Advanced Fibrosis and Hepatocellular Carcinoma in South Texas

COLLABORATORS

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1. BACKGROUND

Hepatocellular carcinoma (HCC) and advanced liver disease appear to affect Texans, particularly in the southern counties, disproportionately compared to the US population. In southern Texas counties, the age-adjusted death rate from HCC is 12 per 100,000, compared to 5.6 per 100,000 nationally (Ramirez *et al*., 2012). In earlier studies, we found a high prevalence of advanced liver disease in a chart review (126/100,000 overall, and 386/100,00 in men (Perez et al., 2004). Significant risk factors reported were history of alcohol use (OR 6.6: 95% CI 4-10.8), and hepatitis (19.3%: 95% 8.0-46.4%). Confidence intervals were large and the analysis left a significant number of patients with no known risk factors, suggesting an important role for "cryptogenic cirrhosis", in other words, non-alcoholic steatohepatitis (NASH). To corroborate these findings, we have an extensively characterized population-based cohort, the Cameron County Hispanic Cohort, which has generated rich data in regards to risk factors for liver disease. In a population such as that in South Texas, where the prevalence of obesity is 50.9% and that of the metabolic syndrome 49.7% (Fisher-Hoch, 2012) we also find the prevalence of hepatic steatosis to be 52% on ultrasound (non-alcoholic fatty liver disease: NAFLD) (Pan *et al*., 2015). These data confirm our impression that this population has a significant burden of NAFLD and most likely also NASH. Indeed we have recently estimated the prevalence of NASH to be between 17 and 63% using a range of diagnostic panels. We hypothesize these high rates to be attributable to the high prevalence of obesity, diabetes and metabolic syndrome (Pan *et al*., 2015). What is now needed is (1) to determine the real prevalence of advanced fibrosis, NASH and HCC, and (2) to understand the risk factors specific to the South Texas population and choose appropriate intervention strategies.

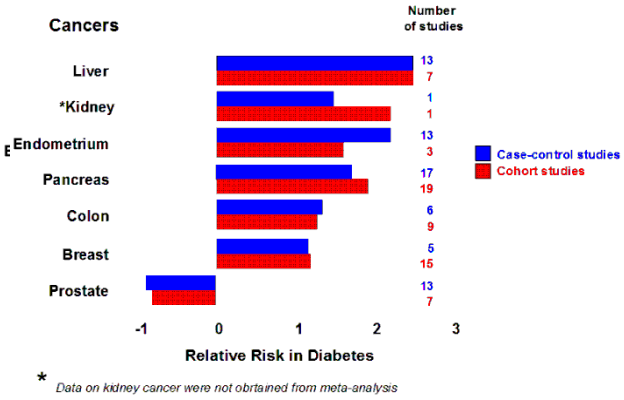
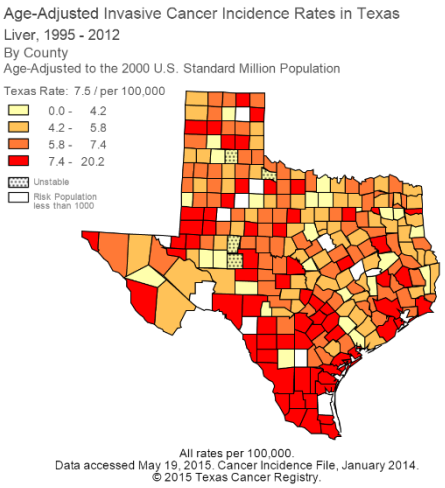


Figure 1. Relative risk for cancer in diabetes: (from Vigneri, et al, Endocrine-Related Cancer (2009)

HCC is the cancer most commonly associated with diabetes (figure 1). Both high body fat and insulin levels are implicated in the pathogenesis of cancer in diabetes, but the mechanisms are unknown. HCC is the fastest growing cause of cancer-related death in the United States (Ries, 2008) with incidence rising over the past 3 decades. From 2005 to 2009, incidence and death rates for HCC rose by 3.7% and 2.3% per year, respectively. The similarity between incidence

and mortality rates reflects the rapidity of death after diagnosis, as most HCC cases are diagnosed at a late stage, presenting with large, multifocal tumors. The overall, 5-year survival is 15%. While the national age-adjusted annual death rate from HCC is 5.6 per 100,000, the age-adjusted death rate in Texas is among the highest in the country reaching 9.3 deaths per 100,000 in 2009. Most strikingly, Hispanics in Texas southern counties have the highest overall rate with over 12 deaths per 100,000 (Fig.2A). ***Therefore, HCC is a leading cause of death among south Texas Hispanics regardless of age or gender despite significant underreporting (Fig.2B) (Ramirez et al., 2012; Albano et al., 2007).***

A



B

	Gender	US SEER Rate (95% CI)	Texas Rate (95% CI)	South Texas Rate (95% CI)
Latinos	Male	11.9 (11.4-12.4)	14.8 (14.2-15.4)	17.3 (16.4-18.2)
	Female	3.8 (2.6-4.1)	5.1 (4.8-5.4)	5.4 (5.0-5.9)
	Total	7.5 (7.2-7.7)	9.5 (9.2-9.8)	10.6 (10.1-11.1)
NLW	Male	4.8 (4.7-4.9)	5.2 (5.1-5.4)	6.0 (5.4-6.5)
	Female	1.3 (1.3-1.4)	1.4 (1.3-1.5)	1.7 (1.4-2.0)
	Total	2.9 (2.8-3.0)	3.1 (3.1-3.2)	3.7 (3.4-4.0)

Fig. 2: (A) Regional distribution of liver cancer death rates; **(B)** Incidence rates of HCC in Hispanics from US SER. Texas and South Texas, 1995-2006 (Ramirez et al, 2012);

Because the prognosis for HCC is poor, and non-invasive means of population screening of fibrosis and HCC are currently not available, novel methods of early detection are crucial. Dr. Laura Beretta's group has previously identified novel biomarkers for the risk prediction of HCC in patients with cirrhosis as well as in the general population. She has also identified biomarkers to predict intermediate and advanced fibrosis in patients with nonalcoholic fatty liver diseases (NAFLD). Further, there is a need to determine the somatic mutations and other genomic alterations in HCC. Since HCC is the final and usually terminal stage in a spectrum of liver injury, we will also be studying the preceding conditions, particularly liver fibrosis and cirrhosis. In this study we aim (1) to determine the etiology and characteristics of hepatic fibrosis and HCC in South Texas counties, (2) evaluate existing and novel biomarkers and diagnostic algorithms for predicting advanced liver disease in this population, (3) define the somatic genomic alterations in HCC in this population and (4) determine the familial genetic risk component of HCC in South Texas Hispanics.

3. HYPOTHESES AND RESEARCH AIMS

3.1 *Hypotheses*

We hypothesize that a large proportion of HCC cases will have a wide range of underlying conditions, including non-alcoholic steatohepatitis (NASH), non-alcoholic and alcoholic cirrhosis, as well as chronic viral hepatitis, or possible combinations of the above. We also hypothesize that the underlying conditions and somatic mutation profiles are different in this population compared to the typical HCC underlying disease pattern in the US.

3.2 *Research Aims*

Aim 1: Characterize and determine risk factors for liver disease and HCC in South Texas

We will obtain retrospective imaging and clinical data for patients from the various providers, clinics, and hospitals in Cameron and Hidalgo counties, linked with registry data from the Texas Cancer Registry, for patients with liver disease or HCC. We will then analyze de-identified data to determine etiology and evaluate risk factors for advanced fibrosis and HCC and their relative contributions to advanced liver disease in this population.

Aim 2: Characterize fibrotic and HCC pathology samples and conduct genomic analyses

We will retrospectively acquire liver pathology samples from the subset of subjects from the chart review that have histologically confirmed fibrosis or HCC at Valley Baptist Medical Center (VBMC) in Brownsville and Harlingen, TX, and at Doctor's Hospital at Renaissance in Edinburg, TX. These samples will be paired with the medical record data before being de-identified. The team at the UTHealth Medical School will analyze de-identified biopsy material to determine characteristics of NAFLD, NASH, and fibrosis. The team at MD Anderson will use this material for HCC analysis, including genomic analysis of HCC tumor cells and determination of the relationship to the host DNA profile. Of particular interest will be generating data on mutation profiles of HCCs with non-viral and non-alcoholic etiology.

Aim 3. Evaluate the performance of existing and novel biomarkers in detecting and predicting fibrosis and HCC in Hispanics, and enroll family members to determine familial genetic profiles.

We will prospectively enroll patients undergoing routine diagnostic liver biopsy, obtaining consent to collect portions of biopsy specimens, additional blood specimens, and medical records to evaluate the sensitivity and specificity of non-invasive predictors for fibrosis and HCC. The pathology samples and associated medical records will also be evaluated for inclusion in analysis for Aim 2. Using the paired blood specimens, we will then correlate biomarkers with clinical data and pathology and determine optimal cut-off scores for each panel of biomarkers. This will be accomplished by developing a diagnostic algorithm with or without previously validated early detection biomarkers. This aim will allow us to address major obstacles to early diagnosis and detection of pathology when disease is more easily reversed.

Aim 4. Evaluate the clustering of risk factors (somatic and genetic) in close relatives of patients with liver disease and HCC and determine high-risk endophenotypes.

During the consent process for Aim 3, we will also obtain consent to contact 1st- or 2nd- degree relatives of each enrolled participant, whom we will invite to complete the standard Cameron County Hispanic Cohort examination at the Clinical Research Unit (CRU). We will additionally invite patients from tertiary care facilities in Cameron and Hidalgo counties who have a confirmed HCC or fibrosis diagnosis to participate, along with their 1st- or 2nd- degree relatives. After consenting, family members of each enrolled patient, together with the patient will attend the CRU for physical examination, medical history, urinalysis, blood specimens (plasma, DNA and RNA); ultrasound measurement, including liver ultrasound, elastography, and Fibroscan; As detailed in HSC-SPH-11-0631. These data will be compiled and used to identify high risk endophenotypes for liver disease and HCC. (Endophenotypes are heritable intermediate traits that are correlated with disease liability. The heritable nature of endophenotypes provides increased power to localize and characterize disease related genes and evaluate the specificity of biomarkers.)

4. METHODS

4.1 Aim 1

This Aim collects and collates data collected during the diagnostic and treatment procedures of patients with liver disease (NAFLD, NASH, fibrosis, or cirrhosis) and HCC from January 1st, 1995 through December 31st, 2015. We will obtain and analyze medical records of patients with diagnosis of liver disease or HCC at a variety of providers, hospitals, clinics, and other institutions in Cameron and Hidalgo counties.

4.1.1 Population and Sample Size

We conservatively estimate gathering 500 records from patients with diagnosed HCC and 2500 records with diagnosed liver disease. Over the study period we therefore predict that we will gather approximately 3000 unique patient records, composed of medical records from various sources. A two group chi-square test with a 0.05 two-sided significance level will have 86% power to detect the difference between the proportion of non-HCC cases with a given characteristic (risk factor, underlying disease), p_1, of 0.10 and the proportion of HCC cases with underlying effects (or other risk factors), p_2, of 0.15.

4.1.2 Acquiring Retrospective Medical Record Data

We will collect complete clinical data from a variety of sources, including clinical histories, laboratory results, imaging reports, and pathology reports. We will also request access to complete name, date of birth, and date of procedures in order to match with other existing medical records from distinct providers in the region. We will run a search for the participant in the Texas Cancer Registry. Pertinent information will be added to the study participant record. These data will be immediately de-identified after reconciling duplicate records and assigning the appropriate study number. Once matched and abstracted, original identifiable data will be destroyed. Any available dynamic imaging data from HCC cases will be reviewed centrally and staged using the OPTN/LIRADs staging system and imaging data (CT, MR, US) from non-HCC

controls will be evaluated in a similar fashion with recording of standardized assessment of features of cirrhosis. The data will be abstracted to a REDCap database, also maintained behind the UTHealth firewall, accessed using approved UTHealth credentials. Only de-identified data will be used in analyses.

4.1.3 Statistical Analyses

Descriptive statistics for the different disease groups will be generated to determine the characteristics of each group, defined by liver diagnosis. Chi-square tests will be used to test for associations of categorical variable, and Students *t*-test or nonparametric tests will be used to test for differences in the values of continuous variables in the case versus control groups. Additionally, multivariable logistic regression models will be used to determine age-adjusted independent effects of specific underlying liver disease and other risk factors on the presence of HCC compared with non-cancerous liver disease.

4.2. AIM 2

This aim is a retrospective case-control analysis of existing pathology material, among patients with histologically confirmed fibrosis or hepatocellular carcinoma. It will be a sub-set of the sample of Aim 1 including only patients from Valley Baptist Medical Center and Doctor's Hospital at Renaissance with existing liver biopsy specimens.

4.2.1 Population and Sample Size

The inclusion criteria for this part of the study will be any patient with histologically confirmed liver fibrosis or HCC at the two participating hospital systems. We estimate that we will be able to collect 150 cases of histologically confirmed HCC (cases), and 450 cases of histologically confirmed fibrosis (controls) during the study period. A two group chi-square test with a 0.05 two-sided significance level will have 80% power to detect the difference between the proportion of controls with other underlying liver conditions (or other risk factors), p_1, of 0.10 and the proportion of cases with other underlying liver condition (or other risk factors), p_2, of 0.19. Other scenarios are presented in Table 1.

Table 1. Additional scenarios in Aim 1.

Controls (n=450) p1	Cases (n=150) p2	Power (alpha=0.05)
0.10	0.19	80%
0.10	0.20	86%
0.10	0.22	94%
0.20	0.30	71%
0.20	0.29	62%
0.20	0.32	84%

4.2.2 Acquiring and processing pathology samples

The Brownsville Regional Campus has established relationships with area hospitals, and we will work directly with the heads of Pathology to identify specimens that meet our inclusion criteria. After identifying and pulling retrospective samples, matching with existing records, and de-identifying the samples, pathology slides will be packed and shipped according to UTHealth protocol to the UT Medical School in Houston. Patient records for this aim will be collated with the rest of the chart review records. De-identified fibrotic pathology samples will be reviewed by a single hepatic pathologist (Dr. Younes, UTHealth) using the METAVIR scoring system, and in patients found to have clinical and histological features of NAFLD, the NAFLD Activity Score will be measured. Suspected or confirmed HCC tumor pathology will be evaluated for histological type, grade, tumor component, and cellularity by Dr. Laura Beretta at Anderson Cancer Center. Non-neoplastic liver parenchyma will be assessed for presence or absence of hepatitis, steatosis, and fibrosis. Once the diagnosis of HCC is confirmed, the percentage of neoplastic cellularity will be evaluated, and tumor and non-neoplastic liver parenchyma will be marked on the slide for microdissection. Additional genomic analyses will be conducted by Dr. Beretta's laboratory at MD Anderson Cancer Center.

4.2.3 Statistical analysis

Used in conjunction with existing medical record data, we expect to be able to analyze a variety of underlying diseases and hypothesized risk factors, and their association with the centrally staged and graded pathology samples. Analyses will proceed as outlined in 4.1.3.

4.3 AIM 3

In Aim 3, we will prospectively collect a blood sample and paired liver histology from consenting patients who have been referred for a pre-procedure blood draw and liver biopsy under the care of physicians seeing patients at Valley Baptist Medical Center or Doctor's Hospital at Renaissance (DHR). These patients, and two of their first- or second-degree relatives, will be invited to complete a clinical exam at the Brownsville or Harlingen Clinical Research Unit.

4.3.1 Population and Sample Size

The inclusion criterion for Aim 3 is any patient scheduled for liver biopsy, or suspected or confirmed HCC or advanced fibrosis. We anticipate obtaining 300 prospective pathology samples with paired blood samples during the study period. Valley Radiology and Associates performs about 200 liver biopsies per year; additionally, among liver biopsies performed by Dr. Jose Luis Almeda's group at DHR, about 50 are confirmed as HCC annually. Based on these data, we can expect to recruit up to 100 participants per year.

4.3.2 Acquiring and processing prospective pathology material and blood sample

We will work with each group to acquire liver pathology and paired blood samples prospectively. Before beginning, we will obtain permission from referring physicians to approach their patients to participate in this research. Before a scheduled liver biopsy, a bilingual (English/Spanish) member of the study team in Brownsville will approach the patient, explain the study and obtain informed consent to participate before the pre-procedure blood draw. The consent will include permission to draw an extra 10 mL of blood to be used for research, and a HIPAA consent to request medical records from VBMC and from referring physicians with related patient data including any imaging which may be available (e.g. MRI, CAT scans). The HIPAA consent will also include permission to search for name, date of birth, and related demographic information to the Texas Cancer Registry and other registries cancer or mortality data, where applicable.

The consent form will specify retention of material including DNA for genetic studies, and their use for future studies. The consent will also specify permission to be contacted for future studies with UTHealth. Finally, the patient will consent to our team contacting family members to enroll in Aim 4. The patient may decline any part of the study despite being enrolled by marking appropriate responses on the form. If the patient consents, a copy will be made and provided to the subject with contact numbers in case of questions or injury according to UTHealth standard protocols required by the IRB. All consent forms have been reviewed and approved by UTHealth Committee for the Protection of Human subjects.

During the blood draw, a member of the Brownsville team will provide the attending phlebotomist one 10 mL lavender-top tube (EDTA) to collect material for research after the clinical tubes have been filled. The phlebotomist will invert the tube 7 to 10 times and then give the sample to the member of the research team. The additional tube will be immediately placed in a biohazard container and transported on foot or in a UTHealth vehicle to one of our Clinical Research Units to be centrifuged. The sample will then be separated into plasma, buffy coat, and red blood cells and immediately frozen in the -80 C freezer at the Clinical Research Unit. All blood samples will be paired with the pathology material and medical records and given a study number. Critical missing laboratory information, such as Hepatitis virus assays, will be generated in the laboratory at the UT School of Public Health in Brownsville before being sent to Dr. Beretta's laboratory at MD Anderson.

After every 20 participants, liver specimens will be packed and shipped to the UTHealth Medical School, and aliquots of red blood cells, buffy coat and plasma will be packed and shipped according to UTHealth protocols to Dr. Beretta's laboratory in Houston for analysis.

At the hospital, a portion of the pathology sample acquired during the scheduled biopsy will be set aside for research and will be processed and analyzed according to 4.2.2 and incorporated into procedures described in Aim 2, if inclusion criteria are met. That is, the sample will be fully characterized as described in Aim 2, and then paired with the blood sample for analysis in Dr. Beretta's laboratory. If fibrosis or HCC is not found, the pathology sample will not be used for the case-control study in Aim 2, but the histology sample and paired blood sample will be used for additional studies in Dr. Beretta's laboratory (see section 4.3.3 below). Over the two year study period, we expect to collect 300 pathology samples with paired blood samples, with up to 150 HCC cases and 150 fibrosis cases.

4.3.3 Laboratory analysis

After being centrally graded and staged (4.2.2) at the UTHealth Medical School, pathology samples will be packed and shipped according to UTHealth protocol and sent to Dr. Beretta's lab at MD Anderson. There, HCC samples will be genotyped and a somatic mutation profile generated. These profiles will be compared with somatic profiles obtained from blood. Biomarkers will be evaluated using frozen plasma samples. This information will later be incorporated into diagnostic algorithms for fibrosis and HCC.

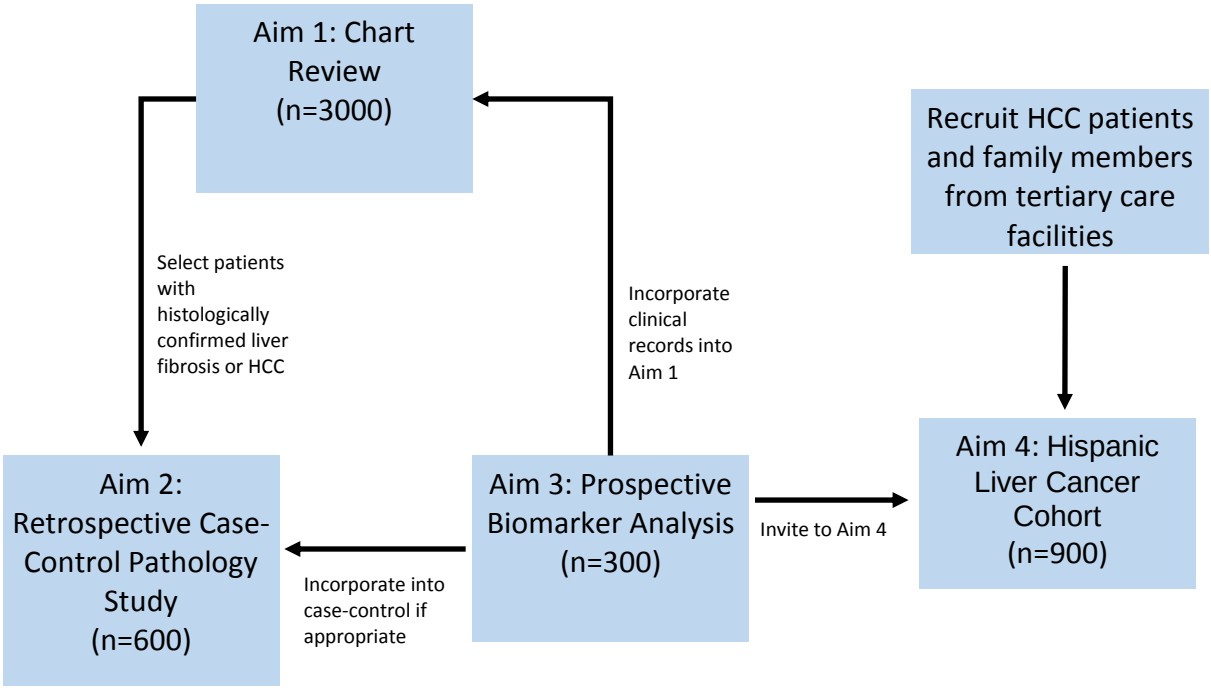
4.4 AIM 4

In Aim 4 (The "Hispanic Liver Cancer Cohort"), we invite prospectively enrolled participants in Aim 3 to complete a clinical exam and ultrasounds at the CRU, and enroll two family members from consenting participants in Aim 3 to complete this exam. We will also invite patients with diagnosed HCC or fibrosis under the care of physicians at tertiary care centers in Cameron and Hidalgo counties, and their 1^{st}- and 2^{nd}- degree relatives. We will generate risk factor profiles, with an emphasis on genetic data, in these families using these clinical data. Participants with diagnosed disease will be invited for follow-ups every year, and their family members will be invited for follow-ups every two years.

4.4.1 Population and Sample Size

We expect to enroll 150 patients with HCC and 150 patients with advanced fibrosis from Aims 3 and 4, and anticipate that we will be able to contact two family members for each of these participants. Thus, for the Hispanic Liver Cancer Cohort we expect to enroll 300 cases plus 600 family members, for an expected total sample size of 900. See Figure 1 for an overview of the study Aims.

Figure 1. Diagram of Study Aims



4.4.2 Recruitment of the Family Cohort participants

We will obtain detailed contact information for up to ten first- and second-degree relatives of each invited patient. The consenting participant will be invited to complete the CRU exam and ultrasounds at a later date and will also be invited for follow up visits (University of Texas Health Science Center Committee for the Protection of Human Subjects protocols HSC-SPH -03-007B and HSC-SPH-11-0631).

Using the provided contact information, we will then attempt to contact the family members listed: first by phone, then by up to five home visits at varying days and times. If contact is successful, the research team will explain that his or her contact information was provided by

the referring participant, and will invite him or her to complete the CRU exam. We will explain the exam briefly, and, if the family member is interested, set an appointment to come to the CRU, complete the consent process, and undergo the clinical exam. The family member will also have the opportunity to refuse to participate or request to be contacted at a later time. If no contact is made after attempts by phone and up to five home visits, then this file will be closed and the team will attempt to contact other family members.

4.4.2 Examination of Family Cohort Participants

After consenting, each family member will complete a detailed physical exam, including anthropometrics, electrocardiogram, bioimpedance analysis, blood draw, urinalysis, and personal and family medical history. This process is detailed in HSC-SPH-03-007B. These participants will be assigned a study number for the Hispanic Liver Cancer Cohort (HLCC) and identifying information will be kept confidential. Blood and urine samples will be obtained as CPHS study HSC-SPH-03-007B. After obtaining blood and urine samples, the samples will be processed and stored in accordance with HSC-SPH-03-007B protocol. Results for the lipid panel, comprehensive metabolic panel, hemoglobin A1c (HbA1c) and complete blood count will be shared with the participant via mail when available, and any questions about the results answered. All participants who complete the CRU exam will also be invited to consent to complete the other studies offered in the Cameron County Hispanic Cohort, including echocardiogram, liver ultrasound, elastography, and fibroscan, carotid and brachial ultrasound, and segmental pressure, examination as detailed in HSC-SPH-11-0631. A DXA examination will be offered as detailed in HSC-SPH-14-0439. All procedures have been incorporated into CPHS protocol HSC-SPH-15-0167.

Anthropometrics: Height and weight was measured at the time of the CRU visit on all subjects. Weight will be measured using a portable scale to the nearest 10^{th} kilogram. In addition, we will measure waist and hip circumference to allow determination of central adiposity. Waist circumference will be obtained at the level of the umbilicus and hip circumference will be obtained at the widest point.

Clinical and Laboratory assessment: Collection of (overnight) fasting blood specimens, totaling 30 ml by venipuncture includes; two 10 ml EDTA vacutainers, one 2.5 ml Pax Gene Tube for RNA extraction, two 2ml EDTA, and one 3.5 ML SST vacutainer tube. For cost efficiency we send 7.5 ml of blood to the Community Reference Laboratory at Valley Baptist Medical Center to run CMP, lipid panel, CBC-Diff, and A1C. Laboratory assays will be performed on HLCC participants. Information on gene expression, inflammatory markers and cytokines will be used in additional data analysis. mRNA is being collected for studies of gene expression, and the DNA from participants is also being sequenced. Systolic and diastolic blood pressure will be determined following standard protocols. Participants will sit quietly for 5

minutes and then have 3 blood pressure determinations using a Welch Allyn vital signs machine. In addition, a resting pulse will be obtained on all subjects using standard protocols. A resting 12-lead standard supine EKG (GE MAC 5500, General Electric), will be performed.

Ultrasound Methods and test: We will perform ultrasound measurements on participants, during routine cohort enrollment and follow-up visits. Carotid ultrasound, brachial arterial reactivity testing and liver/elastography ultrasound and segmental pressure vascular test will be included. All will be performed by trained CRU staff or licensed sonographers using the echosens Fibroscan 502, Siemens Acuson X300, and the Siemens S2000 ultrasound systems; and a VF 13-5 linear array transducer or 5-MHz transducer (Ch5-2, Siemens, Mountain View, CA), and interpreted by our expert co-investigators. For the vascular test we will use Koven Smartdop 30 EX; Ankle Brachial Index (ABI) (CPT93922), Segmental Pressures (CPT 93923, Toe Brachial Index (TBI).

The **carotid ultrasound** protocol follows the guidelines of the American Society of Echocardiography consensus statement on subclinical vascular disease. Both common carotids will be imaged from three different angles for a total of six images. Carotid atheroma will be determined by examining the carotid bulb, its bifurcation and the carotid branch arteries in addition to the common carotid artery. cIMT will be measured using Carotid Analyzer software (Medical Imaging Applications, Coralville, Iowa), a semi-automated border detection program. Measurements will be made at the R-wave of the EKG on a minimum of two clips from each side and results averaged. Carotid atheroma will be defined as an area of wall thickening >50% of the thickness of the surrounding wall.

- The **brachial artery reactivity test** will be performed according to standard procedures. Participants will be asked to abstain from food, consumption of vitamin E or C, and smoking for ≥6 hours before the scan. An occlusion blood pressure cuff will be positioned around the right arm, 2 inches below the antecubital fossa, and the brachial artery of the right arm will be imaged 5 to 9 cm above the antecubital fossa at rest. To induce reactive hyperemia, the brachial artery will be occluded for 5 minutes at an occlusion cuff pressure of 250 mmHg. The occlusion cuff will then be deflated and a 'release' Doppler velocity obtained to verify reactive hyperemia. All brachial artery reactivity studies will be analyzed using a semi-automated border detection software program (Medical Imaging Applications, Coralville, Iowa). FMD will be expressed as the percentage of increase in the brachial artery diameter (media-adventitial interface to the media-adventitial interface) with reactive hyperemia. A change of ≥4% is considered to be significantly greater than natural variability. All measurements will be performed by a single blinded expert reader. In order to monitor intra-reader reproducibility, ultrasound studies from 10% of the participants will be repeated.

- The methods of **liver ultrasound** have been described elsewhere. Briefly, subjects need to be fasting for at least 6 hours prior to ultrasound examination. Liver parenchyma will be examined sub- and intercostally in a decubitus position as well as in modified slightly oblique positions, with the right arm above the head and the right leg stretched, during all respiratory cycles to identify the best approach and to avoid artifacts caused by movement of the thorax. The overall gain, initial gain, and time gain compensation settings will be kept within a narrow range. The liver is considered normal if the echotexture is homogeneous without acoustic attenuation, the portal veins are visible, the diaphragm is well visualized, and echogenicity is similar or slightly higher than the echogenicity of the renal parenchyma. The liver is characterized as fatty when the liver has areas of significantly greater echogenicity than the renal parenchyma, the ultrasound beam is attenuated and the diaphragm is indistinct, or there is blurring of the intrahepatic vessels**.**

- The **liver elastography** will be performed according to the liver ultrasound method described above. It will provide then additional high quality images with higher resolution. The images will provide additional diagnostic information to detect liver steatosis and fibrosis.

- The **Fibroscan is** a non-invasive assessment of liver disease. It will be performed according to the liver ultrasound method described above. The shear wave speed, stiffness and CAP (controlled attenuation rate) values may be used as an aid to clinical management of adult patients with liver disease.

- The **echocardiogram** will be performed following the standard CCHC procedure. Subjects are not required to be fasting; they can take any scheduled medication as instructed by their own physician.

  Echocardiography is an important, simple and noninvasive tool to assess ventricular function, both systolic and diastolic, as well as underlying cardiac structural abnormalities. Development and progression to HF may be modulated by specific triggers of increased CV stress such as inflammation. We will measure metabolic and inflammatory markers, and pericardial fat (a metabolically active ectopic fat depot associated with cardiac remodeling and inflammation). We will quantify pericardial fat using echocardiography, and correlate this with left ventricular (LV) structure and function, abdominal adiposity, markers of inflammation and oxidative stress (hsCRP, adiponectin, interleukin-6, MCP-1, and TNF receptor α), dysmetabolic risk profile, and the prevalence, development, and progression of HF. We will use machine learning algorithms to identify modulators of risk association factors among HF phenotypes. These analyses will be correlated with hepatic outcomes and our endophenotype analysis.

- The **segmental pressure vascular** study will be performed following standard procedure. A specific questionnaire for PAD will be administered before the test. Subjects are not required to be fasting; they can take any scheduled medication as instructed by their own physician. It is performed by placing the patient in the supine position and wrapping pressure cuffs around both arms and both legs at various positions. Blood pressure cuffs will be placed on both arms, feet and big toes, ankle, below the knee, above the knee, and thigh. A total of 12 blood pressure cuffs will be connected to the Doppler one at a time. Blood pressure readings will be taken from both arms. Then, readings will be taken for the right foot and leg. Once the right side is complete, the process will be repeated on the left side. Pressures are taken at both arms, and at each position on the legs by inflating the pressure cuffs past the point where Doppler sounds cease, then slowly deflating the cuffs until Doppler sounds return.

- Following a simple questionnaire about fractures and risk fractures, total and multi-compartment body composition will be measured and calculated by **Dual Energy X-ray absorptiometry (DXA)**. DXA is used to access overall skeletal changes that often occur with age. This will allow for estimation of bone mineral density (BMD), bone mineral content (BMC), total lean mass and fat mass within specific regions, including the visceral depot. Subjects will be required to lie still on the table and the entire body will be scanned. The subject needs to be able to lie flat on his/her back for the duration of the scan without difficulty, pain or shortness of breath. Subjects will be asked to wear a gown and to remove

all metal objects (glasses, jewelry, and cell phones). Subjects will be exposed to a small dose of radiation (10 DXA = 1 chest X-ray, average dose from DXA scan: 10-15 mrem to participants) and the test will last approximately 15 minutes. Radiation doses are less than a single day in sunshine or a flight from Los Angeles to New York. Subjects will not be required to be fasting. The DXA exam will begin with the lumbar spine scan followed by the femur (hip) scan and then by the body composition scan. Lumbar spine L1-l4 and left hip will be scanned following the standard clinical protocol. Right hip will be scanned if any metal implant or previous hip surgery in the left hip. If there is history of metal implants in both hip sides, then only lumbar spine will be scanned. Pregnancy tests will be assessed on all females aged 18-59.

The aim is to determine the evidence for pathological progress of liver and other diseases using ultrasonography and DXA. Ultrasound images will be reviewed and graded in Houston. These examinations will include carotid artery, brachial reflexivity, liver, renal and include other images if appropriate. In addition, participants who complete the CRU exam will be invited for follow up visits. A small incentive will be provided in recognition of their time.

Finally, after the patient visit, we will retrospectively acquire liver pathology (if available) and medical records from the hospitals and tertiary care facilities in which the patient has received care.

4.4.3 Analysis of Family Cohort Samples

Plasma, buffy coat, and red blood cell samples will be processed for extraction and quantification of genetic material and performance of routine hematology, chemistries and other assays, such as hepatitis virus antibodies and adipocytokines. The team at the Brownsville Regional Campus will conduct detailed genetic epidemiologic analysis to determine traits associated with liver disease and liver cancer in this population. The de-identified pathology results of the original participant in Aim 3 will be appended to this dataset in order to determine the particular associations for specific liver pathologies.

5. RISKS, BENEFITS, AND OUTCOMES

5.1 Possible risks to participants

For each aim of the study, there is a potential risk of loss of confidentiality and anonymity. However, all data collection staff routinely receive intensive training related to protection of

confidentiality. To protect this confidentiality, all hard copies of records will be filed in locked cabinets, in a locked area of the Clinical Research Unit at the Brownsville Regional Campus. Electronic records will be kept in password-protected computers behind the UTHealth firewall and only de-identified data will be used for analysis. A master file allowing identification of particular patients to track study progress will be maintained at the UT School of Public Health in Brownsville behind the university firewall, and be accessible only to limited project staff requiring access. All liver specimens will be kept in accordance with UTHealth practices at Drs. Younes, and Beretta's laboratories during the analysis. Afterwards, these specimens will be shipped back to the originating hospital, under the direction of the respective director of pathology.

The risks of taking additional blood (Aim 3) during the pre-procedure blood draw are minimal. A blood draw is a standard of care for liver biopsy, and the blood draw will be conducted by a trained and licensed phlebotomist at the participating hospital. There is a risk of minor discomfort, bleeding, bruising, and fainting during the blood draw.

The risks and benefits of participation in the Hispanic Liver Cancer Cohort are detailed in HSC-SPH-03-007B. Briefly, there is minor risk of physical discomfort, bruising, bleeding, dizziness, or fainting during the blood draw. There is a risk of discomfort or embarrassment while answering medical history questions and when undergoing the anthropometrics, electrocardiogram, and bioimpedance studies. All data are held behind the UT System Firewall, and only de-identified data are used for analyses.

5.2 Potential benefits

There are no individual benefits for the participants in this study, with the exception of participants in the Hispanic Liver Cancer Cohort, who will receive the results from the CRU exam free of charge. All patient data will be de-identified and used for population analysis. All aims of this study, however, will add valuable information to the knowledge base of fibrosis and HCC, especially in health disparity and minority populations in the US. By matching histology with patient records, and aggregating these records, we will be able to analyze the particular risk factors of developing fibrosis, and of progressing from fibrosis to HCC, including genetic risk factors. This is the first project to investigate seriously the disparate burden of end-stage liver disease and HCC in South Texas.

5.3 Outcome

By uniting fragmented portions of a patient's record of liver disease, we will create a more complete picture of each case of liver disease and liver cancer in the population. We will also develop a stronger understanding of the somatic mutation profiles of HCC in this population, as well as the particular biomarkers associated with fibrosis and HCC in Mexican Americans. Finally, in the family cohort design, we will be able to distinguish heritable and environmental determinants of liver disease and hepatocellular carcinoma. Because of the progressive nature of undiagnosed or untreated liver disease, methods for early detection are crucial; once adopted, these new methods of detection have the power to increase diagnoses and slow or halt the progression of liver disease.

6. Timeline

We expect to be able to collect this number of samples in 3 years, completing data collection in June 2019. The total sample size for Aim 1 will be composed of both retrospective and prospective samples. Staging and grading of pathology samples, as well as genomic analysis and the work with biomarkers, will take place continuously during data collection. Statistical analyses will begin once all data has been collected and generated. We expect to complete the project and have drafted manuscripts for publication by early 2019.

Reference List

Albano,J.D., Ward,E., Jemal,A., Anderson,R., Cokkinides,V.E., Murray,T., Henley,J., Liff,J., and Thun,M.J. (2007). Cancer mortality in the United States by education level and race. J. Natl. Cancer Inst. 99, 1384-1394.

Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002 Jan 16;39(2):257-65.

Pan,J.J., Fisher-Hoch,S.P., Chen,C., Feldstein,A.E., McCormick,J.B., Rahbar,M.H., Beretta,L., and Fallon,M.B. (2015). Burden of nonalcoholic fatty liver disease and advanced fibrosis in a Texas Hispanic community cohort. World Journal of Hepatology ESPS Manuscript No: 17035.

Perez, A., Anzaldua, M., Mccormick, J., and Fisher-Hoch, S. (2004). High frequency of chronic end-stage liver disease and hepatocellular carcinoma in a Hispanic population. J. Gastroenterol. Hepatol. 19, 289–295.

http://www.koven.com/PAD_Segmentals.htm

Ramirez,A.G., Weiss,N.S., Holden,A.E., Suarez,L., Cooper,S.P., Munoz,E., and Naylor,S.L. (2012). Incidence and risk factors for hepatocellular carcinoma in Texas Latinos: implications for prevention research. PLoS. One. 7, e35573.

Ries, L.A.G., Melbert, D., Krapcho, M., Stinchcomb. D.G., Howlader, N., Horner, M.J., Mariotto, A., Miller, B.A., Feuer, E.J., Altekruse, S.F., Lewis, D.R., Clegg, L., Eisner, M.P., Reichman, M., Edwards, B.K. 2008 SEER Cancer Statistics Review, 1975-2005. National Cancer Institute. <http://seer.cancer.gov/archive/csr/1975_2005/>.

Sorensen KE, Celermajer DS, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Thomas O, et al. Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. Br Heart J 1995 Sep;74(3):247-53.

Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008 Feb;21(2):93-111.

Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011 Jan;140(1):124-31.