. It's easy to spend hours in front of the screen, but your body needs to move around more

Aim for 60 minutes a day of physical activity. Start with half an hour and slowly increase. You can do 5 or 10 minutes at a time but it's a good idea to have 30 continuous minutes of an activity that makes you break a sweat.

Activity ideas:

- Sign up for PE, a team, martial arts, or dance class.
- Walk and talk. If you enjoy telephone calls with a friend, invite the friend to walk and talk instead.
- Dance to the music. Put on your favorite music and dance for half an hour.
- If you work part-time, pick a job where you do some moving. Mow lawns or wait tables rather than run a cash register or (worse) cook French fries.

If you feel discouraged, talk to your doctor. He or she can suggest additional help, such as a meeting with a dietitian.

Other handouts are available.

"Just enough for you"
http://www.niddk.nih.gov/health/nutrit/pubs/justenuff/justenough.htm

"Helping your overweight child"
http://www.niddk.nih.gov/health/nutrit/pubs/helpchld.htm

"Take charge of your health"
http://www.niddk.nih.gov/health/nutrit/pubs/winteen/index.htm

FATTY LIVER IN CHILDREN

Jean Pappas Molleston and Sarah E Barlow

What is fatty liver?

Fat can sometimes accumulate in the liver in a condition called fatty liver, "hepatic steatosis", or "NAFLD" (Non-alcoholic fatty liver disease). If the liver is being injured by the fat, causing abnormal blood tests or damage to the liver tissue, the condition is called "steatohepatitis" or "**NASH**" (Non-alcoholic steatohepatitis). Note: the "non-alcoholic" term means that the liver abnormality is NOT from drinking excess alcohol. The "hepatitis" term means that the liver is injured, not that there is a virus.

What causes fatty liver?

Most commonly, fatty liver is seen in obese patients. Diabetics, patients with cholesterol or triglyceride problems, and some women with a syndrome called "polycystic ovary syndrome" are at increased risk, as well. A few medicines, such as prednisone, have also been associated with fat in the liver. New research suggests that insulin, a hormone that helps control blood sugar, doesn't work well in obese patients; this "insulin resistance" may contribute to the liver problem in NAFLD. A few patients with fatty liver are not overweight; the cause of their NAFLD is unknown.

What does the liver do?

The liver performs many important functions in the body. It helps make proteins that make the blood clot and keep fluid within blood vessels. It helps get rid of wastes, including bilirubin, a substance which makes people jaundiced (yellow in color) when it accumulates. The liver helps the body use sugars, proteins, and fats from the diet. It also helps process some drugs.

What tests can be done to tell how my liver is doing?

Your doctor will do a physical examination, during which she/he measures liver and spleen size by pressing on the abdomen; she/he will also look for dark skin around the neck that some NAFLD patients have. Your doctor may obtain an ultrasound, CT, or MRI; these x-ray tests can often detect excess fat in the liver. Blood work measures liver enzymes including ALT and AST; elevations in liver enzymes suggest damage to liver cells. Blood tests may be done to exclude other causes of liver disease and to look at possible underlying causes of fatty liver. Sometimes, a liver biopsy is done so that a tiny piece of liver can be examined under a microscope to evaluate liver injury and scarring.

What will happen to my liver if I have NASH?

Doctors are researching outcome in patients who have fatty liver. In some patients, there is no significant damage to the liver from fat. In others, however, scarring and even cirrhosis (severe scarring) of the liver can occur, usually after many years.

What can I do to help my liver?

1. Gradual weight loss, via diet and exercise, is the most reasonable approach to NAFLD at this time. Research is underway to evaluate potential drug therapy to help.
2. Avoid alcohol, and other drugs or substances that can damage the liver.
3. Ask your doctor about immunization for Hepatitis A, to protect your liver from further injury if you happen to be exposed to that virus.
4. If you have diabetes or elevated cholesterol/triglycerides, controlling these conditions with diet and medicines can help your liver.

HELP YOUR LIVER BY HEALTHY LIVING

Extra body fat contributes to NASH. These days, many Americans are overweight. If your doctor has told you that your weight is higher than is healthy, then these guidelines will help you balance how much you eat and how active you are so that you will gradually lose the extra weight.
Remember, the extra weight came on slowly and slow reduction is the safest road - aim for 2 to 4 pounds a month.

Practice healthy habits

- It takes over 6 months to make a behavior a habit.
- Set a goal, keep track on a calendar, and then reward yourself for success! For example, if you dance 5 times a week for 3 weeks, pick out a new CD.
- Get help from your family: if everyone in the home is living healthier, you'll feel more motivated and won't face so many temptations.

Healthy eating changes:

The food guide pyramid is your best guide.
Eat five fruits and vegetables a day: for example, strawberries on your cereal, carrots and half a banana with your lunch, celery and peanut butter for snack, and a salad with dinner.
Don't drink your calories. Do you drink soda or juice when you are thirsty? Switch to water or calorie-free drinks.
Choose naturally low-fat foods, like chicken, bread, fruits and vegetables. Don't fry. Less than 30% of your calories should come from fat. Avoid fast food; burgers, fries, and pizza are full of fat.
Watch out for portion size. People feel satisfied when their plate is empty, so put smaller servings on your plate.
Eat at the table with the television off, even for snacks.
But drink plenty of low fat (1% or skim) milk. It's a great way to build bones, essential when you are a teenager.
Eat breakfast every day. Even a small breakfast will keep you from getting too hungry and overeating after school.