EFFECT OF OBESITY ON HEPATIC STEATOSIS AND FIBROSIS IN A HEPATITIS-C INFECTED POPULATION

A Thesis

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CERTIFICATE OF APPROVAL

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ABSTRACT

Recently, the media has been saturated with reports of America's new "obesity epidemic". The deleterious effects of obesity on health have also been well publicized: obesity contributes to the development of high blood pressure, high cholesterol, hyperlipidemia, diabetes, osteoarthritis, and obstructive sleep apnea, among other problems[1]. Obesity is also a very strong risk factor for coronary heart disease. A recent study of the Nurses' Health Cohort, consisting of over 88,000 American women, showed that each one unit of BMI increment translated into an 8% increased risk of coronary heart disease.[2] In fact, studies have shown that moderate weight loss can be as effective as an antihypertensive pill in lowering blood pressure[3].

Although most of the public is aware of some of the medical risks associated with obesity, few people are aware of the fact that long-standing obesity can also result in liver failure: obese individuals can develop hepatic steatosis, in which fat is deposited in the liver. Hepatic steatosis represents the first along a continuum of conditions that make up the term "nonalcoholic fatty liver disease" (NAFLD). Initially, fat infiltrates the liver, a condition called "steatosis", which can eventually result in inflammation, referred to as "non-alcoholic steatohepatitis" (NASH), scarring, and eventually cirrhosis independent of other recognized causes of liver failure such as heavy alcohol use and chronic viral hepatitis[4].

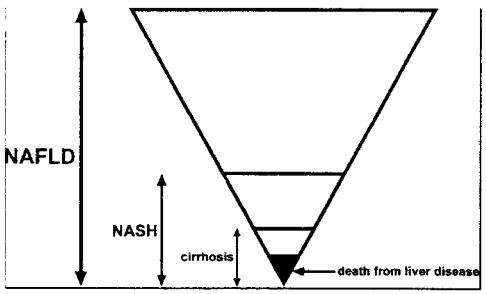

Figure 1: NAFLD pyramid: About 20% of adults have excess fat in the liver, shown here as an inverted pyramid. Of these people, 10% to 15% have NASH and 20% of those with NASH are at risk for developing cirrhosis. Up to 30% to 40% of those with NASH cirrhosis will die from end-stage liver disease[4].

The purpose of this investigation was to examine the association between chronic hepatitis C infection and obesity with advanced hepatic fibrosis and steatosis in a community population infected with hepatitis C. A secondary goal was to also examine any other significant risk factors for the two outcomes. This information can serve to educate affected individuals about their health and treatment options.

Advanced steatosis was found to be positively, independently associated with obesity, advanced fibrosis, and multiple metabolic co-morbidities. Advanced fibrosis was found to be positively, independently associated with advanced steatosis, male gender, site, and age.

BACKGROUND

Obesity and Hepatic Steatosis

It is estimated that one quarter (900,000) of Americans are obese[5], one of the highest rates in the world. Although the reasons for this epidemic are multi-fold, they distill down to two factors: exercising less and eating more, via bigger portions and higher caloric foods. The annual costs of obesity are enormous. In 2003, the direct health care costs of obesity were $96 million, resulting in an annual average of $732 health care dollars per person.[6] However, a hidden cost of obesity unknown to much of the American public is non-alcoholic fatty liver disease (NAFLD), which has an estimated prevalence of 20% (50 million) among the general adult American population[4], and up to 75% in patients with obesity and type II diabetes mellitus.[7] Twenty per cent of the NAFLD population will have steatohepatitis (two million) and 10% (200,000) of these will progress to cirrhosis.[8] NAFLD is conjectured to be the cause of 14% of liver transplants in the US.[9]

Hepatic steatosis, the process of fatty replacement of liver parenchyma, can also result from toxic insults to the liver, such as medications, alcohol, or hepatitis C genotype 3.[10] Drug-induced steatosis is seen with long-term use (more than six months) of prescription medications such as corticosteroids, estrogens, tamoxifen, and methotrexate, of which the latter can exacerbate steatotic-induced hepatic fibrosis. Unfortunately, discontinuation of the hepatotoxic substance may not affect the progression of hepatic steatosis to fibrosis.[11] However, steatosis from medications is rare. Obesity likely remains the most common cause of hepatic steatosis due to its widespread prevalence.

Another pathologic finding on liver biopsy is hepatic fibrosis, representing scarring of the liver parenchyma. A variety of insults can induce hepatic fibrosis, including heavy alcohol use, chronic viral hepatitis, and biliary obstruction from autoimmune diseases such primary biliary cirrhosis or primary sclerosing cholangitis. Fibrosis is a progressive process, which can be accelerated by factors such as age, duration of viral hepatitis infection, and degree of inflammation on biopsy.[4] Once the hepatic parenchyma is completely replaced with fibrosis, a condition called cirrhosis, hepatic failure can occur, manifested as clotting difficulties, protein malnutrition, and changes in mental status (hepatic encephalopathy). As some of these etiologies of fibrosis also cause steatosis, it is important to evaluate the relationship between the two.

To understand the cause and potential treatments for hepatic steatosis and its sequelae, it is necessary to understand the "metabolic syndrome": the triad of dyslipidemia, insulin resistance, and obesity.[12] Obesity is defined by the medical community as a body mass index* (BMI) of 30 or greater.[13]

Although prevalent in only 4.6% of normal weight Americans, 22.4% and 59.6% of over-weight and obese persons, respectively, will have the metabolic syndrome. This corresponds to at least 27 million American adults. In general, the prevalence of the metabolic syndrome increases with age, but a growing number of adolescents who are obese are being diagnosed with type 2 diabetes, a criterion for the metabolic syndrome. Overall, between 1995 and 2000, the prevalence of obesity increased from 55.9% to 64.5% of the American population. Women and certain ethnic groups, such as Hispanics, are more susceptible to the metabolic syndrome. In fact, half of all cardiovascular events in women may be related to metabolic syndrome. Surprisingly, African-American men

*BMI = weight in kilograms / (height in cm^2)

have a lower occurrence of the metabolic syndrome than Caucasian men, due to lower prevalence of hyperlipidemia, despite higher prevalence of hypertension and diabetes. The prevalence of metabolic syndrome is higher among African-American and Hispanic women than men.[12]

Commonly, the metabolic syndrome also results in hepatic steatosis. A common pathway for each of the metabolic co-morbidities that comprise the metabolic syndrome is increased body fat. As fat is deposited subcutaneously, it is also deposited *viscerally*, within surrounding tissues and organs, such as the liver, causing hepatic steatosis. Increased visceral fat is seen clinically as "central obesity", resulting in a disproportionately high waist-to-hip ratio.[4] Increased visceral fat also promotes insulin resistance, which can progress to the development of type 2 diabetes mellitus. Resultant dyslipidemia causes triglycerides to accumulate in the liver.[4]

The association of steatosis and dyslipidemia is further strengthened by observing the effect of steatosis among patients with hereditary lipid disorders, such as familial hypobetalipoproteinemia (FHBL). FHBL patients have similarly deranged regulation and transport of triglycerides as do patients with hepatic steatosis. A study among these patients versus controls showed that liver fat percentage was positively correlated with BMI and waist circumference. Despite five-fold differences in liver fat percentage in cases versus controls, mean values for obesity and insulin indexes were similar. Thus, for similar degrees of obesity, FHBL subjects had more hepatic fat.[14]

It is hypothesized that mitochondrial injury causes steatosis because of impaired beta-oxidation of fatty acids, which generates reactive oxygen species (radicals) and ATP depletion. The excess amount of hepatic fat leads to an abundance of free fatty acids,

which may be peroxidized into free radicals. These free radicals are harmful to hepatocytes and produce inflammatory cytokines and, eventually, fibrosis. Other theories of how steatosis causes liver injury result from experience in animal studies. In these experiments, steatosis results in decreased adenosine triphosphate (ATP) production, the source of energy of cells, probably as a result of mitochondrial damage and disturbance of blood flow through liver sinusoids.[11] Other contributing factors may include Kupffer cell dysfunction and leukocyte adhesion. Fatty hepatocytes have reduced tolerance against ischemic injury with a predominant necrotic form of cell death.[15] In addition, the ability of hepatocytes to regenerate after major tissue loss is impaired in the steatotic liver. In hepatitis C, specific core proteins of virus genotype 3 have also been postulated to independently promote hepatic steatosis.[16]

Although hepatic steatosis itself is often benign, it represents the first stage of a continuum of disease. In patients with a high burden of hepatic steatosis, a condition called "steatohepatitis" can occur, in which the liver becomes inflamed, similar to the inflammation seen in chronic hepatitis C infection or acute alcoholic hepatitis. When steatohepatitis occurs in the absence of alcohol, it is called "non-alcoholic steatohepatitis" (NASH). Obesity-induced steatosis can progress to non-alcoholic steatohepatitis (NASH) and, eventually, cirrhosis. It is unknown why some cases of steatosis progress, but risk factors include: age, diabetes, obesity; an elevated ratio of the liver enzymes alanine aminotransferase to aspartate transaminase (AST/ALT), and hepatic histology.[4] In a recent study of 48 morbidly obese nondrinkers (BMI of 60) undergoing gastric bypass, 65% had moderate to severe steatosis, 33% had evidence of NASH, and 12% had advanced fibrosis. There was a very strong association between diabetes and NASH

(odds ratio of 128) or severe fibrosis (odds ratio of 75). Age, sex, BMI, and fasting triglyceride levels were not associated with the presence of NASH or advanced fibrosis once adjusted for diabetes.[17] However, the lack of association after adjusting for such a strongly associated risk factor may be due to the small sample size.

Abdominal ultrasonography and elevated liver enzymes (ALT, AST, GGT) have been used to detect the presence of hepatic steatosis, but are neither very specific nor sensitive tests. The gold standard is a liver biopsy. It is also possible to quantify hepatic fat content on abdominal CT scans in Hounsfield units or fast hepatic MRI scan using a modified Dixon technique.[18] In a study of twenty-two obese pediatric patients, twenty-one had an elevated hepatic fat fraction on fast hepatic MRI scans. Among the seven with a hepatic fat fraction less than or equal to 18%, all had normal serum ALT. However, twelve of the remaining thirteen subjects with fat fraction of greater than 18% had moderately elevated serum ALT. However, there was no correlation between hepatic fat fraction and age, BMI, or serum AST. This may be due to the fact that AST is found in other places in the body, such as muscle and red blood cells, whereas ALT is chiefly found in the liver. This suggests that BMI itself is an insensitive measure of hepatic fat fraction and that using elevated liver enzymes, especially AST, is likely an insufficient measure in the presence of a low fraction of hepatic fat.[19]

Treatments for hepatic steatosis are aimed at reversing the insulin resistance via diabetic and lipid-lowering agents, as well as antioxidative substances such as vitamin E, although the latter has yet been shown to be beneficial. Of course, modifying the initial factor, obesity, is the ultimate goal, although this is notoriously difficult to achieve, much less maintain. To further complicate the situation, rapid weight loss can exacerbate

hepatic damage. Some studies have suggested that ursodeoxycholic acid is able to reduce serum levels of hepatic enzymes in patients with nonalcoholic fatty liver disease, but this effect has not been shown to modify liver fat content. A Brazilian study showed that treatment with ursodeoxycholic acid reduces hepatic enzymes in patients with NAFLD, but had no effect on the amount of liver fat content.[20] Currently, no treatment is known to reverse hepatic damage from NASH or NAFLD, apart from minimizing other risk factors.

Hepatitis C

Another very common liver disease is chronic hepatitis C infection. An estimated 2.7 million Americans are chronically infected with hepatitis C, in addition to nearly another million who have been infected in the past.[21]. Since the advent of blood screening tests in early 1990's, the majority of incident infections are from intravenous drug use (IVDU),[21] making hepatitis C an enormous social and public health problem, with the burden of disease falling most heavily on those with lower socioeconomic status. The annual financial burden of hepatitis C infection has been estimated to be at a minimum of $600 million a year[22], and was estimated as $15 billion in 2000;[23] the vast majority of this sum was spent treating complications from liver failure. Both these estimates may be conservative since they do not include costs related to pain and suffering and the value of care provided by family members. The year 2000 estimate is comparable to the $18 billion estimate for annual cost of asthma, a much more publicized and less stigmatized disease.[24]

Unlike hepatitis B, the incidence of which has been dropping due to increasing vaccination rates in high-risk populations, no vaccine exists for hepatitis C. Current prevention strategies for reducing the incidence of hepatitis C include providing intravenous drug users information about needle exchange programs and sterilization techniques, as well as discouraging needle sharing. Antiviral treatment for hepatitis C has been improving over the years, but remains expensive and rife with morbidities, including teratogenicity. Thus, therapy is reserved for a select population who are likely to be compliant with therapy and to be able to afford the cost; likely non-compliance is considered to be an absolute contraindication for treatment.[25] The treatment dropout rate averages 15-20% due to drug side effects, such as depression, insomnia, and low blood cell counts.[26] Furthermore, even among those who are able to complete a full course of treatment, usually 48 weeks of daily ribavirin pills and weekly injections of pegylated interferon, a sustained viral response (SVR) is successfully reached in a minority of cases. A recent study reported a SVR in 41% in non-cirrhotic and 34% in cirrhotic patients with genotype 1, and 79% (24 weeks) and 72% for non-cirrhotics and cirrhotics, respectively, with genotype 2 or 3.[27]

The natural history of chronic HCV infection is a gradual progression of hepatic fibrosis, eventually resulting in cirrhosis in 10%-20% of patients. An estimated 1%-5% of those infected with HCV will go on to develop hepatocellular carcinoma, a cancer with very low five-year survival rates, despite advances in therapy.[28,29] Once a patient has developed decompensated cirrhosis, a liver transplant is the only option for survival. As a result of the growing number of cases of cirrhosis due to hepatitis C, hepatitis C has become the leading indication for liver transplantation in the United States.[30] Criteria for

eligibility for a liver transplant are even stricter than those for treatment. Therefore, once infected, finding ways to slow the progression to cirrhosis holds the greatest hope for reducing morbidity and mortality from HCV.

No specific factors have been found to explain why 15% of incident infections resolve without treatment, whereas the other 85% have chronic viremia. However, once chronically infected, a number of factors have been shown to independently increase the risk of developing liver failure (4-8% of incident infections). These factors include ongoing use of alcohol, male gender, age at time of infection (over 40), and co-infection with hepatitis B or HIV.[31, 32,33]

Obesity has also been considered as a risk factor for progression of hepatic fibrosis. Studies have shown that obesity is associated with both hepatic steatosis *and* fibrosis in chronic hepatitis C infection.[10, 34] Further, obesity is associated with a poorer response to interferon-based therapies.[35,36] Studies have also shown that HIV co-infection decreases rates of sustained viral response to interferon-based HCV therapy.[37] In fact, antiretrovirals themselves can be hepatotoxic and are known to cause hepatic steatosis.[38] This presents even more dilemmas for the practitioner who must maximize the health of the patient associated with the two infections.

There is large overlap between hepatitis C virus (HCV) and steatosis. Half of those chronically infected with hepatitis C are estimated to have hepatic steatosis.[39] Although hepatic steatosis is usually due to obesity-induced NAFLD, recent research has implicated the core protein of HCV genotype 3 as being independently associated with the development of steatosis. Murine models have shown that hepatic overexpression of HCV core protein interferes with the hepatic assembly and secretion of triglyceride-rich

very low-density lipoproteins (VLDL) via inhibition of the activity of the VLDL transfer protein.[40] This evidence is further strengthened by the observation that antiviral therapy can reverse histologic steatosis among those infected with genotype 3. It is hypothesized that specific viral sequences of genotype 3 promote lipid accumulation in hepatocytes, as seen in animal studies.[39,40] Although genotype 1 (predominantly subtype 1a, followed by 1b) is by far the most prevalent genotype in the United States (65-75%), there is a significant proportion (about 15%) of genotype 2 (subtype 2b, followed by 2a), trailed by genotype 3a at about 7-8%. Other genotypes, such as 4 and 6, are found much less commonly in the United States, but are the predominant genotypes in areas such as the Middle East and Egypt.[41]

Steatosis is also associated with an increased risk of developing hepatocellular carcinoma (HCC) for those with chronic HCV infection related cirrhosis.[42] Japanese investigators found that the cumulative incidence rates of HCC were 24%, 51%, and 63% at 5 years, 10 years, and 15 years, respectively, in a study of 161 Japanese chronic hepatitis C patients. In multivariate analysis, hepatic steatosis, age, cirrhosis, and lack of antiviral treatment were independent risk factors for HCC. Furthermore, hepatic steatosis was correlated with BMI, serum ALT levels, and triglyceride levels.[42]

As chronic hepatitis C is estimated to affect 2% of the U.S. population, and that 30% of Americans are considered overweight or obese, it is important to consider the combined effects of obesity and chronic hepatitis C infection on fibrosis and steatosis of the liver. Public scrutiny has resulted in increased research for effective interventions to reduce morbidity and mortality for the two diseases. This presents a particular challenge as existing treatments are imperfect and relatively ineffectual. For this reason, current

efforts are focused on primary prevention. If obesity is found to be a risk factor for liver failure, diet and weight loss programs could provide an inexpensive and health-preserving treatment, not only for preventing liver failure, but also to decrease the morbidity of hepatitis C infection, increase response to interferon therapies, and carry the added benefits of decreased insulin resistance, risk of cardiovascular disease, and improving osteoarthritis and hyperlipidemia.

This purpose of this study is to examine whether obesity is associated with hepatic steatosis and fibrosis among patients with chronic hepatitis C infection, thus suggesting a method of secondary prevention for progression to hepatic fibrosis from hepatitis C. This study may be the first American community-based study correlating liver biopsy-based steatosis and fibrosis data with BMI. Therefore, the impact of a non-invasive intervention for hepatitis C that effectively slows progression to liver failure would be enormous.

OBJECTIVES

This study is a secondary analysis of the existing Chronic Liver Disease database of newly diagnosed chronic liver disease patients from three different communities (Portland, Oregon; Oakland, California; and New Haven, Connecticut), in collaboration with the Centers for Disease Control and Prevention in Atlanta, Georgia.

The purposes of this investigation are to:

1. Describe the demographics and prevalent morbidities of a hepatitis C-infected population subset of the Chronic Liver Disease database

2. Analyze a hepatitis C-infected subset of patients from the Chronic Liver Disease database for risk factors for hepatic steatosis and fibrosis on liver biopsy, including obesity

RESEARCH DESIGN AND METHODS

Data Access

This investigation is a cross-sectional study of risk factors for hepatic steatosis and fibrosis among a community-based population of individuals chronically infected with hepatitis C. The data source is a subset of the Chronic Liver Disease study (CLD) database from the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia.[43] Subjects were identified during the calendar year 1998-2001 by active surveillance at community gastroenterology clinics and a selected group of primary care clinics. An eligible case was defined as a new adult chronic liver disease case diagnosed at various community sites and referred to a gastroenterologist (Appendix A: Case Definitions for the CLD study). The three participating sites were the Hepatology Research Clinic at OHSU (Oregon Health & Science University) in Portland, Oregon; Yale University in New Haven, Connecticut; and the Kaiser Permanente group in Alameda County, California. Other collaborators included members of the CDC and the Oregon Department of Human Services. The study protocols for each institution were approved by the Institutional Review Boards of all participating and collaborating institutions.

Trained research interviewers administered a survey to eligible subjects who agreed to participate. Interview data gathered included demographics, medical histories, hepatitis history, and recreational drug and alcohol use (Appendix B: CLD Study Patient Interview Form). Relevant clinical data obtained from review of the patients' medical records from the gastroenterologists' offices were recorded on the clinical extraction form by the interviewers. Subjects also underwent serum testing for laboratory values, as

well as hepatitis C virus genotype. All subjects also underwent liver biopsies, usually within a year prior to their interview. Study pathologists who were blinded to subject clinical history reviewed all available liver biopsies, independent of previous reading by outside pathologists. Data were gathered on a standardized pathology scoring sheet. Among the variables scored were hepatic steatosis, fibrosis, and inflammation The pathologists also recorded the likely etiology(ies) for the liver damage seen on histology (i.e. hepatitis C-induce damage only or mixed hepatitis C- and alcohol damage) (Appendix C: CLD study pathology scoring sheet for liver biopsies)..

Power and sample size

The CLD database contains 2039 subjects. 1593 subjects were ineligible for further analysis for our study based on a number of exclusion criteria: missing final diagnosis (120), HIV co-infection (169), diagnosed outside of study period of 1999-2001 (40), not referred to a gastroenterologist (656), not HCV-infected (389), no pathology score sheet (204), and insufficient tissue on biopsy (15), leaving 446 subjects for analysis. Since we were working with a fixed sample size (analyzed as 450), we calculated the odds ratios that would be detectable via logistic regression analysis, at a set alpha level of 0.05, given that 10% of non-obese subjects had advanced steatosis and 40% of non-obese subjects had advanced fibrosis, via PASS®. Obesity was defined as a BMI $\geq$ 30, advanced steatosis as a grade of 2+ or higher, and advanced fibrosis as stage 2 or higher.

For advanced steatosis, using an alpha level of 0.05 and 80% power, and assuming that the baseline prevalence of advanced steatosis is 10% in the non-obese population, we would be able to detect odds ratios of 2.217 and higher, which

corresponds to at least 19.8% prevalence of advanced steatosis in the obese subjects (which make up 33% of the population under study). For advanced fibrosis, using an alpha level of 0.05 and 80% power and assuming that the baseline prevalence of advanced fibrosis of 40% in the non-obese population, we would be able to detect odds ratios of 1.760 and higher, which corresponds to at least 53.9% prevalence of advanced fibrosis in the obese subjects.

Data Management

For our secondary analysis, all hepatitis C patients with liver biopsy results were considered. Selected items from the survey were extracted from the database. The data of interest fell into four categories: (1) demographic information; (2) medical history; (3) pathology data; and (4) duration and mode of hepatitis C infection. The CDC screened the main database for all patients infected with hepatitis C. The subset consisted of 450 subjects with study ID numbers only and no personal identifiers. The raw database was transmitted from Microsoft Excel format and converted to SPSS format. SPSS software versions 11.0 – 14.0 (SPSS Inc., 2004) and Microsoft Excel 2000 versions (Microsoft Corporation, 2002) were used for database management and statistical analysis.

The two outcome variables of interest were advanced grade of steatosis and advanced stage of fibrosis on liver biopsy. The main co-variate of interest was body mass index, calculated by self-reported weight and height during the interview intake, as opposed to the date of the liver biopsy, which usually predated this by about a year. This was the only measure available to compute BMI. Other independent variables considered were site, gender, race and ethnicity, number of metabolic syndrome co-morbidities

(hypetension, hyperlipidemia, and diabetes), history of significant use (six months or more in the ten years prior to interview) of medications known to cause steatosis (estrogens, immunomodulators, antiretrovirals), grade of inflammation on biopsy, final histologic diagnosis on pathology, history of heavy alcohol use (>60 grams daily for men; >30 grams daily for women), route of HCV infection (intravenous drug use or other), HCV genotype, age, years lapsed between liver biopsy and interview, and estimated duration of HCV infection.

Variable Coding

Distributions of each variable were carefully considered via graphic representations, frequency tables, and measures of spread to determine the most appropriate scale to be used for modeling outcomes of interest: continuous, categorical, or binary. Scaling of continuous variables was further assessed in analytic models via orthogonal polynomial contrasts, a statistical technique for assessing trends in regression coefficients.[44] All non-continuous variables were scaled as binary or categorical where appropriate, based on frequencies and scaling. In most cases, categorical variables were dichotomized in order to avoid small cell sizes in subsequent analyses. In most cases, the frequency of one category was large enough to justify collapsing of the remaining categories into an "other" category. If an individual was missing values for a specific variable, the variable was coded as missing, but the subject was still included in the database. Only duration of infection and route of infection had more than 5% missing values. A summary of the final variable scaling can be found in Table 1: Scaling of Variables on page 46.

Advanced, or clinically relevant, steatosis was one of the two main outcomes of interest. On the pathology form, steatosis was graded from 0 to 3, where grades 0 or 1 were considered "not advanced" and grades 2 or greater were considered "advanced" (corresponding to 30% or greater steatosis). Advanced fibrosis was the second main outcome of interest in this study. Fibrosis has five stages: 0 (none), 1 (portal fibrosis), 2 (periportal fibrosis), 3 (bridging fibrosis), or 4 (cirrhosis). For purposes of analysis, any fibrosis score of 2 or above was considered to be "advanced" fibrosis. These divisions are used in much of the hepatology literature.[10,45] Also, any subject who was determined to be "cirrhotic" by study doctors, even if their biopsies were read as stage 3 or less, were changed to "cirrhosis" (stage 4) on their fibrosis score, affecting two subjects with stage two fibrosis and five subjects with stage three fibrosis. All these subjects had advanced fibrosis; thus, coding was not affected. One clinically non-cirrhotic subject had missing fibrosis data, but was still included for subsequent analyses, such as demographic data.

One of the pathologic hallmarks of chronic hepatitis C infection is histologic inflammation on liver biopsy. Inflammation has five grades: 0 (none or minimal), 1 (portal only), 2 (mild interface hepatitis), 3 (moderate interface hepatitis), or 4 (severe interface hepatitis). For purposes of analysis, similar to the division made for steatosis and fibrosis, any inflammation score of 2 or above was considered to be "advanced" inflammation. Presence or absence of steatohepatitis on biopsy was also recorded.

Body mass index (BMI) was analyzed as a dichotomous [obese ($\geq$30) versus not obese (<30)], continuous, and three-category variable (normal weight, overweight, and obese). Although all methods of scaling BMI were found to be significantly associated with the outcomes of interest, BMI was dichotomized for model building purposes. This

is the standard division used in clinical literature, specifically in cardiovascular literature as a cardiac risk factor. One subject was missing BMI data. One subject was trans-gendered; this subject was coded as "missing". Subjects were asked during their interview if they were of Hispanic or Latino origin (ethnicity), and then asked to choose one or more racial categories that best describe them. The five choices for racial categories were: white, black/African American, American Indian/ Alaska Native, Asian, or native Hawaiian/Pacific Islander. For reporting reasons, any subject who listed American Indian/Alaska Native (AI/AN) as a race, regardless of ethnicity or other races chosen, was coded as AI/AN. Age was considered as a continuous and as a categorical variable (under 30, 30-49, 50+), but ultimately was left as a continuous variable in analytical models.

Study pathologists also had to determine the predominant diagnosis responsible for pathologic abnormalities on liver biopsy. The choices were hepatitis C or a mixed picture (hepatitis C plus alcohol, hepatitis C plus NASH, hepatitis C plus hepatitis B, etc). This variable was also dichotomized (HCV only versus HCV mixed) to maximize power during model building. During the interview, subjects were asked about history of potential HCV exposures, including intravenous drug use (IVDU), blood or blood product transfusions, sexual or household contact with an individual with known HCV infection, and exposure as a health care worker (HCW) via contact with potentially infected blood or bodily fluids. If a subject reported ever injecting recreational drugs, he or she was considered to have been infected by this route. The remaining flow chart for hierarchy of likely route of HCV infection is illustrated in Figure 1: Hierarchy of HCV Infection on page 59. Duration of infection in years was another variable calculated by

this information; this was left as continuous for analysis. All other routes of infection, or if route was not known or missing, were coded as "unknown". Data on exposure was missing from fifty-three subjects. For analysis, data regarding route of HCV infection were dichotomized into IV drug use and other.

The interviewers asked subjects if they had any medical problems, to be chosen from a pre-determined list, presented in lay terms to the subject. For the purposes of this study, the only diagnoses of interest were those that comprise the metabolic syndrome, specifically, diabetes, hypertension, and hyperlipidemia. Since self-reported diagnoses may not be completely accurate, study interviewers also reviewed patient charts from the participating gastroenterologist and recorded any co-morbidities listed on a chart extraction form. The patient report and chart report were compared; if either the subject or the chart reported a diagnosis, it was coded as present. During the interview process, subjects reported if they had any medical conditions in the past five years. For simplicity of interpretation, only the presence of multiple co-morbidities was used in regression analysis. Medication history was also gathered in a similar manner. For purposes of the study, only medications known to cause steatosis were examined. This was defined as six months or more of use within the past ten years of a medication known to cause steatosis (estrogens, immunomodulators, and antiretrovirals).

During the interview, data regarding alcohol use and abuse were gathered via a rigorous, validated instrument first developed by Harvey Skinner, PhD.[46] First, individuals were asked if they had ever consumed any form of alcohol. If they answered yes, they were asked if there was ever a period in their lives when they consumed at least one drink per month, and if so, what age they were. This established their first "drinking

period". They were asked about the number of drinks (sizes shown) per typical drinking day, the number of days per month they drank, the maximum number of drinks on an occasion, and what type of alcohol consumed. This data was gathered for each "drinking period", defined as a time in which the subject's drinking habits differed significantly from other time periods in their life. History of DUI arrests (driving under the influence) was also gathered. These data were put into a standardized algorithm[46] that assigned each subject into one of three categories of heavy drinking: fewer than five years, between five and ten years, and ten years or greater.

Another potential confounder we examined was the time between biopsy and interview date. Obviously, the longer the duration between these measures, the more likely it is that the histology may evolve. Although many measures will remain static with time, the main outcome variables (steatosis and fibrosis), as well as the main independent variable, body mass index, could significantly change. Therefore, the association between time lapse and outcome was examined. Most eligible subjects were identified by having abnormal liver biopsies and were subsequently enrolled into the study. Therefore, most of the liver biopsies occurred before the interview. In some cases, the interview occurred years after the biopsy. Further, twenty-six biopsy dates were missing. Time lapse was evaluated in its original, continuous form, as well as categorized versions, approximating frequencies tertiles (interview occurred before biopsy to biopsy occurring < 6months before interview, biopsy occurred 6 mo to 1 year before interview, biopsy occurred $\geq$ 1 year before interview). We determined that the relationship to outcomes of interest was best described using the continuous variable. Site was also examined to account for potential population heterogeneity between sites.

Finally, as genotype 3 HCV infection is known to independently cause hepatic steatosis via a viral-mediated mechanism, we analyzed the association of genotype to steatosis and fibrosis. We dichotomized the variable into genotype 3 versus other, which included the 167 missing observations (37% of the total sample), in order to maximize sensitivity.

ANALYSIS

(1) Descriptive statistics

Appropriate descriptive statistics, including frequencies, mean, range, and standard deviation, were computed for all variables.

(2) Bivariate correlations

Next, bivariate correlations between all variables (outcome and co-variates) were analyzed. Multicollinearity was considered likely if the Pearson's correlation value between two variables was significant at the 0.01 level and was greater than or equal to 0.15. In cases of likely multicollinearity, the most clinically relevant variable was kept in for modeling and the others removed.

(3) Model building strategy

The association between each risk factor and outcome was analyzed via simple logistic regression for each dependent variable: clinically advanced steatosis and fibrosis. Both outcome variables were dichotomous, with scores of 2 or greater considered "clinically relevant" and less than 2 considered "not clinically relevant". Variables that were statistically significant ($p \leq 0.25$) were considered to be candidates for a multiple logistic regression model, as described in Applied Logistic Regression by Hosmer and Lemeshow.[43] Other variables, such as those mentioned in prior literature, or those thought to be likely confounding variables, were considered candidates for multiple logistic regression model as well.

Candidate variables were entered in a forward, step-wise approach, starting with the most significant variable on simple logistic regression analysis. Variables significant at the 0.05-level were kept in the model; all others were removed.

Once a main effects model was obtained, interactions between all the co-variates in this candidate model were checked for significance (p-value ≤ 0.05 based on the Wald statistic) in the final multiple logistic regression model. Any significant interactions were added to the final model. Odds ratios and 95% confidence intervals (CI) were reported for all variables in the simple and multiple logistic regression models. The odds ratio was obtained by exponentiating the co-variate's coefficient in the multiple logistic regression model (i.e. e^{β}).

Next, confounding between all co-variates that were candidates for the final model was assessed. Confounding was considered if the odds ratio of a co-variate changed by more than 10% in either direction with the addition of another co-variate to the existing variables in the model. In general, odds ratios for a variable will decrease in magnitude with the addition of other variables since part of the association is being explained by the presence of another variable that is associated with the variable of interest.

RESULTS

Descriptive statistics

A summary of frequencies, measures of spread, and other descriptive statistics for both outcome variables and co-variates can be found in Table 2: Frequencies and Descriptive Statistics, on page 52. As site was correlated with fibrosis (p-value of Pearson's chi-square statistic: 0.002), a cross-tabulation table was created, in which the Connecticut site was found to be positively associated with advanced fibrosis (Table 3: Cross-tabulation and chi-square test for site versus advanced fibrosis on page 53). Other inter-site differences found were that Oregon had a significantly longer mean time lapse between interview and biopsy, and California had a significantly older mean subject age (Table 4: ANOVA test for differences in mean time lapse, age, and duration of infection among sites on page 53).

Outcome variables

The first outcome variable considered was advanced steatosis, defined as 2+ or greater. The majority of the subjects did not have advanced steatosis (84.2%: 379/450). In contrast, only 64.1% (68/106) of obese subjects did not have advanced steatosis. Only 26 subjects (5.8%) had steatohepatitis. 86% of these cases occurred in subjects with advanced steatosis. The second outcome variable considered was advanced fibrosis, defined as stage 2 or greater. Two-thirds of the subjects did not have advanced fibrosis (299/450). Sixty-four subjects were deemed cirrhotic histologically (stage 4 fibrosis). However, 71 patients (15.7%) were considered clinically cirrhotic, implying that seven

patients who were below cirrhotic stage pathologically were actually considered clinically cirrhotic.

Co-variates

Body mass index (BMI) was the main co-variate of interest. The study population was roughly evenly divided between the three categories of BMI: normal (<25), overweight (25-29), and obese (≥30). The majority of the subjects also had clinically significant inflammation [81.4% (362/450)] and a final pathologic diagnosis of hepatitis C-induced damage only [66.4% (299/450)]. The next most common diagnosis was hepatitis C mixed with alcohol: 32.2% (145/450). As expected, the majority of HCV infections, 70.6% (314/445), were presumed to be from IV drug use.

The majority of the subjects were male [62.5% (282/450)] and white non-Hispanic [73.4% (331/451)]. Both the black/non-Hispanic category and the Hispanic category had 44 subjects each (9.3%). Five percent (22/451) of the subjects reported American Indian/Alaska Native as their ethnicity, and another thirteen subjects were collapsed into an "other" category. This latter category included Asian subjects (5/451) and those of mixed, non-AI/AN race or unsure race and ethnicity (8/451). The mean age was 45.74 years, with a standard deviation of 7.63 and a range of 19.2 to 76.2 years.

There was quite a bit of discordance between the subjects' reports of co-morbidities and chart extraction. Of those subjects with co-morbidities, only 40 patient reports matched the clinical extraction form (40/177: 23%). Adding the chart extraction form information resulted in 54 newly-captured diagnoses among 52 subjects. Chart abstraction data resulted in 118 total diagnoses among 100 subjects, 66 fewer than patient

report, which reported 184 diagnoses among 135 subjects, suggesting that patient history was more sensitive than chart history (See Table 5: Differences in reported metabolic co-morbidities between patient report and chart abstraction method on page 53).

. The range of time elapsed between biopsy and interview was the biopsy occurring 5.5 months after the interview date to the biopsy occurring 4.6 years before the interview date. The liver biopsy took place a mean of one year before the interview date, with a standard deviation of 0.79. Fifty-four per cent of the subjects (239/443) used alcohol heavily for fewer than five years; this category included those who reported never drinking. 12.2% (54/443) drank for five years or more, but fewer than ten years; and 33.9% (150/443) drank heavily for ten years or more. Eight subjects had missing alcohol histories.

Bivariate Correlations

Correlations were assessed between all variable pairs, whether outcome variables or co-variates. A high degree of correlation between two independent variables suggests multicollinearity, whereas high correlation between an independent variable and an outcome variable implies that the independent variable could overwhelm the association of the outcome with other independent variables in a multiple logistic regression model. In these cases, one of the two correlated variables would be chosen as a candidate for the models, based on either strength of association with the outcome variable of interest on simple logistic regression analysis, or due to biologic importance. Correlations with co-variates and outcome variables were also examined to assess for associations on simple analysis. Due to the sample size, it was decided that only Pearson's correlations of 0.15

or greater that were significant at the 0.01-level were considered. The results are found in Table 6: Pearson correlations for all variables on page 54.

Steatohepatitis and clinically advanced steatosis were highly correlated (Pearson's correlation of 0.46). As a result of this significant correlation, and its low frequency, steatohepatitis was not considered further. Age was highly correlated with duration of HCV infection (Pearson's correlation: 0.50, significant at the 0.001 level). Therefore, the decision was made to use whichever variable was most significant with the outcome variable of interest: duration of infection was used in the steatosis model, and age in the fibrosis model. Inflammation was strongly associated with fibrosis (Pearson's correlation: 0.45, significant at the 0.01-level). This represents how active the subject's HCV infection is. Therefore, inflammation was removed as a candidate for modeling for advanced fibrosis. Final pathologic diagnosis (hepatitis C only versus mixed) showed strong multicollinearity with alcohol abuse (Pearson's correlation: 0.77). Only final diagnosis was a candidate variable for multiple logistic regression analysis since it was significantly associated with both outcomes, and alcohol use was not.

Although many statistically significant correlations were not large enough or clinically significant enough to prompt removal from the model, they did suggest potential confounding. For example, male gender was associated with advanced fibrosis (Pearson's correlation: 0.199; p-value of Pearson's Chi-square statistic: 0.001), IV drug use as route of HCV infection (Pearson's correlation: 0.19; p-value of Pearson's Chi-square statistic: < 0.001), as well as duration of infection (quartiles) (Pearson's correlation: 0.21; p-value of Pearson's Chi-square statistic: p-value 0.003). (Figure 2: Gender and Advanced fibrosis on page 60). Also, based on correlation values, a "black

race versus other" variable was created to assess the relationship with metabolic co-morbidities. Black race was significantly correlated (p-value of Pearson's Chi-square statistic of <0.001) with hypertension, presence of any metabolic co-morbidity, and number of co-morbidities (Table 7: Chi-square and bivariate correlation results for select variables, page 55).

Simple Logistic Regression Results

Each candidate co-variate was analyzed for its association with each of the outcome variables: clinically advanced fibrosis and steatosis. A summary of these results can be found in column two (crude odds ratio) of Table 8: Summary of Simple and Multiple Logistic Regression Analysis for Advanced steatosis on page 56 and Table 9: Summary of Simple and Multiple Logistic Regression Analysis for Advanced fibrosis on page 57.

Advanced Steatosis

Variables significantly associated with steatosis included: advanced fibrosis, obesity, multiple metabolic co-morbidities, advanced inflammation, white non-Hispanic race/ethnicity, male gender, final pathologic diagnosis, genotype, and duration of infection. Variables that were not significant (p-value > 0.25) on univariate analysis included: route of infection, age, site, alcohol abuse history (binary and categorical), steatotic medication history, and time lapse between biopsy and interview.

Advanced Fibrosis

Variables significantly associated with fibrosis included: advanced steatosis, site, age, duration of infection, male gender, obesity, and final pathologic diagnosis. Variables that were not significant (p-value > 0.25) on univariate analysis with advanced fibrosis included: multiple metabolic co-morbidities, time lapse between biopsy and interview, white non-Hispanic race/ethnicity, alcohol abuse history (binary and categorical), and route of infection. Due to a stronger association with advanced fibrosis, it was decided to use age and remove the duration of infection variable from further analysis. On later analysis, it was found that duration of infection was not significant in the final multiple logistic regression model, although age was, confirming that age was a better variable to model.

Main Effects Model Results

All candidate variables were placed in a model in order to assess significance and potential confounding. The results are summarized below and in column three (preliminary main effects model) of Table 8: Summary of Simple and Multiple Logistic Regression Analysis for Advanced Steatosis on page 56 and Table 9: Summary of Simple and Multiple Logistic Regression Analysis for Advanced Fibrosis on page 57.

Steatosis

Advanced fibrosis, obesity, multiple co-morbidities, and final pathologic diagnosis were all significant ($p \leq 0.11$) in the main effects model for advanced steatosis. Advanced

inflammation, race/ethnicity, duration of infection, gender, and genotype were not significant ($p \geq 0.36$).

Fibrosis

Advanced steatosis, site, age, and gender were all significant ($p \leq 0.028$) in the main effects model for advanced fibrosis. Duration of infection, obesity, and final diagnosis were not significant ($p \geq 0.21$).

Multiple Logistic Regression Results

Co-variates that were significantly associated with each of the outcome variables were entered in decreasing order of significance into a multivariate model. Only those whose Wald statistic had a p-value of ≤ 0.05 were kept in the final model, unless the variable was of biologic or other special significance, such as obesity, which was our main risk factor, or if it was a significant confounder. The results are summarized below and in column four (multivariate model) of Table 8: Summary of Simple and Multiple Logistic Regression Analysis for Advanced Steatosis on page 56 and Table 9: Summary of Simple and Multiple Logistic Regression Analysis for Advanced fibrosis on page 57.

Advanced steatosis

The only variables significant ($p \leq 0.05$) in the final multivariate model for advanced steatosis were advanced fibrosis (odds ratio [OR]: 3.85, 95% confidence interval [CI]: 1.82 – 8.13), obesity (OR: 2.85, 95% CI: 1.66 – 4.89), and the presence of multiple metabolic co-morbidities (OR: 2.36, 95% CI: 1.19 – 4.69). Race/ethnicity, male gender,

advanced inflammation, final pathologic diagnosis, genotype, and duration of infection were no longer significant in the multivariate model. As hypothesized, obesity remained significant in the final model.

Advanced Fibrosis

The only variables significant ($p \leq 0.05$) in the final multivariate model for advanced fibrosis were advanced steatosis (OR: 4.37, 95% CI: 2.05 – 9.34), male gender (OR: 1.62, 95% CI: 1.06 – 2.50), age (OR: 1.08 per year, 95% CI: 1.05 – 1.11), and site (OR: 0.33, 95% CI: 0.18 – 0.60 for CA versus CT; OR: 0.41, 95% CI: 0.25 – 0.66, for OR versus CT).

Obesity, final pathologic diagnosis and duration of infection were no longer significant in the multivariate model for advanced fibrosis. Therefore, our variable of interest, BMI (indicated by obesity) was not significant in the final model. However, due to its special significance as our main risk factor of interest, it was added to the final model (OR: 1.31, 95% CI: 0.82 – 2.11, p-value 0.26). The effect on the odds ratios of the variables of interest are reported below and in column six (final model) of Table 9: Summary of simple and multiple logistic regression analysis for advanced fibrosis on page 57: advanced steatosis (OR 3.97, 95% CI 1.84 – 8.60), male gender (OR 1.65, 95% CI 1.07 – 2.55), age (OR 1.08 per year, 95% CI 1.05 – 1.11), and site (OR 0.33, 95% CI 0.19 – 0.60 for CA versus CT; OR 0.40, 95% CI 0.25 – 0.66, for OR versus CT). Finally, steatohepatitis, which showed very high correlation with steatosis, was added into the final model, both with and without steatosis. In the absence of steatosis, steatohepatitis was found to be positively associated with advanced fibrosis, although the odds ratio

(2.665) was only marginally significant (p-value 0.088). However, it was no longer significant when steatosis was added into the model (p-value of 0.980). Steatosis remained highly significant (p-value of 0.001). This confirms that steatosis exhibits multicollinearity with steatohepatitis and confirms the validity of our decision to remove it from analysis in favor of steatosis.

Interactions

Steatosis

The interactions between fibrosis and multiple co-morbidites, as well as obesity and multiple co-morbidities were not significant (Table 10: Interaction terms for final multiple logistic regression model for advanced steatosis on page 58). The interaction term between obesity and fibrosis could not be assessed in the logistic regression models due to an empty cell: there was no obese subject with advanced steatosis, and not advanced fibrosis (Table 11: cross-tabulation of advanced steatosis and advanced fibrosis by obesity on page 58). Therefore, to approximately assess the significance of the interaction (without adjustment for other variables in the logistic regression model), a contingency table was created using the continuity correction (adding 0.5 to each cell) and computing the continuity-corrected chi-square statistic and corresponding p-value. The results illustrated a highly significant association ($p < 0.001$, χ^2 statistic: 98.3. This relationship was confirmed by the test of homogeneity of the odds ratios on cross-tabulation; the Breslow-Day χ^2 statistic was also significant at the 0.001 level, confirming that the odds ratios in the stratified table for fibrosis given steatosis were significantly

different, based on whether the subjects were obese or not. In other words, obesity is an "effect modifier" of the relationship between fibrosis and steatosis.

We found that, among non-obese people, the odds of having advanced steatosis were 42.6 times higher for subjects having advanced fibrosis versus those without advanced fibrosis. Among obese subjects, the odds of having advanced steatosis given advanced fibrosis were only 1.32 times that given not advanced fibrosis.

Fibrosis

No interactions between variables in the final fibrosis model were significant at the 0.05-level (Table 12: Interaction terms for final multiple logistic regression model for advanced fibrosis on page 58).

Confounding

Although not all of the variables that were significant on simple logistic regression analysis were significant in the final multivariate model, the effect of these non-significant variables on the parameter estimates of the variables in the final model was assessed. Although the focus was on the variables in the final model, we explored some cases of confounding of variables not significant in the final model.

Steatosis

Duration of infection exhibited the most confounding among those considered in the model for advanced steatosis. The effects of adding duration of infection on the odds ratios of the co-variates were: an increase of 13% for obesity on univariate analysis (from

3.16 to 3.57) and by 17% in the final model (from 2.85 to 3.32), a decrease of 13% for advanced fibrosis on univariate analysis (from 4.03 to 3.51) and by 11% in the final model (from 3.85 to 3.43), and a decrease of 15% for multiple metabolic co-morbidities on univariate analysis (from 2.74 to 2.32) and by 19% in the final model (from 2.36 to 1.91). Further, although genotype did not end up in the final model, the addition of duration of infection decreased the odds ratio of genotype on univariate analysis by 22% (from 1.78 to 1.27). Because of these effects, the final model for steatosis is reported with and without duration of infection (Table 7: summary of simple and multiple logistic regression analysis for advanced steatosis for all candidate variables, page 55).

In addition to duration of infection, obesity was found to be a confounder. The effect of adding obesity on the odds ratios of the co-variates on univariate analysis was a decrease of 21% for advanced fibrosis (from 4.03 to 3.20) and of 16% for multiple metabolic co-morbidities (from 2.74 to 2.30). The only other significant confounder of the three co-variates significant on multiple logistic regression analysis was age: it *increased* the odds ratio for multiple metabolic co-morbidities by 15% (from 2.74 to 3.15). Although it did not end up in the final model, inflammation was significantly confounded by fibrosis: adding fibrosis to inflammation on univariate analysis decreased the odds ratio by 30% (from 1.49 to 1.03).

Fibrosis

Duration of infection was also a confounder in the model for advanced fibrosis. Adding duration of infection to advanced steatosis on univariate analysis with fibrosis decreased the odds ratio for steatosis by 13% (from 4.03 to 3.50). However, because age was much

more highly associated with advanced fibrosis, duration of infection was discarded in favor of age for the final model. The only other confounding relationship was that between obesity and steatosis. Adding advanced steatosis to obesity on univariate analysis decreased the odds ratio for obesity by 15% (from 1.46 to 1.24). Similarly, adding obesity to the final multiple logistic regression model decreased the odds ratio for advanced steatosis by 9% (from 4.37 to 3.97). Although this did not meet the criterion we used to establish confounding (>10% change in odds ratio), because obesity was our main risk factor of interest, the final multivariate model for advanced fibrosis was reported with and without obesity.

DISCUSSION

The chief aim of this study was to examine the effect of obesity on advanced steatosis and fibrosis on liver biopsy among a hepatitis C-infected population. Obesity was indeed associated with steatosis, and less strongly with fibrosis, on simple logistic regression analysis. In the multiple logistic regression models, obesity remained independently associated only with advanced steatosis but not with advanced fibrosis. However, because obesity was the main risk factor of interest, the fibrosis model was reported with and without obesity.

Advanced steatosis was also independently associated with advanced fibrosis and multiple metabolic co-morbidities. Obesity was found to be a significant effect modifier of the relationship between steatosis and fibrosis. Duration of infection was a significant confounder and thus was reported in the final model. Advanced fibrosis was independently associated with advanced steatosis, site, age, and male gender. The implications of these findings will be discussed below. Steatotic medication use, time lapse, alcohol use, and route of infection were not associated with either outcome variable, even on univariate analysis.

Because of the fact that the two outcome variables were significantly associated with each other, another possible modeling approach would have been to remove either co-variate from the other model. However, the decision was made to report all variables of interest in the model in order to most accurately explore the risk factors of the greatest significance, both clinically and statistically. In fact, during statistical analysis, removing steatosis and fibrosis from the corresponding final model did not significantly change the odds ratios for the other co-variates.

Steatosis

Obesity, advanced fibrosis, and having multiple metabolic co-morbidities were all independently associated with advanced steatosis. Due to the biologic pathways discussed in the background section of this paper, it was expected that obesity, the main risk factor of interest, would be independently associated with advanced steatosis. This finding demonstrates that obesity is associated with significant histologic changes (i.e. steatosis) on liver biopsy in patients with chronic hepatitis C infection. It was also expected that subjects with other metabolic co-morbidities (hypertension, diabetes, and/or hyperlipidemia) would be more likely to be obese and to have advanced steatosis on liver biopsy. Although no significant association was seen with the presence of a single co-morbidity, subjects with two or more metabolic co-morbidities were much more likely to have advanced steatosis on liver biopsy, independent of BMI and advanced fibrosis.

Duration of infection was found to be the most significant confounder in the model for advanced steatosis, affecting the odds ratios of every variable when added to the final model. Addition of duration of infection increased the odds of having significant steatosis given obesity by 13% on univariate analysis and by 17% when added to the final model, implying that duration of infection was a negative confounder of the relationship between obesity and steatosis. In other words, the true measure of effect of obesity on steatosis is blunted when duration of infection is not taken into account. Interestingly, obesity was associated with neither age nor duration of infection. Duration of infection showed weak association with steatosis (p-value 0.162 on univariate analysis), but age did not show any association. This significance of this finding is unclear. Conversely, duration of infection weakened (i.e. positively confounded) the association between

steatosis and fibrosis, as well as between steatosis and multiple metabolic co-morbidities. One interesting point is that age, which is obviously very highly correlated with duration of infection (Pearson's correlation value of 0.494, significant at > 0.001 level), had the *opposite* effect on the odds ratios for fibrosis and multiple co-morbidities in final model for steatosis: it increased them (i.e. was a negative confounder). However, this increase was only significant for multiple co-morbidities (OR 2.74 to 3.15). Another finding was that although inflammation was associated with steatosis on univariate analysis, it was no longer significant after the addition of fibrosis to the model.

Hepatitis C virus genotype 3 is known to cause viral-mediated steatosis. Therefore, we expected to see an association between advanced steatosis and genotype 3 versus other genotypes. Although genotype 3 was weakly associated with advanced steatosis on univariate analysis (p-value 0.239), this significance disappeared in both the main effects model, as well as the final multivariate model, as well as by the addition of duration of infection. However, this lack of significance is most likely due to the fact that only 6% of the sample had genotype 3a and that 37% subjects were missing genotype information. Our genotype 3 prevalence approximates that of 7.4% found in the NHANES III population study. Genotype was analyzed as a bivariate variable in multiple ways (genotype 3 versus other/mixed/missing, genotype 3/mixed versus other/missing, genotype 3/missing versus other/mixed), none of which significantly changed the results. It is unlikely that a differential misclassification occurred: i.e. that those missing genotype information were more or less likely to be genotype 3. Therefore, this missing information on genotype would bias our results towards the null, as does our final classification of the variable into genotype 3 versus an "other and missing" category. We

confirmed that our study was underpowered to detect a significant difference in advanced steatosis between genotype 3 and other genotypes via PASS® software: assuming that genotype 3 has a 10% prevalence and that 50% of non-genotype 3 HCV subjects have advanced steatosis, using an alpha level of 0.05 and 80% power, we would need 760 subjects to detect an odds ratio of 2 ($\geq$ 67% of genotype 3 patients have significant steatosis) and 2155 subjects to detect an odds ratio of 1.5 ($\geq$ 60% of genotype 3 patients have significant steatosis).

Obesity, our main risk factor, was found to be both a positive confounder *and* an effect modifier of the relationship between the two outcomes: advanced steatosis and fibrosis. The association between advanced steatosis and advanced fibrosis was weakened when obesity was taken into account. Table 10 ("Cross-tabulation of advanced steatosis and advanced fibrosis by obesity", page 58), demonstrates that the effect of steatosis on fibrosis is much more pronounced in the non-obese subjects than the obese subjects. Also, obesity was a significant positive confounder of the relationship between steatosis and metabolic co-morbidities.

Surprisingly, alcohol use was not associated with advanced steatosis, despite the fact that acute alcohol use itself is a major cause of hepatic steatosis. The lack of association is most likely due to the fact that all the study subjects were under medical care and had been strongly advised to avoid alcohol use. Therefore, a previous history of alcohol use was less likely to impact present steatosis, which results more from recent alcohol use.

Fibrosis

Advanced steatosis, site, age, and male gender were also independently associated with fibrosis. Obesity, although associated with fibrosis on simple logistic regression analysis, was no longer significant when the other risk factors were put into the model. However, because of its importance as our main risk factor, the final model was reported with and without obesity. Adding obesity resulted in a 9% decrease in the odds ratio for advanced steatosis; thus, obesity is a positive confounder of the relationship between steatosis and fibrosis, as mentioned previously. Also, adding steatosis to obesity in the fibrosis model resulted in a significant decrease in the odds ratio for obesity. The only other significant confounder in the fibrosis model was, again, duration of infection, which positively confounded the relationship between steatosis and fibrosis.

Again, a history of either moderate or significant alcohol abuse was not related to advanced fibrosis, even on univariate analysis, despite the fact that alcohol abuse is one of the leading causes of hepatic fibrosis and cirrhosis. Potential reasons for this were discussed in the "steatosis" section above, but may be because damage from previous alcohol abuse is overwhelmed by the ongoing damage by chronic hepatitis C infection. Alternatively, because alcohol information was reported by subjects, they may have underestimated their alcohol history, which could bias our results towards the null and explain our inability to find an association, an example of **recall bias**, despite the rigor our methodology used. However, although alcohol use was not a significant variable, it is possible that final pathologic diagnosis is in fact a better proxy variable for detecting the effect of past alcohol use on hepatic histology than patient report, as it is a more objective measure. It was positively, though weakly, associated with both advanced

steatosis (p-value 0.134) and advanced fibrosis (p-value 0.110) on simple logistic regression analysis. Although it was no longer significant in the final models for either outcome, it was still significant (p-value of 0.11) in the main effects model for steatosis.

Gender is a common confounder in epidemiologic studies[48]. In this study, males were much more likely to be infected by IV drug use as females (OR 2.28, p-value <0.001). This may reflect that since 33% of females compared to 10% of males were infected by routes other than IV drug use (transfusions, contact with a known infected person, or as a health care worker), females may be more inclined to seek out their infection status earlier. Thus, the lead time before their HCV diagnosis is shorter, and they will have less fibrosis. This is supported by the finding that although male subjects were only marginally older than female (mean age 46.3 versus 44.8, ANOVA p-value 0.049), their duration of HCV infection was significantly longer (mean 26.8 versus 23.3 years, ANOVA p-value <0.001). Similar to other studies, male gender was also associated with more severe fibrosis in chronic HCV.[34] Estrogen exposure may be protective: parity has been found to be associated with less hepatic fibrosis. Further, animal studies have shown HRT to reverse steatotic phenotype.[47]

Site was strongly associated with advanced fibrosis. As seen in Table 5: Cross tabulation for site versus advanced fibrosis on page 52, Connecticut had a much higher percentage of subjects with advanced fibrosis than Oregon or California. Although duration of infection did not differ significantly between sites, California, on average, had significantly older subjects, and Oregon had a significantly longer time lapse between biopsy and interview. Neither of these factors, however, would likely explain why Connecticut subjects had more advanced fibrosis. In fact, based on age alone, California

subjects should have more advanced fibrosis. The potential reason becomes clear when we examine site differences in alcohol use: 37.9% of Connecticut subjects reported 10 years or greater significant alcohol use, compared to only 34.4% of Oregon subjects and 20.5% of California subjects ($\chi 2$ test statistic p-value of 0.019). To confirm, we then examined differences in final diagnosis on pathology, which serves as a proxy for significant previous alcohol use: 36.6% of Connecticut subjects had final diagnoses of HCV and alcohol, compared to 33.3% of Oregon subjects, and only 16.5% of California subjects ($\chi 2$ test statistic p-value of 0.008). In fact, site ended up significantly associated with fibrosis in our final model, likely due to these factors. The only other variable that differed significantly between sites was genotype. Oregon subjects were much more likely to be genotype 3 (10.3%) than either California (8.2%) or Connecticut (1.7%) (X2 test statistic p-value of 0.001). However, this is unlikely to explain the difference in fibrosis levels, especially since there was no significant difference in the prevalence of advanced steatosis between the sites.

Another possible explanation for the differences in fibrosis between the sites is that California's subjects came from a Kaiser Permanente managed care group, which tended to enroll an older, employed, and more educated population compared to the New Haven center, which enrolled patients from a more urban population, including Medicaid and county health department patients. This could explain why the Kaiser patients were older and reported significantly less alcohol use. There was no significant difference in route of infection among sites.

Age was also independently associated with fibrosis, which is corroborated by previous research[34] and biologic knowledge: over time, the constant insult of chronic

hepatitis C infection to the liver results in hepatic fibrosis. This is the theory behind using interferon, an endogenous immunodefense mechanism released by the body to fight off viruses, as antiviral therapy for chronic hepatitis C infection. Because age was much more strongly associated with duration of infection (which was not significant in the multiple logistic regression model), duration of infection was removed from the fibrosis model for further analysis in favor of age. Again, age and duration of infection confounded variables in the fibrosis model in opposite directions: age increased the odds ratio for steatosis on univariate analysis with fibrosis, although not significantly (OR 4.03 to 4.23), whereas duration of infection significantly decreased it (OR 4.03 to 3.50).

Limitations

This is a **cross-sectional study** and thus carries all the pitfalls of interpreting direction of causality. This is extremely important to remember in interpreting results of our study. We found that our outcomes, steatosis and fibrosis, were independently associated with each other; however, we cannot say that steatosis *causes* fibrosis, or the other way around. Further, we found obesity to be independently associated with advanced steatosis. Again, we cannot say that obesity causes steatosis. In this case, reverse causation is biologically implausible, i.e. that hepatic steatosis could cause obesity. Regardless, we do not know the duration of any of our main outcomes or risk factors, i.e. we do not know if steatosis occurred before or after the development of either fibrosis or obesity.

Complicating the situation further is the fact that **body mass index** was measured a mean of one year after liver biopsy, the source of the data on fibrosis, steatosis,

inflammation, and final pathologic diagnosis. One could conceive that when subjects learn that they have advanced fibrosis or steatosis, they might be motivated to lose weight, thus having a lower BMI on interview day than on the day of the liver biopsy. Another interesting fact is that massive, rapid weight loss is actually known to *increase* hepatic steatosis, although this would likely affect a very small proportion of our population, if any at all. Further, another possibility for having falsely depressed BMI data is the tendency for patients to report themselves taller and thinner than in actuality. This has been documented in studies such as one among adolescents that found that, although self-reported and measured weight and height were highly correlated, subjects underreported body weight by an average of 0.52 kg; this effect was even stronger in the overweight and obese categories.[49] However, despite these potential underestimations of BMI, two-thirds of our population was still overweight or obese, and underrepresenting BMI would only have served to bias our results towards the null. Although uncontrolled ascites could have also overestimated true BMI, this is unlikely due to the low prevalence of cirrhosis in the population. Another problem in measurement is the **time lapse** between the liver biopsy and the time of the interview, when BMI data were gathered. The potential effects on BMI were discussed above. Fortunately, neither outcome variable was correlated with time lapse on simple logistic regression analysis.

As with most epidemiologic studies, this study had some missing values, most specifically those related to HCV infection: 13% of subjects were missing data on duration of infection and 11% for likely route of infection. Also, 23% of patients did not know names of medications that were used for at least six months during the ten years prior to interview; thus, they could have met criteria for being a steatotic medication. This

offers the potential for **recall bias.** Again, however, since all missing medication were dichotomized into the "other" or "no" category, this would only serve to bias our results towards the null, and would not explain finding an association These missing data could have been responsible for a Type II error, in which we did not have enough power to detect a significant difference in the outcomes. A similar argument was made above to suggest why genotype 3 was not found to be associated with steatosis.

A potential argument could be made that self-reported disease diagnoses are a source of potential bias; however, these were confirmed by gastroenterology clinic records. Unfortunately, the concordance rate between patient report and chart report was quite low. Because of the fact that we coded the condition as present if either source reported it, we could have overreported the presence of metabolic co-morbidites. If this were somehow associated with advanced steatosis or fibrosis, we would be committing a Type I error. In fact, the presence of multiple metabolic co-morbidities was independently associated with advanced steatosis. However, this potential error may be balanced out by the unknown number of subjects who are in the pre-clinical or even clinical stage of the disease but not yet been diagnosed.

Finally, the study population's race and ethnicity was overwhelmingly white and non-Hispanic, making the results not generalizable to minority populations.

Future Directions

At the time of writing, a recruitment effort was underway for a five year follow-up for all subjects enrolled in the CLD study. The results of this study would offer longitudinal data regarding the effect of BMI and weight loss on the effect of progression

of fibrosis and steatosis in this population. Further, this study could have been improved by having genotype information on all subjects. Another potential future study could assess whether tight control of diabetes, hypertension, and hyperlipidemia is predictive of steatosis. Finally, this study could be more generalizable if completed in the general population.

PUBLIC HEALTH IMPLICATIONS

Some of the strengths of this study are its size and appropriate power, a large female population, similarly aged female and male subgroups, and that detailed information on duration and source of HCV infection, alcohol abuse, medical history, and medication history were obtained via validated CDC algorithms by trained interviewers. Although previous studies have also examined this question, most of these studies have been European, hospital-based studies. This investigation is unique in the fact that its hepatitis C population is community-based, and many patients had complete liver biopsy results. Further, it reproduces findings from other studies regarding risk factors for steatosis and fibrosis, which supports the validity of our findings.

This investigation further elucidates how obesity clinically affects liver histology in the face of chronic HCV infection. Our results suggest that obesity is associated with advanced steatosis, which is, in turn, independently associated with advanced fibrosis. As chronic hepatitis C infection causes gradual progression to fibrosis, in the absence of pharmaceutical therapy, modifying risk factors that increase progression to fibrosis is essential. Since risk factors such as age, duration of infection, and gender are not modifiable, finding target risk factors that are becomes essential. The results of this study strengthens the argument that obesity worsens liver damage in patients with chronic hepatitis C infection and suggest a role for weight loss as a treatment modality in these patients. This is especially true in light of the findings that metabolic co-morbidities, for which obesity is a very strong risk factor, are themselves independently associated with steatosis.

SUMMARY AND CONCLUSIONS

The effects of obesity on health are far-reaching, affecting almost every organ system, sometimes indirectly through metabolic co-morbidities associated with obesity: diabetes, hypertension, and hyperlipidemia are among the most common causes of myocardial infarctions, stroke, renal failure, vision problems, and vascular disease. Further, we are beginning to understand that obesity has a very influential role in liver disease as well. Obesity is the primary cause of NAFLD, America's most common liver disease. This study found that, even in the setting of chronic hepatitis C infection, obesity is independently associated with advanced steatosis, as was having two or more metabolic co-morbidities, which are caused by obesity. Therefore, obesity delivers a "double whammy" of damage to the liver to produce steatosis. Further, advanced steatosis was associated with advanced fibrosis. Although age and male gender were also associated with significant fibrosis, only steatosis is a *modifiable* risk factor.

Hepatitis C infection is a progressive disease that, over time, progresses to cirrhosis and ultimately, death, either from complications of cirrhosis or the development of hepatocellular carcinoma. Therefore, apart from pharmaceutical therapy, which is expensive, difficult, and long, helping obese patients with chronic HCV infection to lose weight, as well as controlling hypertension, diabetes, and hyperlipidemia, can prevent or improve steatosis, and thus, improve their chances of not progressing to fibrosis. Health care providers should implement weight management counseling for all target patients.

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Table 1: Scaling of Outcome Variables and Co-Variates, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

Type of Variable	Variable	Categories
Binary	Obese	Yes / No
	Advanced fibrosis	≥ 2 / < 2
	Advanced steatosis	≥ 2 / < 2
	Advanced inflammation	≥ 2 / < 2
	Gender	Male / Female
	Race/Ethnicity	White non-Hispanic / Other
	Route of infection	IVDU / Other
	Use of steatotic medications	Yes / No
	Path diagnosis	HCV only / Other
	Any metabolic co-morbidity	Yes / No
Continuous	Age	--
	Duration of infection	--
	Time lapse between BMI and biopsy	--
Categorical	Alcohol abuse	<5, 5-9, >10 years
	Site	CA, CT, OR

Table 2: Frequencies & Descriptive Statistics for Outcome Variables & Co-Variates, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

Category	Variable (units) (n=450)		N / %
Outcome Variables	**Steatosis**[#]**:**	Not advanced: 0	223 (49.6%)
		1+	156 (34.7%)
		Advanced : 2+	60 (13.3%)
		3+	11 (2.4%)
	Fibrosis[#]**:**	Not advanced: 0 (none)	33 (7.3%)
		1 (portal)	117 (26.1%)
		Advanced : 2 (periportal)	161 (35.9%)
		3 (bridging)	67 (14.9%)
		4 (cirrhosis)	71 (15.8%)
Co-variates (binary or categorical)	**BMI**[#]**:** (kg/cm^2)	Normal (< 25)	147 (31.1%)
		Overweight (25 – 29)	172 (36.4%)
		Obese (≥ 30)	153 (32.4%)
	Site:	California	85 (18.9%)
		Connecticut	233 (51.8%)
		Oregon	132 (29.3%)
	Male/female		296/176
	Race/ Ethnicity:[#]	White/non-Hispanic	348 (73.6%)
		Black/non-Hispanic	44 (9.3%)
		Hispanic	44 (9.3%)
		American Indian/Alaska Native	22 (4.7%)
		Other (Asian, mixed, unsure)	15 (3.2%)
	Metabolic co-morbidities:	Hypertension	114 (25.4%)
		Diabetes	64 (14.3%)
		Hyperlipidemia	60 (13.4%)
	Number of co-morbidities:	1	125 (27.8%)
		2 or 3	53 (11.8%)
	Use of steatotic medications:	Yes	74 (16.5%)
		No	232 (71.9%)
		Unknown	52 (11.6%)
	Inflammation:[#]	Not advanced: 0	5 (1.1%)
		1	83 (17.5%)
		Advanced 2	297 (62.8%)
		3	84 (17.8%)
		4	4 (0.8%)
	Final Pathologic Diagnosis:	HCV only	282 (59.6%)
		Mixed (HCV plus other)	191 (40.4%)
	Heavy Alcohol Use: (years)	< 5	238 (53%)
		5 – 9	54 (12%)
		10+	151 (34.1%)
	Route of HCV Infection:	Intravenous drug use	332 (70.2%)
		Other	141 (29.8%)
	HCV Genotype:	3a	25 (6%)
		Other/mixed (1a/b, 2a/b, 4)	257 (57%)
		Missing	167 (37%)
Co-variates (continuous)			**Mean (standard deviation), Range**
	Body mass index (kg / in^2)		28.34 (6.12), 15.9 – 62.1
	Age (years) (n=450)		45.9 (7.66), 19.2 – 76.2
	Time lapse (# yrs interview occurred after biopsy) (n=446)		0.98 (0.79), -0.46 – 4.55
	Duration of infection (years)		25.5 (8.03), 5—50

[#]**Binary outcome variables in regression models (categories collapsed)**

Table 3: Cross-tabulation and Chi-square Test for Site versus Advanced Fibrosis, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

Site	Not advanced fibrosis N (%)	Advanced fibrosis N (%)	Row Totals	p-value of Pearson's χ^2 statistic
CT	60 (26%)	172 (74%)	232	0.002
CA	35 (41%)	50 (59%)	85	
OR	55 (42%)	76 (58%)	131	
Column Totals	**150 (33.5%)**	**298 (66.5%)**	**448**	

Table 4: Differences in Time Lapse between Liver Biopsy and Patient Interview, Age, and Duration of Infection among Sites, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

Site	Mean time lapse (years)	Mean age (years)	Mean duration of infection (years)
CT	0.81	44.40	25.70
CA	1.06	49.05	26.08
OR	1.25	45.93	24.79
ANOVA p-value	**<0.001**	**<0.001**	0.487

Table 5: Differences in Reported Metabolic Co-morbidities between Patient Report and Chart Abstraction Method, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

Reported Co-morbidities	Patient Interview only No. (%)	Chart abstraction only No. (%)	Combined (if reported either place) No. (%)
Hypertension	101 (22%)	47 (10%)	114 (25%)
Diabetes	36 (8%)	48 (11%)	64 (14%)
Hyperlipidemia	47 (10%)	23 (5%)	60 (13%)
None	297 (66%)	350 (78%)	271 (60%)

Table 6: Pearson's Bivariate Correlations between Outcome Variables and Selected Co-variates, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

	Duration of HCV Infection	Steatosis	Fibrosis	1+ metabolic co-morbidities	Alcohol Use	Steatotic Rx Use
Male	0.212**		0.157**		0.107*	-0.457**
White				0.184**		
Duration of HCV Infection			0.105*			-0.146*
Obese		0.205**		0.219**		
Steatosis			0.190**	0.123**		
Final Diag					0.833**	-0.146*
Steatotic Rx Use					-0.134*	

**significant at 0.01
*significant at 0.05

Table 7: Chi-square and Bivariate Correlation Results for Select Variables, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

	Pearson's χ^2 Statistic	p-value of χ^2 statistic	Pearson correlation	p-value of Pearson correlation
Male gender versus IVDU	14.963	<0.001	--	--
Male gender versus duration of infection	13.727	0.003	--	--
Black race versus hypertension	12.911	<0.001	0.169	<0.001
Black race versus any co-morbidity	16.062	<0.001	0.189	<0.001
Black race versus # of co-morbidities	16.987	<0.001	0.187	<0.001
Site versus advanced fibrosis	12.778	0.002	--	--

Table 8: Summary of Simple and Multiple Logistic Regression Analysis for Advanced Steatosis for All Candidate Variables, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

Variable Name	Crude odds ratio* (95% CI)	Preliminary Main Effects Model**	Multivariate Model[#]	p-value[#]	Final model[+]	p-value[#]
Advanced fibrosis						
No	1.00 (--)	1.00 (--)	1.00 (--)	<0.001	1.00 (--)	0.002
Yes	4.03 (1.94 – 8.37)	**3.24 (1.42 – 7.41)**	3.85 (1.82 – 8.13)		3.43 (1.59 – 7.37)	
Obese						
No	1.00 (--)	1.00 (--)	1.00 (--)	<0.001	1.00 (--)	<0.001
Yes	3.16 (1.87 – 5.34)	**3.68 (2.00 – 6.77)**	2.85 (1.66 – 4.89)		3.32 (1.84 – 5.98)	
Multiple co-morbidities						
0 or 1	1.00 (--)	1.00 (--)	1.00 (--)	0.014	1.00 (--)	0.100
2 or 3	2.74 (1.43 – 5.26)	**1.97 (0.91 – 4.29)**	2.36 (1.19 – 4.69)		1.91 (0.88 – 4.11)	
Advanced inflammation						
No	1.00 (--)	1.00 (--)	--	--	--	--
Yes	1.49 (1.00 – 2.20)	1.02 (0.61 – 1.71)				
Race/Ethnicity						
Other	1.00 (--)	1.00 (--)	--	--	--	--
White non-Hispanic	1.54 (0.89 – 2.65)	1.15 (0.59 – 2.24)				
Final diagnosis						
HCV only	1.00 (--)	1.00 (--)	--	--	--	
Mixed	1.49 (0.88 – 2.51)	**1.65 (0.90 – 3.03)**				
Duration of infection per year	1.03 (0.99 – 1.06)	1.00 (0.97 – 1.04)	--	--	1.00 (--) 1.01 (0.97 – 1.05)	0.662
Gender						
Female	1.00 (--)	1.00 (--)	--	--	--	--
Male	1.43 (0.82 – 2.49)	1.36 (0.70 – 2.65)				
Genotype						
Other/missing	1.00 (--)	1.00 (--)	--	--	--	--
3a	1.78 (0.68 – 4.62)	1.38 (0.42 – 4.54)				

*obtained from simple logistic regression model; in increasing order of p-values

**obtained from multiple logistic regression model with all effects entered simultaneously; those significant at p <0.11 are bolded

[#]obtained from multiple logistic regression model

^overall p-value of Wald statistic in multiple logistic regression model

[+]includes significant confounders

Table 9: Summary of Simple and Multiple Logistic Regression Analysis for Advanced Fibrosis for All Candidate Variables, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

Variable Name	Crude Odds ratio (95% CI)*	Preliminary Main Effects Model**	Multivariate Model#	p-value^	Final model+	p-value#
Advanced steatosis						
No	1.00 (--)	**1.00 (--)**	1.00 (--)	<0.001	1.00 (--)	<0.001
Yes	4.03 (1.94 – 8.37)	**3.88 (1.79 – 8.41)**	4.37 (2.05 – 9.34)		3.97 (1.84 – 8.60)	
Site						
CT	1.00 (--)	**1.00 (--)**	1.00 (--)	<0.001	1.00 (--)	<0.001
CA	0.50 (0.30 – 0.84)	**0.34 (0.19 – 0.62)**	0.33 (0.18 – 0.60)		0.33 (0.19 – 0.60)	
OR	0.48 (0.31 – 0.76)	**0.40 (0.25 – 0.66)**	0.41 (0.25 – 0.66)		0.40 (0.25 – 0.66)	
Age						
per year	1.06 (1.03 – 1.09)	**1.08 (1.05 – 1.11)**	1.08 (1.05 – 1.11)	<0.001	1.08 (1.05 – 1.11)	<0.001
Duration of infection per year	1.03 (1.00 – 1.06)	--	--	--	--	--
Gender						
Female	1.00 (--)	**1.00 (--)**	1.00 (--)	0.028	1.00 (--)	0.023
Male	1.96 (1.31, 2.94)	**1.64 (1.06 – 2.52)**	1.62 (1.06 – 2.50)		1.65 (1.07 – 2.55)	
Obese						
No	1.00 (--)	1.00 (--)	--	--	1.00 (--)	0.264
Yes	1.46 (0.95 – 2.25)	1.31 (0.82 – 2.11)			1.31 (0.82 – 2.11)	
Final diagnosis						
HCV Only	1.00 (--)	1.00 (--)	--	--	--	--
Mixed	1.41 (0.92 – 2.17)	1.17 (0.74 – 1.86)				

*obtained from simple logistic regression model; in increasing order of p-values

**obtained from multiple logistic regression model with all effects entered simultaneously; those significant at p <0.028 are bolded

#obtained from multiple logistic regression model

^overall p-value of Wald statistic in multiple logistic regression model

+includes significant confounders

Table 10: Interactions for Final Multiple Logistic Model for Advanced Steatosis, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

Interaction	Value of Test Statistic	p-value
Fibrosis x obesity	96.2 (χ^2)*	<0.001
Fibrosis x # co-morbidities	0.516 (Wald)	0.773
Obesity x # co-morbidites	3.902 (Wald)	0.142

*Calculated by contingency table with continuity correction of 1

Table 11: Cross-tabulation of Advanced Steatosis and Advanced Fibrosis by Obesity, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

	Not advanced fibrosis	Advanced fibrosis	Row N (%)	Odds ratio[#]
Not obese				
Not advanced steatosis	110 (40%)	163 (60%)	273	42.6
Advanced steatosis	0 (0%)	31 (100%)	31	
Column subtotal N (%)	**110 (73%)**	**194 (65%)**	**304 (68%)**	
Obese				
Not advanced steatosis	31 (30%)	74 (70%)	105	1.32
Advanced steatosis	9 (24%)	29 (76%)	38	
Column subtotal N (%)	**40 (27%)**	**103 (35%)**	**143 (32%)**	
Total	**150 (34%)**	**297 (66%)**	**447***	

*BMI data missing from one subject

[#]Using continuity correction factor of 0.5

Table 12: Interactions for Final Multiple Logistic Model for Advanced Fibrosis, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

Interaction	Wald Statistic	p-value for Wald Statistic
Steatosis x male gender	0.006	0.936
Steatosis x age	1.261	0.261
Steatosis x site	0.843	0.656
Male gender x age	0.150	0.698
Male gender x site	0.108	0.947
Age x site	1.802	0.406

Figure 1: Hierarchy for Establishing Duration and Likely Route of HCV Infection, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006

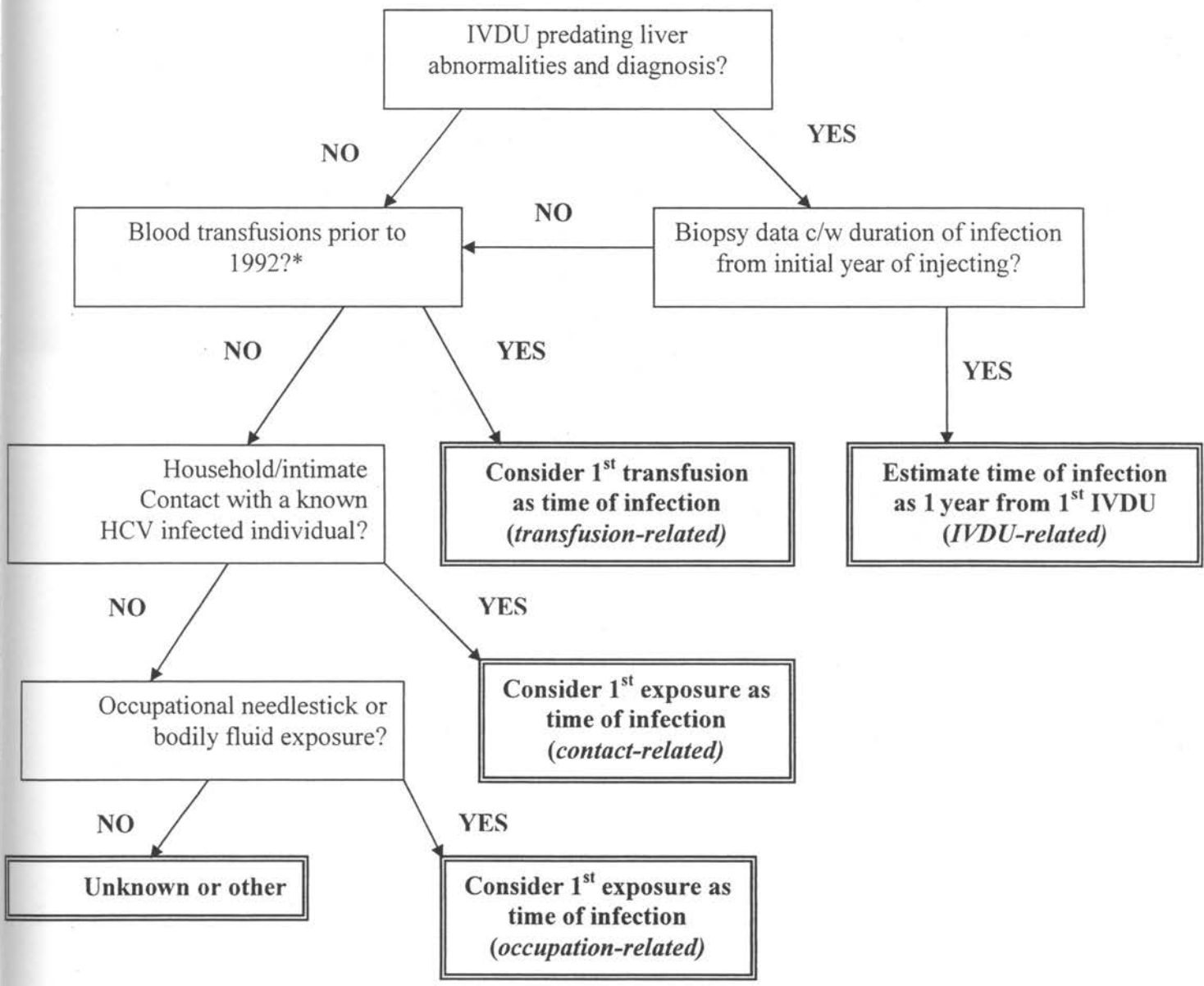
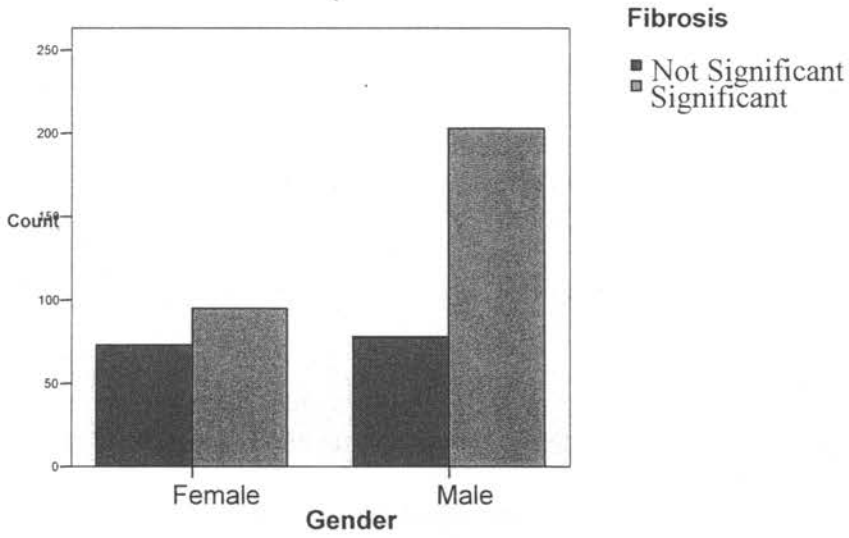


Figure 2: Histogram of Advanced fibrosis by Gender, Effect of Obesity on Hepatic Steatosis and Fibrosis Study, 2006



Appendix A: Case Definitions for CLD Study

1. **"Chronic liver disease"** was defined for the purposes of this study as[1]:
   a. abnormal liver function tests detected over a period of six months in one of the following combinations:
      i. alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) with or without abnormal alkaline phosphatase and bilirubin;

      -OR-

      ii. normal ALT/AST with abnormal alkaline phosphatase or bilirubin and elevated gamma-glutamyl-transferase (GGT) or 5'- nucleotidase;

- OR -

   b. pathology seen on liver biopsy, including findings of cirrhosis, fibrosis or chronic hepatitis;

- OR -

   c. abnormal findings on imaging studies, including nodularity or evidence of portal hypertension;

- OR -

   d. occurrence of a "diagnostic clinical event", such as variceal bleeding, evidence of portal hypertension found on endoscopy, encephalopathy, or portal hypertensive ascites.

2. **"Newly Diagnosed Cases" (incident cases):** an eligible subject must have received a new diagnosis of chronic liver disease that meets the above criteria during the calendar year 2000. To be counted as a newly diagnosed case and be included in our epidemiologic investigation of CLD, a patient must start the period of "becoming chronic" sometime in the six months before or after January 1, 2000 and pass the six-month benchmark of chronicity during the year 2000. We will define a year 2000 newly diagnosed case as a patient[2]:
   a. who seeks medical care in the year 2000;
   b. with abnormal LFTs, hepatopathology, hepatic imaging or a "diagnostic clinical event" first recorded between July 1, 1999 and June 30, 2000;
   c. that persist for a six month period ending anytime between January 1 and December 31, 2000.

3. **"Pre-existing Cases" (prevalent cases):** CLD prevalence data was gathered for calendar year 2000. We define a pre-existing case as:
   a. a patient who seeks medical care in 2000;
   b. with abnormal LFTs for at least six months before January 1, 2000

[1] This case definition was jointly developed and is currently employed by CDC and EIP sites engaged in CLD surveillance.

[2] Patients with abnormal LFTs first recorded after July 1, 2000 may become newly diagnosed CLD cases in the year 2001 if LFT abnormalities persist for six months to some point during the year 2001.

Appendix B: CLD study patient interview form

CHRONIC LIVER DISEASE SURVEILLANCE PROJECT

Initial Evaluation

V 2001 B

This section to be completed by the interviewer and separated from the rest of the survey

Patient ID#: _ _ _ _ _ _ _ _ _ _ Patient DOB: _ _ _ _ _ _
M M D D Y Y

Patient Name: _____

Patient Address: _____ Street

_____ Town and zip

Practice Code: _ _ _

Evaluator Initials: _ _ _ _

Blood collected for PCR ___Y ___N Date drawn: _ _ _ _ _ _
M M D D Y Y

ID# _ _ _ _ _ _ _ _ _ _

Site: (CIRCLE ONE) **CT** **CA** **OR**

Evaluation Date: _ _/_ _/_ _ _ _
M M D D Y Y Y Y

Draw Date: _ _/_ _/_ _ _ _
M M D D Y Y Y Y

Index Date*: _ _/_ _/_ _ _ _
M M D D Y Y Y Y

*Date patient met case definition

I'd like to begin by asking some questions about yourself.

1. What is your date of birth? _ _/_ _/_ _
M M Y Y Y Y

(9's if month and/or year is unknown)

2. **A.** What country were you born in? ..... ___ ___ ___

(See FIPS code card-Insert 3 digit code; 840=USA, 999=unknown)

STOP **if 840, go to question #3A**

B. What year did you come to the United States to live? ..... ___ ___ ___ ___
Y Y Y Y

(9999=unknown)

3. **A.** Were both of your parents born in the United States?

(Check one) ..... 1 ___ Yes 2 ___ No 9 ___ Unsure

STOP **if Yes, go to question #4**

B. What country was your mother born in? ..... ____ ____ ____
(use FIPS codes; 999=unknown)

C. What country was your father born in? ..... ____ ____ ____
(use FIPS codes; 999=unknown)

4. What is your gender?
(Check one) ..... 1 ____ Male 2 ____ Female 3 ____ Transgender

5. What is your height? ..... ____ feet ____ inches

6. What is your current weight? ___ ___ ___ pounds

Clinical Data

Now I would like to ask some questions about the problem you currently have with your liver. I will refer to this condition as chronic liver disease, by which I mean an ongoing, or chronic condition that affects the liver. Many other terms might be used to describe this condition, such as chronic hepatitis, chronic active hepatitis, or cirrhosis of the liver.

7. A. What type of liver problem do you have? ..... Hepatitis C 1 ____
(Allow patient to answer. Check all that apply)
Hepatitis B 2 ____
Cirrhosis 4 ____
Liver Cancer 5 ____
Alcoholic Liver Disease 6 ____
Autoimmune Hepatitis 7 ____
Hemochromatosis 8 ____
Wilson's Disease 9 ____
Fatty Liver 11 ____
Abnormal Liver Test 12 ____
Primary Biliary Cirrhosis (PBC) 13 ____
Primary Sclerosing Cholangitis (PSC) 14 ____
None / Don't know 15 ____
Medication/Drugs 16 ____
Other 10 ____
Specify: _____

For the next question, I want you to think back to when your liver problem was first discovered. (-pause-) I will read a list of ways that liver problems are usually discovered. When you hear a situation that is most like yours, let me know.

8. A. A concern about my liver was first raised by: *(Read list and check one)*
tests done during a routine physical 1 ___
life insurance testing 2 ___
being seen by a doctor in an office, clinic or hospital for another medical problem 3 ___
a condition that I have which might lead to chronic liver disease (e.g. HBV, HCV, alcoholism) 4 ___
Having symptoms such as fatigue, abdominal pain, jaundice 5 ___

tests done after a blood transfusion 6 ___
having an abnormal test result during a blood donation 7 ___
seeing a doctor because of an announcement or advertisement 8 ___
Having a family member with hemochromatosis 9 ___
Other 10 ___
Having a family member/spouse/partner with hepatitis 11 ___
Response to a needlestick 12 ___
I/Friend/Other thought it was a good idea 13 ___
Specify Other: _____

9. A. When did this happen? ..... __ __ __ __
Y Y Y Y

B. Which of these symptoms, if any, did you have at that time?
(*Read list. Check all that apply)* ..... fatigue 1 ___
..... jaundice 2 ___
..... leg swelling 7 ___
..... vomiting blood or rectal bleeding 12 ___
..... itching 14 ___
..... loss of libido or impotence 15 ___
..... none 17 ___

10. A. Have you been seen by a gastroenterologist (specialist) for your liver problem? ..... 1____ Yes 2____ No

if No, go to question #12

B. When did you first see a gastroenterologist (specialist) for a liver problem? ..... __ __/__ __ __ __
M M Y Y Y Y

11. I am now going to read some of the common reasons why people first see a gastroenterologist. When you hear a reason most like yours, let me know. When you saw the gastroenterologist (specialist) for the first time, did you go because:
(Read list and check one) ..... A physician or hospital suggested it 1 ___
You decided on your own to go 2 ___
A friend, family member or spouse suggested you should go 3 ___
A lay organization (for example, a community screening program, the American Liver Foundation) suggested you should go 4 ___
You were seen by the gastroenterologist (specialist) while in the hospital 5 ___
Other 6 ___
Specify Other: _____

12. A. Are you currently having any of these symptoms? *(Check all that apply)* ..... fatigue 1 ___
..... jaundice 2 ___
..... leg swelling 7 ___

.....vomiting blood or rectal bleeding 12 ___
.....itching 14 ___
.....loss of libido or impotence 15 ___
.....none 17 ___

13. A. Have you ever had acute hepatitis or yellow jaundice? By this I mean specifically an illness that lasted from a few weeks to a month or so, during which your skin might have turned yellow, you might have had dark urine, felttired, and may have felt nauseated, lost your appetite, had abdominal pain, or vomited.
(Check one) ..... 1 ___Yes 2 ___No 9____ Not sure/don't know

STOP **if No or Not sure, go to question #14**

B.

What type of acute hepatitis was it? *(Check all that apply)*	What year did this occur? *(9999=unknown)*
1 ___ hepatitis A (infectious hepatitis)	_____(YYYY)
2 ___ hepatitis B (serum hepatitis)	_____(YYYY)
3 ___ hepatitis C	_____(YYYY)
4 ___ hepatitis nonA – nonB	_____(YYYY)
5 ___ other: _____	_____(YYYY)
6 ___ alcoholic hepatitis	_____(YYYY)
9 ___ don't know/not sure	_____(YYYY)

14. How many times have you been hospitalized because you became ill from a liver problem, not including hospitalizations for testing purposes only.
____ ____ times *(99=Don't know)*

STOP **if 0 or 99, go to question #15A**

Starting with your first hospitalization for liver disease and going forward to your most recent:

What year were you hospitalized?	What was the main reason for being hospitalized? *(check one)*	
___ ___ ___ ___ Y Y Y Y	6__ Variceal bleed 3__ Infection in Abdominal Fluid 4__ Jaundice 2__ Mouth bleed 9__ Rectum bleed	1__ Confusion 5__ Fluid in legs or abdomen 8__ Vomiting 7__ Pain 10__ Other:
___ ___ ___ ___ Y Y Y Y	6__ Variceal bleed 3__ Infection in Abdominal Fluid 4__ Jaundice 2__ Mouth bleed 9__ Rectum bleed	1__ Confusion 5__ Fluid in legs or abdomen 8__ Vomiting 7__ Pain 10__ Other:
___ ___ ___ ___ Y Y Y Y	6__ Variceal bleed 3__ Infection in Abdominal Fluid 4__ Jaundice 2__ Mouth bleed 9__ Rectum bleed	1__ Confusion 5__ Fluid in legs or abdomen 8__ Vomiting 7__ Pain 10__ Other:

15. **A.** In your lifetime, have you ever received a transfusion of blood or blood products (e.g. platelets, plasma)?
(Check one) .... 1___Yes 2___No 9___don't know

STOP **if No or Don't Know, go to question #16**

B. How many times have you had a transfusion of blood products? .... ___ ___ times

(99=Don't Know)

C. When was the first transfusion you ever received? .... ___ ___ ___ ___
Y Y Y Y
(9's if unknown)

D. When was the most recent transfusion you received? .... ___ ___ ___ ___
Y Y Y Y
(9's if unknown)

16. Do you have a blood disorder (e.g. hemophilia) that ever required you to take a clotting factor?
(Check one) .... 1 ___Yes 2 ___No 9 ___ don't know

17. How many times have you had a liver biopsy, that is, removal of a piece of liver tissue? .... ___ ___ times
(99= Don't Know)

STOP **if 0 or 99, go to question #18A**

Starting with the most recent biopsy:

Where was it performed? *Hospital and City*	When was it done? *(9's if unknown)*
	__ __/__ __ __ __ M M Y Y Y Y
	__ __/__ __ __ __ M M Y Y Y Y
	__ __/__ __ __ __ M M Y Y Y Y

INTERVIEWER USE ONLY

18. A. Have you ever received medication or some other therapy from a physician for your liver problem?
(Check one) ..... 1 ___Yes 2 ___No 9 ___ don't know

..... STOP **if No or Don't Know, go to question #19**

B. Starting with the most recent treatment:

Did you ever receive any of these treatments for your liver disease? *(Read choices and check all that apply)*	What year did you start? *(9999=unknown)*	How many months did it last? *(99=unknown)*
1 ___ Interferon (such as IntronA, Roferon, Inferon, PEG Interferon)	__ __ __ __ Y Y Y Y __ __ __ __ Y Y Y Y	__ __ months __ __ months
2 ___ Ribavirin	__ __ __ __ Y Y Y Y __ __ __ __ Y Y Y Y	__ __ months __ __ months
3 ___Chelation (such as Penicillamine)	__ __ __ __ Y Y Y Y	__ __ months
4 ___ Phlebotomy (that is, having blood taken for therapeutic purposes)	__ __ __ __ Y Y Y Y __ __ __ __ Y Y Y Y	__ __ months __ __ months

5 ___ Steroids (such as Prednisone, Prednisolone, Deltizone)	___ ___ ___ ___ Y Y Y Y ___ ___ ___ ___ Y Y Y Y	___ ___ months ___ ___ months
6 ___ Ursodiol (such as Actigall, Urso)	___ ___ ___ ___ Y Y Y Y ___ ___ ___ ___ Y Y Y Y	___ ___ months ___ ___ months
8 ___ Lamivudine	___ ___ ___ ___ Y Y Y Y ___ ___ ___ ___ Y Y Y Y	___ ___ months ___ ___ months
9 ___ Methotrexate	___ ___ ___ ___ Y Y Y Y ___ ___ ___ ___ Y Y Y Y	___ ___ months ___ ___ months
10 ___ Imuran	___ ___ ___ ___ Y Y Y Y ___ ___ ___ ___ Y Y Y Y	___ ___ months ___ ___ months

19. Are you currently using any of the following therapies or changes in lifestyle specifically for your liver disease?

(Read list. Check if yes, for all that apply)

1 ___acupuncture
2 ___herbal medicine
3 ___vitamins or other supplements
4 ___homeopathy
5 ___chiropractic
6 ___other alternative or complementary therapies
7 ___ decrease or cessation of smoking
8 ___ increased amount of sleep
9 ___ reduction of stress
10 ___change in amount of exercise
11 ___change in diet
12 ___decrease or cessation of drinking alcohol
13 ___support group for liver problems
14 ___prayer/faith

20. The hepatitis A vaccine first became available in the United .....States in 1995 and is given in a two dose series. Have you ever received this vaccine? *(Check one)*.....1____Yes 2____No 9____unknown

If Yes, what year did you receive it? ..... ___ ___ ___ ___
Y Y Y Y

(9999=unknown)

21. The hepatitis B vaccine has been available for over 10 years and is given in a three dose series. Have you ever received this vaccine?
(Check one) ..... 1___Yes 2___No 9___unknown

If Yes, what year did you receive it? ..... ___ ___ ___ ___
Y Y Y Y

(9999=unknown)

Co-Morbidity and Additional Epidemiologic Data
Now we'll talk about health related issues other than your liver disease.

22. In the past 5 years have you had any of the following conditions?
(Read list, check all that apply)

CONDITION

1 ___ Low Blood Count *(Anemia)*
2 ___ Asthma
3 ___ Stroke or TIA *(Cerebrovascular Disease)*
4 ___ COPD *(Chronic Obstructive Pulmonary Disease)*
5 ___ Heart attack or Angina *(Coronary Artery Disease)*
6 ___ Heart Failure *(Congestive Heart Failure)*
7 ___ High blood pressure *(Hypertension)*
10 ___ Overactive/Hyper or Underactive/HypoThyroid *(Thyroid Disease)*
11 ___ Crohn's Disease
12 ___ Diabetes
13 ___ Ulcers *(Peptic Ulcer Disease)*
14 ___ Psoriasis
15 ___ Colitis *(Ulcerative colitis)*
16 ___ Cancer, not of the liver *(Extra-hepatic Malignancy)*
17 ___ High Cholesterol or Triglycerides *(Hyperlipidemia)*

23. Have you ever received abdominal radiation treatment for cancer? *(Check one)* ..... 1___Yes 2___No
24. How many times have you been hospitalized for reasons other than your liver, including childbirth? ..... __ __ times
(99= Don't Know)

25. How many times have you been pregnant for at least 7 months? __ __ times *(99= Don't Know)*

26. A. Have you ever had surgery? *(Check one)* 1 ___Yes 2___ No

if No, go to question #27A

B.

How many times have you had:	What year did you have your first:
Inpatient surgery? ___ ___ ___ ***(999=DK)***	**Inpatient surgery? ___ ___ ___ ___** ***(9999=DK)***
Outpatient surgery? ___ ___ ___ ***(999=DK)***	**Outpatient surgery? ___ ___ ___ ___** ***(9999=DK)***

27. A. Approximately how many times have you had oral surgery or extensive dental work?

By extensive dental work I mean more than routine exams, cleaning, and fillings. ..... ____ ____ times
(99=Don't Know)

if 0 or 99, go to question #28A

B. Did you have oral surgery or extensive dental work done in 1988 or earlier?
(Check one) ..... 1___Yes 2___No

C. Have you had oral surgery or extensive dental work done from 1989 until now?
(Check one) ..... 1___Yes 2___No

28. A. Have you ever received general anesthesia? This type of anesthesia puts you to sleep throughout the procedure and you typically wake up in a recovery area.
(Check one) ..... 1___Yes 2___No

if No, go to question #29 Intro

B. What type of procedure did you have general anesthesia for? Was the procedure:
(Read list and check one) ..... Medical ____1
Dental ____2
Both ____3
Don't Know ___9

Licit Drug Section

This next section deals with medications you may have taken at any time in your life for medical reasons.

29. Have you ever taken a medication that was prescribed by a doctor for any of the following....
(Read list. Check all where subject answers Yes)

Condition Code *(use # on history chart)*

1 ____	Acne or other Skin Conditions	
2 ____	Allergy, Skin Rashes	
3 ____	Anabolic Steroid Therapy for weight gain or muscle growth	
4 ____	Arthritis, Rheumatoid conditions, Inflammation, Autoimmune Disease, or After a Transplant	
5 ____	Cancer or blood disorders	
6 ____	Cholesterol	
7 ____	Depression or Mental Health	
8 ____	Diabetes	*If Yes, go to appropriate Cue Card*
9 ____	Heart or Blood Pressure	

10 ____ HIV disease
11 ____ Hormone Therapy
12 ____ Infection
13 ____ Lung Disease (including Asthma)
14 ____ Muscle Relaxant
15 ____ Oral Contraceptives
16 ____ Seizures or other neurological conditions
17 ____ Thyroid conditions

Record cue card answers on Rx Medication History

The next question asks about medications you may have taken for common conditions. Most of these medications can be purchased over the counter (that is, without a doctor's prescription), but some may be prescribed by a physician.

30. In the past 24 months, have you taken any medications either daily or frequently (2-3 times per week) for at least one month for....
(Read list. Check all where subject answers Yes)

18 ____ Colds, Allergies, Cough or Flu
19 ____ Headache, Pain, Inflammation, Menstrual Symptoms, Injury
20 ____ Sleep
9 ____ To prevent Heart disease, cancer or other diseases

If Yes, go to appropriate Cue Card
Record cue card answers on OTC Medication History

The next questions focus on the use of herbal and vitamin supplements.

31. Have you ever taken any vitamins daily or frequently (2-3 times per week) for at least one month?

(Check one)..... 1___Yes 2___No 9___Don't Know

STOP **if Yes, go to Vitamin Cue Card. Record Cue Card answers on Vitamin History.**

32. Have you ever taken herbal supplements or extracts?

(Check one)..... 1___Yes 2___No 9___Don't Know

STOP **if Yes, go to Herb Cue Card. Record Cue Card answers on Herb History.**

33.

A. Have you participated in any research studies in the past year that have included additional therapies, medications or treatments? *(Check one)*..... 1___Yes 2___No

..... STOP **if No, go to question #34A**

B. What was the treatment you received? _____

C. Are you still taking this treatment? *(Check one)*..... 1___Yes 2___No

The next couple of questions have been shown to be sensitive to some people. They are about tattoos and body piercing you may currently have or have received in the past. Your honest answers are appreciated.

34. A. In your lifetime, have you ever been tattooed? *(Check one)*..... 1 ___Yes 2 ___No

STOP if No, go to question #35A

B. What year did you first get tattooed? ..... ___ ___ ___ ___
Y Y Y Y
(9999=unknown)

C. What year did you last get tattooed? ..... ___ ___ ___ ___
Y Y Y Y
(9999=unknown)

D. How many times have you been tattooed? ..... ___ ___ times
(99=Don't know)

E. Where have you gone to get a tattoo: ..... street kiosk/booth or stand 1 ___
(Check all that apply)
home 2 ___
bar 3 ___
party 4 ___
jail 5 ___
professional store 6 ___
military housing 8 ___
outside US 9 ___

F. Who did your tattoo(s)? Was it: ..... a professional tattoo artist 1 ___
(Check all that apply)
a friend/acquaintance 2 ___
a family member 3 ___
self 5 ___

35. A. In your lifetime, have you had any body piercing, not including pierced ears?
(Check one) ..... 1 ___Yes 2 ___No

STOP if No, go to question #36A

B. What year did you first get a body piercing? ..... ___ ___ ___ ___
Y Y Y Y
(9999=unknown)

C. What year did you last get a body piercing? ..... ___ ___ ___ ___
Y Y Y Y
(9999=unknown)

D. How many times have you had your body pierced? ..... ___ ___ times
(99=Don't know)

E. Where have you gone to get pierced: ..... street kiosk/booth or stand 1 ___
(Check all that apply) ..... home 2 ___
bar 3 ___
party 4 ___
jail 5 ___
professional store 6 ___

military housing 8 ___
outside US 9 ___

F. Who did your piercing(s)? Was it: ..... a piercing professional 1 ___
(Check all that apply) a friend/acquaintance 2 ___
a family member 3 ___
self 5 ___

36. A. Have you ever received any of the following medical procedures outside of this country?
Blood Transfusion (1)
Surgery (2)
Dental Work (3)
Injection/Shot (4)
Suturing/Stitches (5)
Hospitalization (6)
(Check one) ..... 1 ___ Yes 2 ___ No

STOP if No, go to question #37

B.

Procedure *(Use codes from 36A)*	Where was this done? *(Use FIPS codes; 999=unknown)*	What year was it? *(9999=unknown, if range of years given, enter earliest year)*	Did this occur on a US military base or other US-operated facility?
___	___ ___ ___	___ ___ ___ ___	1 ___ Yes 2 ___ No
___	___ ___ ___	___ ___ ___ ___	1 ___ Yes 2 ___ No
___	___ ___ ___	___ ___ ___ ___	1 ___ Yes 2 ___ No
___	___ ___ ___	___ ___ ___ ___	1 ___ Yes 2 ___ No

37. In your lifetime, how many times have you donated blood?

(Check one) ..... 1 ___ None
2 ___ 1-9
3 ___ 10-19
4 ___ 20-29
5 ___ 30+

38 A. Have you ever been told that you could not donate blood?

(Check one) ..... 1 ___ Yes 2 ___ No

if No, go to question #39A

B. When were you told you could not donate blood? ..... __ __ __ __
Y Y Y Y
(9's if unknown)

C. What was the primary reason given for not donating blood? ..... Positive for a marker of hepatitis 1 ___
(Allow subject to respond and check one) Elevated liver function test 2 ___
Other 6 ___
Don't Know 7 ___

39. A. Before you were diagnosed with chronic liver disease, were you or anyone living in your household ever associated with a hemodialysis or kidney transplant unit?
(Check one) ..... 1 ___Yes 2 ___No

if No, go to question #40

B. Were you a: *(Read list and check one)* ..... Patient 1 ___
Employee 2 ___
Household contact of patient or employee 3 ___

C. What was the first year you were associated with a hemodialysis or kidney transplant unit in this way? ..... __ __ __ __
Y Y Y Y
(9's if unknown)

40. For this question I want you to think about your family's history with liver problems. By family, I mean parents, grandparents, brothers, sisters, spouses or partners and children.

To your knowledge, has anyone in your family ever had: *(Read list)*	*(Check one)*	What was their relationship to you? *(Check all that apply)*
Hepatitis B (10)	1__ Yes 2__ No 9__ Not Sure	1 __ Parent 4 __ Spouse/Partner 2 __ Grandparent 5 __ Child 3 __ Sibling
Hepatitis C (11)	1__ Yes 2__ No 9__ Not Sure	1 __ Parent 4 __ Spouse/Partner 2 __ Grandparent 5 __ Child 3 __ Sibling
Cirrhosis/Chronic Liver Disease (4)	1__ Yes 2__ No 9__ Not Sure	1 __ Parent 4 __ Spouse/Partner 2 __ Grandparent 5 __ Child 3 __ Sibling
Liver Cancer (5)	1__ Yes 2__ No 9__ Not Sure	1 __ Parent 4 __ Spouse/Partner 2 __ Grandparent 5 __ Child 3 __ Sibling
Alcoholism (12)	1__ Yes 2__ No 9__ Not Sure	1 __ Parent 4 __ Spouse/Partner 2 __ Grandparent 5 __ Child 3 __ Sibling
Hemochromatosis (7) (High iron in the blood)	1__ Yes 2__ No 9__ Not Sure	1 __ Parent 4 __ Spouse/Partner 2 __ Grandparent 5 __ Child 3 __ Sibling

In this next section, I'll be asking a few questions about some of the jobs you have had in the past. Please remember that all information collected on this survey is for research purposes only and will be kept confidential.

41. A. Have you ever served in the U.S. armed forces? *(check one)* ..... 1 ___Yes 2 ___No

if No, go to question #42

B. When did you serve? ..... __ __ __ __ to __ __ __ __
Y Y Y Y Y Y Y Y
(9's if unknown)

B. Did you ever serve outside the US?
(Check one)..... 1 ____Yes 2____No 9 ____ Not sure

if No, go to question #42

D. Which countries did you visit as a member of the armed forces? ..... ____ ____ ____
(See FIPS code card-Insert 3 digit code; 999=unknown)

____ ____ ____
(See FIPS code card-Insert 3 digit code; 999=unknown)

Were you ever in combat? *(Check one)* 1 ____Yes 2____No 9 ____ Not sure

If Yes, Did you have contact with blood in combat? *(Check one)* 1 ____Yes 2____No 9 ____ Not sure

42. *(Show Job Cue Card)*

Now I'm going to show you a list of jobs. Please tell me which group best describes the job you worked at for most of your life.

(Job Code) ..... ____ ____

(See Job code card-Insert 2 digit code; 99=unknown)

43. These are substances that you may have worked with either as part of your job or as a hobby. For each substance, tell me if you worked with it before your liver disease was identified, either on the job or with a hobby for 10 hours a week or more.

*(Read list and **check one column** for each item)*

	1 Yes, occup.	2 Yes, hobby	3 Both	4 No (neither)
carbon tetrachloride	_____	_____	_____	_____
1,1,1-trichloroethane	_____	_____	_____	_____
1,1,2,2 tetrachloroethane	_____	_____	_____	_____

44. A. While working at any job, either at present or in the past, did you ever have any exposure or direct contact with human blood?
(Check one)..... 1____ Yes 2 ____No 9____ Unsure

STOP **if No, go to question #45A**

B. What kind of job was it? Were you working as a: *(check "Y" for all that apply, "N" if not)*

1. Health care worker (e.g., nurse, phlebotomist, EMT) ..... 1___Yes 2___No
2. Public safety worker (e.g., fire fighter, police) ..... 1___Yes 2___No
3. Laboratory worker ..... 1___Yes 2___No
4. Other ..... 1___Yes 2___No

C. Were you ever stuck by a needle at this job? *(Check one)* ..... 1___Yes 2___No

If Yes, When was the first time this happened? ..... ___ ___ ___ ___
Y Y Y Y
(9's if unknown)

D. Were you ever cut while on this job? *(Check one)* ..... 1____Yes 2____No

If Yes, When was the first time this happened? ..... ___ ___ ___ ___
Y Y Y Y
(9's if unknown)

45. A. Before you were diagnosed with liver disease, were you or anyone living in your household ever associated with an institution, sheltered workshop or

group home for the developmentally disabled?
(Check one) ..... 1 ____Yes 2 ____No

 **if No, go to question #46A**

B. Were you a: ..... Resident 1 ___
(Read list and check all that apply) Employee 2 ___
Household contact of a resident or employee 3 __

C. For what period of time were you associated with the
developmentally disabled in this way? From: ..... ___ ___ ___ ___ to ___ ___ ___ ___
Y Y Y Y Y Y Y Y
(9's if unknown)

46. A. Before you were diagnosed with liver disease, were you or anyone living in your household ever associated with a detention facility, jail or prison for at least one week?
(Check one) ..... 1____Yes 2____No

 **if No, go to question #47**

B. Were you an: ..... Inmate 1 ___
(check one) Employee 2 ___
Household contact of an inmate or employee 3 ___

C. What was the first year you were associated with a jail in this way? ..... ___ ___ ___ ___
Y Y Y Y
(9's if unknown)

What was the last year you were associated with a jail in this way? ..... ___ ___ ___ ___
Y Y Y Y
(9's if unknown)

C. What is the total number of years you were associated with a jail in this way?..... ___ ___
(9's if unknown, round up. Enter 0 if < 6 mos)

The next set of questions ask you to rate your health status.

47. Would you say your health in general is excellent, very good, good, fair, or poor?..... Excellent 1 ____
(Check one) Very good 2 ____
Good 3 ____
Fair 4 ____
Poor 5 ____

48. Compared to a year ago, how would you rate your health in general now:
(Read list and check one) ..... Much better than a year ago 1____
Somewhat better than a year ago 2____

About the same as a year ago 3____
Somewhat worse than a year ago 4____
Much worse than a year ago 5____

49. Thinking about your physical health, which included physical illness and injury, for how many days during the past 30 days, was your **physical health** not good? ____ ____days *(99if unknown)*

50. Thinking about your mental health, which includes stress, depression and problems with emotions, for how many days during the past 30 days was your **mental health** not good? ____ ____days *(99if unknown)*

51. During the past 30 days, for about how many days did poor physical **or** mental health keep you from doing your usual activities, such as self-care, work or recreation? ____ ____days *(99if unknown)*

Economic and Household Data
Now I would like to ask you a few questions about yourself and your household.
52. Are you of Hispanic or Latino origin?
(Check one) ..... 1 ___ Yes 2 ___ No 9___Unsure

53. A. *(Show Race cue card)*
Please select one or more of the following categories to best describe your race:
(Check all that apply) ..... White 1 ____
Black or African American 2 ____
American Indian or Alaska Native 3 ____
Asian 4 ____
Native Hawaiian or other Pacific Islander 5 ____

B. *(If Asian)* Which of the following best describes your origin? *(Read and Check one)*
Cambodian 1 ____
Chinese 2 ____
Indian 3 ____
Japanese 4 ____
Korean 5 ____
Malaysian 6 ____
Pakistani 7 ____
Filipino 8 ____
Thai 9 ____
Vietnamese 10 ____
Other 11 ____
Specify other: _____

54. What type of housing do you live in?
(read list and check one)
House/ single family dwelling 1 ____
Mobile home 2 ____
Apartment or multi-family dwelling 3 ____
Dormitory 4 ____
Nursing home 5 ____

Shelter/homeless 6 ____
Other arrangements 7 ____
Specify Other: _____
Unknown/ not sure 9 ____

55. A. What is the highest grade or year of school you completed?
(Read if necessary and check one).....Never attended school or only attended kindergarten 1 ____
Grades 1 through 8 (Elementary) 2 ____
Grades 9 through 11 (Some high school) 3 ____
Grade 12 or GED (High school graduate) 4 ____
College 1 year to 3 years (Some college or technical school) 5 ____
College 4 years or more (College graduate) 6 ____
Don't Know 9 ____

B. What is the highest grade or years of schooling completed by anyone in your household?
(Read if necessary and check one)..... Never attended school or only attended kindergarten 1 ____
Grades 1 through 8 (Elementary) 2 ____
Grades 9 through 11 (Some high school) 3 ____
Grade 12 or GED (High school graduate) 4 ____
College 1 year to 3 years (Some college or technical school) 5 ____
College 4 years or more (College graduate) 6 ____
Don't Know 9 ____

56. How would you best describe your current employment status? Are you:.....Employed full-time 1____
(Read list and check one)
Employed part-time 2____
A Homemaker (housewife/husband) 3____
Unemployed 4____
If Yes, When were you unemployed? *(9's if unknown)* ___ ___ ___ ___
Y Y Y Y
Disabled 5____
If Yes, When were you disabled? *(9's if unknown)* ___ ___ ___ ___
Y Y Y Y

Retired 6____
If Yes, When did you retire? *(9's if unknown)*___ ___ ___ ___
Y Y Y Y
A Student 7____

57. Are you currently covered by medical insurance? *(Check one)*..... 1 ____ Yes 2 ____ No

if No, go to question #58

What type of medical insurance is it?
(check all that apply).....Private 1 ____
Managed Care (HMO, PPO, POS) 2 ____
Medicaid or state assistance 3 ____

Medicare 4 ____
Veterans 6 ____

Does your insurance plan require you to see a primary care physician in order to be referred to a specialist? *(Check one)* 1 ____ Yes 2 ____ No

58. *(Show Income cue card)*
Using the income categories shown on this card, what was the total combined family income during the past 12 months? Include all sources of income, including wages, salaries, pensions, and insurance payments.
(Check one)

$15,000 or less 1 ____
More than $15,000, but less than $30,000 2 ____
At least $30,000, but less than $50,000 3 ____
At least $50,000, but less than $100,000 4 ____
$100,000 or more 5 ____
Refused 7 ____
Don't know/ Not sure 9 ____

Now I need to ask you some questions about your use of alcohol, drugs and sexual practices. Let me remind you that all information you share with me here will be kept confidential. It will only be seen by myself and a few project staff members. This information will not be shared with your doctor, and will not affect the care you receive. You may decline to answer any question or questions.

A. 59. Have you ever consumed any form of alcohol?
(Check one) ..... 1____Yes 2____No

STOP **if No, go to question #68-Intro**

A. 60. Was there ever a period in your life when you consumed at least one drink per month?
(Check one) ..... 1____Yes 2____No

STOP **if No, go to question #68-Intro**

The next questions deal with drinking experiences. I'd like to start with the year that you first began drinking regularly, meaning at least one drink a month, and work forward to the present.

A. 61. Let's start with the first year that you began to have at least one drink per month and work forward to the present. Please think about the first year that you began to have at least one drink per month. How old were you?
Record the age to one decimal point on the answer sheet.

Now think to when your drinking behavior was different in a significant way from this time. This could be the next six months or perhaps 2 or 5 years later. Bear in mind any events in your life that changed that may have altered your drinking habits.
Fill in the age ranges for each stage under the "Age Range" column

(Establish when the person's drinking behavior first changed in a significant way from that recorded under First Stage. Since the drinking history is aimed at major trends , some judgment will be necessary in differentiating important from minor changes in drinking patterns.)

Now that we've established these periods in your life, I'd like to ask you some specific questions about your drinking history. We'll start with the period that you first began drinking regularly and work forward to the present. Please give me information as accurately as you can about what type of beverage you were drinking, how much, and how often.

A. 62. *(Show Drink Size cue cards)*
How many drinks would you have on a typical drinking occasion (drinking day)? Please look at the drink sizes on these cards to determine (the equivalent of) how many drinks of this size you would have per day.
Record the typical number of drinks next to "# of drinks" in the "Quantity" column.

A. 63. How many days per month would you generally drink at this level? (i.e. average drinks)
Record the number of days next to "# of days" in the "Frequency" column.

A. 64. What is the most or maximum number of drinks you would have in any one drinking occasion?
Record the number of drinks next to "maximum" in the "Quantity" column
Note: this is the maximum number that the person actually would drink, not an estimate of his/her potential capacity.

A. 65. How many days per month would you generally drink at this level?
Record the number of days next to "maximum" in the "Frequency" column.

A. 66. What type of beverage would you usually consume in an average month?
Record the relative percentages of beer, liquor or wine in the "Type" column
(This section should add up to 100%)

Now think to when you were ____ years old *(the next drinking period).*
*Repeat **A. 62 - A. 66** for each period*

67. Have you ever been arrested for driving under the influence of alcohol?
(Check one) ..... 1____Yes 2_____No 9 ____Refused

Now I am going to ask you about your past experience with recreational drugs. These are drugs that are not medically prescribed and may be smoked, snorted, inhaled, injected or otherwise ingested.

A. Have you ever used needles to inject recreational drugs? This includes injecting even once a long time ago.
(Check one) ..... 1 ____Yes 2 ____No 9 ____Can't remember/Refused

STOP **if No or Can't remember, go to question #69A**

B. What was the year the first time you injected drugs? ..... __ __ __ __
Y Y Y Y
(9999 if unknown)

C. Not including this year, in what year did you last inject drugs? ..... __ __ __ __
Y Y Y Y
(9999 if unknown)

69. A. Have you ever used recreational drugs, but not by injecting them?
(Check one) ..... 1 ____Yes 2 ____No 9 ____Can't remember/Refused

STOP **if No or Can't remember, go to question #70A**

A2. Did this include drugs other than marijuana?..... 1 ____Yes 2 ____No 9 ____Can't remember/Refused

B. How did you use recreational drugs? ..... Snort 1 ____
(Read list and check all that apply)
Smoke 2 ____
Free base 3 ____
Eat or Swallow 4 ____
Other 5 ____
Specify Other: _____

C. What was the year the first time you used recreational drugs? ..... __ __ __ __
(This includes marijuana) Y Y Y Y
(9999 if unknown)

D. Not including this year, in what year did you last use recreational drugs? ..... __ __ __ __
Y Y Y Y
(9999 if unknown)

70. A. Have you ever snorted cocaine? *(Check one)* .....1 ____Yes 2 ____ No 9 ____don't remember/Refused

STOP **if No or Don't Remember, go to question #71- Intro**

B. What was the year the first time you snorted cocaine?
__ __ __ __
Y Y Y Y
(9999 if unknown)

C. Not including this year, in what year did you last snort cocaine? ..... __ __ __ __
Y Y Y Y
(9999 if unknown)

We're almost done. This section asks a few questions about your reproductive health and behaviors.
Some of the questions may be sensitive, but I need you to be as accurate as possible with your responses.

71. Have you ever been diagnosed by a doctor with any sexually transmitted disease, such as gonorrhea, syphilis, chlamydia or genital herpes?
(Check one).....1 ____ Yes 2 ____No 9____ Declined answer

72. What is your best estimate of the total number of persons of the opposite sex That you've had sexual intercourse with in your lifetime?
..... ____ ____ _____ number of persons
(999=declined answer; if answer is ≥999 enter 998)

STOP **if subject is a Woman, go to question #74**

73. A. Have you ever had sexual intercourse with a person of the same sex?
(Check one).....1 ___Yes 2 ___No 9____Declined answer

if No or Declined Answer, go to question #74

B. What is your best estimate of the total number of persons of the same sex with whom you've had sexual intercourse in your lifetime?
..... ____ ____ ____ number of persons
(999=declined answer; if answer ≥999 enter 998)

74. Before you were diagnosed with chronic liver disease, as far as you know, did you ever have sexual or household contact with someone who:

A. Had hepatitis before or at the time of contact
(Check one) ..... 1 ___Yes 2 ___No 9 ___Declined answer

if No, go to question #74B

Was this a sexual or household contact?
(Check one—if contact is both household AND sexual, check sexual) ..... 1 ____ Household 2 ____ Sexual
What year did you first have contact with this person? ..... ____ ____ ____ ____
..... Y Y Y Y
(9999 if unknown)

B. Had ever used needles to inject recreational drugs
(Check one) ..... 1 ___Yes 2 ___No 9 ___Declined answer

if No, go to question #74C

Was this a sexual or household contact?
(Check one—if contact is both household AND sexual, check sexual) ..... 1 ____ Household 2 ____ Sexual
What year did you first have contact with this person? ..... ____ ____ ____ ____
Y Y Y Y
(9999 if unknown)

C. Had hemophilia or other blood coagulation disorder
(Check one) ..... 1 ___Yes 2 ___No 9 ___Declined answer

if No, go to #75 Intro

Was this a sexual or household contact?
(Check one—if contact is both household AND sexual, check sexual) ..... 1 ____ Household 2 ____ Sexual
What year did you first have contact with this person? ..... ____ ____ ____ ____
Y Y Y Y
(9999 if unknown)

We're near the end, I have only a couple more questions.

75. Would you be interested in participating in future research projects?
(Check one) ..... 1 ___Yes 2 ___No 9 ___Don't Know

76. Would you like any educational materials on the liver?

(Check one) ..... 1 ___Yes 2 ___No 9 ___Don't Know

Closing Statement: **Okay, that's the last question. Thank you very much for your time. Before we finish, are there any questions you would like to ask?_**

Appendix C: CLD study pathology score sheet

Histologic Features

Architecture/Fibrosis | **Hepatic Lobules**

Stage:
- 0 - No fibrosis
- 1 - Portal fibrosis
- 2 - Periportal fibrosis
- 3 - Bridging fibrosis
- 4 - Cirrhosis

0-No inflammation/necrosis___
1-Inflammation, no necrosis___
2-Focal necrosis/acidophil bodies___
3-Severe focal cell damage____
4-Bridging necrosis____

Portal Tracts

Bile ducts: (check if present)
- Injury ____
- Chronic inflammation _____
- Proliferation _____
- Duct loss _____
- Bile plugs ducts____ Ductules____
- Cholangitis ____ Pericholangitis____

Vessels: normal/abnormal

Inflammation: (0-4+)
- 0-None or minimal _____
- 1-Portal only_____
- 2-Mild interface hepatitis_____
- 3-Moderate Interface hepatitis _____
- 4-Severe Interface hepatitis_____

Eosinophils (0-3+)_____

Steatosis: (0-3+)
- Macrovesicular __
- Microvesicular __

Steatohepatitis (yes/no) ____
Neutrophils:yes/no
Peri-central vein fibrosis: yes/no
Sinusoidal fibrosis: yes/no
Mallory Hyaline: yes/no
Central sclerosis: yes/no
Ground glass change yes/no

Cholestasis (check if present)
- Canalicular ___
- Hepatocellular ____

Iron Deposition (if stain done)
- Parenchymal (0-4+)___
- Kupffer cell: yes/no

Miscellaneous Findings and Comments D-PAS globules: (if done) yes/no

Central zone: (check if present)
Congestion _ sinusoidal dilatation _
Veno-occlusive disease __ Thrombi__

Pathologic Diagnosis

Hepatitis	**Cholestasis**	**Steatosis**	**Iron Deposition**	**Metabolic Disorder**	**Malignancy**
Hep C ___	Drug reaction _	Steatohepatitis ___	Secondary ___	Wilson's __	Primary ___
Hep B ___	Vanishing bile duct syndrome _	NASH ___	Primary ___	Glycogen storage disease ___	Secondary ___
Autoimmune ___	PBC __	Alcoholic ___	Unknown ___	Alpha-1 antitripsin deficiency ___	
Drug reaction _	PSC __	Nondiagnostic ___			
Other					
Nondiagnostic _	Nondiagnostic ___			Nondiagnostic ___	

Stage___ Grade___ (where applicable)

Appendix D: Abbreviations Used, Effect of Obesity on Hepatic Steatosis and Fibrosis, 2006

AI/AN: American Indian / Alaskan native

ALT: aspartate aminotransferase

AST: alanine aminotransferase

ATP: adenosine triphosphate

BMI: body mass index

CI: confidence interval

CLD: chronic liver disease

HCV: hepatitis C virus

HCW: health care worker

IVDU: intravenous drug use

NAFLD: non-alcoholic fatty liver disease

NASH: non-alcoholic steatohepatitis

OR: odds ratio