THE WOMEN'S INTERAGENCY HIV STUDY
SECTION 41: FIBROSCAN STUDY PROTOCOL

A. BACKGROUND

Liver disease is a leading cause of morbidity and mortality in HIV-infected persons.[1-3] Coinfection with hepatitis C virus (HCV) is a primary cause of liver disease and has been associated with accelerated progression to cirrhosis compared to HCV infection alone.[4-6] While sex differences in liver fibrosis progression have been reported in HCV-monoinfected persons,[7,8] the rate of liver fibrosis progression is thought to accelerate in women after the menopausal transition and approach that of men. Furthermore, liver fibrosis may not be limited to only those with HCV infection. Recent published studies show that HIV monoinfection is also associated with increased liver fibrosis compared to those with neither HIV nor HCV infection.[9,10] About half of U.S. HIV-infected persons are now over the age of 50, and therefore, an understanding of the mechanism by which the menopausal transition affects liver fibrosis is critical. Estrogen depletion is thought to be associated with upregulation of inflammatory cytokines,[11] increased visceral adiposity and worsened metabolic parameters, which are also risk factors for hepatic steatosis (or fatty liver).[12] HIV and HCV infection are also associated with immune activation and inflammation, and changes in adipose tissue and metabolic parameters.[13-19] These inflammatory and metabolic consequences may be key risk factors for an acceleration in liver steatosis and fibrosis progression during the menopausal transition in HIV-infected women with underlying HCV-associated liver injury, and possibly in women with HIV alone. To adequately examine these associations, a measure of liver fibrosis that is acceptable to patients is needed. While liver biopsy is the clinical gold standard to assess fibrosis, it is invasive and often contraindicated in HIV-infected persons. Transient elastography (TE), also known commercially as Fibroscan®, is a non-invasive ultrasound-based imaging technique that visualizes a larger area of the liver than biopsy. With the addition of the Continuous Attenuation Parameter (CAP) software to the new FDA-approved TE, we will be able to quantify steatosis and fibrosis *simultaneously* to address our aims in women with HIV monoinfection, HIV/HCV coinfection, HCV monoinfection, and neither infection (control women).

From October 2010 to April 2013, a cross-sectional TE study was established at three WIHS sites: San Francisco, Chicago, and Washington, D.C., and 381 women with HIV monoinfection, HCV monoinfection, HIV/HCV coinfection, and neither infection were scanned. 338 (or 88%) had valid TE scan data. Those with an invalid scan had a higher median BMI than those with a valid scan. The new FDA-approved Fibroscan® 502 Touch device includes both a medium (M) probe and an extra-large (XL) probe, which will allow for acquisition of valid scans in women with high BMI.

The overall goal of the LIVRA study is to define the contribution of HIV, HCV, the menopausal transition and associated changes in visceral obesity, gut microbial translocation markers, and inflammation to steatosis and fibrosis progression. This is a prospective study of the simultaneous assessment of steatosis and fibrosis using transient elastography (Fibroscan®) with the Controlled Attenuation Parameter (CAP) software (which allows the additional measurement of steatosis) at two time points three years

apart. In HCV-infected women who start HCV treatment during the study period, Fibroscan® should be performed every six months over three study visits (or 18 months) after the study visit they report taking HCV medications and again at the 3-year follow up from the first baseline scan with Fibroscan® and CAP.

Beginning October 1, 2016, we propose longitudinal analyses to examine the following aims:

AIM 1: To examine the association of HIV, HCV, the menopausal transition and associated metabolic and inflammatory mediators with *liver steatosis and liver fibrosis progression* (measured using TE with the CAP assessment) in women with HIV/HCV coinfection, HCV and HIV monoinfection, and neither infection.

AIM 2: investigates the effects of visceral obesity, gut microbial translocation and inflammation on steatosis progression during the menopausal transition.

AIM 3: determines the effects of the menopausal transition and its onset on fibrosis progression.

AIM 4: To examine the association of HCV treatment with change in liver fibrosis measured using TE prior to HCV treatment initiation and then at the 6, 12, and 18-month intervals in women with HCV infection.

B. **STUDY POPULATION**

We propose to enroll 1,650 WIHS participants from 10 WIHS sites (300 women were enrolled as part of the NIH supplement to WIHS; the remaining 1,350 women will be enrolled as part of the LIVRA R01).

Start date will vary by WIHS site depending on when the Fibroscan® operator is certified, but we expect all WIHS sites to be enrolling into LIVRA by Visit 45 (starting October 2016). Baseline visits should be completed over a 6 to 18 month period or over 3 visits.

Study Enrollment Targets:

sites	Number of individual participants covered by R01 for 1st visit
UNC	100
DC	200
Chicago	100
UAB	100
UMISS	100
Emory	200
Miami	100
Bronx	150
Brooklyn	100
UCSF	200

1. **INCLUSION CRITERIA**

    (1) Women ages 35 to 60.

    (2) HIV-monoinfected, HIV/HCV-coinfected, HCV-monoinfected, and with neither infection (control). HCV infection is defined via detection of HCV RNA.

    (3) Not receiving anti-HCV therapy at time of enrollment.

2. **EXCLUSION CRITERIA**

    (1) Women examined semiannually at a WIHS clinic without a TE device capable of CAP assessment.

    (2) Current pregnancy (as determined by a urine pregnancy test at time of consent).

    (3) Implantable device (i.e., cardiac defibrillator).

3. PROCEDURE

Participants who agree to undergo the Fibroscan® must fast (no eating or drinking anything other than water) for at least three hours before the procedure. The Fibroscan® generally takes no more than 20 minutes. The Fibroscan® needs to be done within 60 days of the Core visit.

(1) LIVRA baseline and 3-year follow up studies. Table 2 below shows the approved start dates for the study by WIHS site and operator.

(2) For HCV-infected participants who start HCV therapy in between the LIVRA baseline and 3-year follow up, bring participants back every six months over three CORE visits (or 18 months). Every effort should be made to perform Fibroscan® during their CORE visit.

- If a participant misses a CORE visit and therefore only has, for example, one or two post-HCV treatment scans over the 18-month period, you do not need to bring them back for one or two more scans if it is beyond the 18-month window; however, please ensure that the participant returns for the LIVRA 3-year follow up based on the date of her baseline LIVRA scan.

- If a participant missed her CORE visit, but is willing to return for a Fibroscan at a non-CORE visit, bring her back if it is within the 18-month period.

For participants who underwent Fibroscan®, but a valid scan could not be completed, **do not delete** the scan from the Fibroscan® database.

The **Fibroscan® Participation Notification Form (FSNOTI)** should be completed for:

LIVRA Study Start Date *(based on Operator Certification Date; last updated 9/27/2016):*

Sites	*operator*	Start Date
UNC	TNJ	4/7/16
	DAC	4/27/16
	LT	pending
DC	MSD	11/17/15
	DAM	7/6/16
	YRP	pending
Chicago	SM	7/1/16
	CT	7/1/16
UAB	ZC	pending
	AWT	
UMISS	VM	pending
	MB	
	TB	
Emory	KJS	2/10/16
	AGF	2/29/16
	AGK	3/2/16
Miami	Y	10/21/15
Bronx	SL	pending
Brooklyn	B	pending
UCSF	MB	2013

(1) Every participant screened for Fibroscan®, e.g., when it is the first baseline visit at which TE with CAP is performed and the 3-year follow-up visit;

(2) HCV-infected participants who will be undergoing additional Fibroscan® measurements outside of the baseline and 3-year follow up visit because of HCV treatment initiation. Enter "YES" to HCV6, HCV12, or HCV18 to indicate whether the Fibroscan is for the immediate CORE visit after the participant reports starting HCV therapy, or the CORE visit 12 months after the visit where the participant reports starting HCV therapy, or the CORE visit 18 months after the visit where the participant reports starting HCV therapy.

C. INTRODUCTION TO THE FIBROSCAN®

The Fibroscan® 502 Touch is a FDA-approved ultrasound-based device that uses vibration-controlled transient elastography (VCTE) to non-invasively assess liver stiffness (LS), an estimate of liver fibrosis, measured in kilopascal (kPa). All WIHS sites will use this device along with the CAP software to measure liver stiffness and steatosis for the Fibroscan® Study. During examination, the center of the probe creates a single sheer wave that travels longitudinally through the liver, ideally at its longest point. The shear wave velocity is determined in kPa using an algorithm, as is the degree of ultrasound attenuation by liver fat, which is expressed in decibels/meter (dB/m). Steatosis and liver stiffness are measured simultaneously in the same region of interest[21].

D. WIHS FIBROSCAN® QUALITY ASSURANCE PROCEDURES

The purpose of this protocol is to standardize the Fibroscan® scanning acquisition procedures across the clinical WIHS sites. The success of the study will depend on several factors, including the qualification of the scan operators, adherence to the specifications for scan acquisition as set forth in this protocol, and good lines of communication among the TE Quality Control (QC) Center at UCSF, the WIHS Data Management and Analysis Center (WDMAC), and investigators and participants at the WIHS clinical sites.

This protocol builds upon (rather than replaces) the operator training and documentation provided by Echosens North American (formerly Sandhill Scientific, Inc). It is expected that each technologist performing scan acquisition is competent with the scanning system employed at his/her study site. In addition, the material in this protocol should be read and understood before beginning to scan participants for the WIHS Fibroscan® Study. Beyond initial certification by Echosens North America, operators are required to pass the quality assurance standards outlined in this protocol before initiating actual scans on WIHS participants.

WIHS sites should maintain an annual service contract that includes annual probe calibration by Echsosens North America. If your Fibroscan was purchased as a clinical scanner by your institution/medical center, then the service contract should be handled by the institution/medical center. If your Fibroscan was purchased through research grant funds, then the cost of the annual service contract will be covered by the LIVRA Study R01 grant. Sites will be responsible for coordinating probe calibration and shipping probes overnight inside their respective cases with Echosens North America representatives. When the device indicates that probe calibration is needed, please contact EchoSens North America, Inc. at 866-90-LIVER or 866-905-4837 or Email: ESNAservice@echosens.com. At the time of shipping, please ensure that the probe cases are surrounded by sufficient padding within a large box to ensure that the probe is not damaged. **Forward copies of the calibration certificates to WDMAC.**

E. WIHS TRAINING REQUIREMENTS

Some studies indicate that 50 to 100 scans must be performed before an operator is sufficiently trained to consistently acquire valid scans.[22] Because of the longitudinal nature of the WIHS Fibroscan® Study, it is especially important that quality scans are obtained throughout the follow-up period. Therefore, before approval to acquire actual

Study scans in WIHS, operators must complete at least 50 scans and submit PDF files of the scan output/images for review by the TE QC team. These scans may be performed on other WIHS staff members or WIHS participants, but ideally should consist of scans performed on women with a wide range of body types and within the age range of eligible WIHS participants. Training scans should be entered into the machine as described in Section G.

Prior to beginning the WIHS Fibroscan® training requirements, each site must email WDMAC a list of operators who have been trained formally by a representative from Sandhill Scientific, Inc. or Echosens North American, and who will also perform the WIHS Fibroscan® Study scans. **A copy of each operator's training certificate must also be mailed to WDMAC upon receipt.**

The schedule and requirements for the 50 WIHS training scans are as follows:

Scans	Guidelines	Training Goals
1-10	**Subjects**: WIHS staff, discrete subjects **Parameters**: RECOMMENDED: IQR<30% of median, >60% of scans deemed valid by device REQUIRED: 10 valid scans obtained per participant	• Get comfortable with positioning • Familiarize with Fibroscan® screen and available tools
11-25	**Subjects**: Varied body types and ages, discrete subjects **Parameters**: RECOMMENDED: IQR<30% of median, >60% of scans deemed valid by device REQUIRED: 10 valid scans obtained per participant	• Observe how age and abdominal fat affect Fibroscan® acquisition • Work on consistently achieving valid assessments
→After 25th scan, submit PDF/Excel files to **UCSF Box** for review; receive feedback report, check-in call optional		
26-40	**Subjects**: Varied body types and ages, discrete subjects **Parameters**: REQUIRED: IQR<30% of median, >60% of scans deemed valid by device, 10 valid scans per participant	• Focus on achieving valid assessments consistently • Utilize deletion to meet parameter requirements
→After 40th scan, submit PDF/Excel files to **UCSF Box** for review; receive feedback report, check-in call optional		
41-50	**Subjects**: Varied body types and ages, up to 5 scans can be repeated subjects from first 40 scans **Parameters**: REQUIRED: IQR<30% of median, >60% of scans deemed valid by device, 10 valid scans	• Consistently achieve valid assessments
→After 50th scan, submit PDF/Excel files to **UCSF Box** for review; feedback report will be provided		

After the first 25 scans are obtained, each operator will submit PDF files of the 25 scan images and an Excel file of the exported scan data to **UCSF Box**.

Notify Jennifer Cohen at jennifer.cohen@ucsf.edu of the name and email address of the person(s) responsible for submitting the data to arrange access to UCSF Box. Files will then be sent to the TE QC team led by Drs. Phyllis Tien and Marion Peters and coordinated by Heather Freasier at heather.freasier@va.gov.

After completion of the review of the first 25 scans, the TE QC team will provide a written report and may coordinate a brief phone call with the operator to discuss any issues that were encountered on review of images. The TE QC team or upon site request may refer operators to a nearby WIHS site with a certified TE operator for more hands-on training and observation.

A similar procedure will be followed after the next 15 scan images are acquired, and again once the last 10 scan images are acquired (See Table above). After completion of 50 scans, the final report will indicate whether: (1) the operator is certified to begin scans on WIHS participants for the WIHS Fibroscan® Study, (2) an additional 20 scans are needed for review; and/or (3) a site visit is warranted.

For site visits, at least 5 volunteers/participants with a range of ages and body habitus types should be identified by the site. A certified Fibroscan® trainer will observe the operator perform scans on these participants and provide feedback. A review of the data transfer and storage procedures will also be performed at the visit. Operators will be notified whether they are approved and certified to begin performing Fibroscans for the study by a member of the TE QC team.

MAINTAINING CERTIFICATION DURING STUDY

Fibroscan® data exported from the device in an Excel file will be sent to WDMAC at the end of every semiannual core visit. WDMAC will then request that PDF images of scan data be sent via **UCSF Box** for review by the TE QC team from the following groups:

(1) All participants who have a median LS value >9.3kPa.

(2) 10% of participants with LS values <9.3kPa. WDMAC will randomly select these participants.

Each operator is required to perform at least 20 scans per calendar year to maintain certification.

F.   SETTING UP THE FIBROSCAN AND PROBES

This section describes how to set up the Fibroscan® for new patient acquisition. Be sure to read and familiarize yourself with the material in this section before scanning WIHS participants.

Setting up the Fibroscan® machine

- Keep Fibroscan® device in a safe place that involves minimal movement between its storage and active scanning locations. The M (medium) and XL (extra-large) probes may be stored on the machine, if they can safely be stored together. Utmost care is needed when handling the Fibroscan® probes, especially the probe's transducer (the end with the black cylinder).

- The left hand side of the machine also has two storage pockets. Store the ultrasound gel bottle upside down, in the flat-based slot. See picture to the right for sample set-up:

- After the probes are removed from their cases, place the probe with the **transducer-up** into the probe pockets on the right-hand side of the machine, then follow their cords to the back of the machine, where there are two plug-in slots.
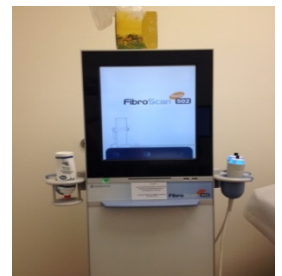
Preparing the machine for new acquisition

- Wheel the Fibroscan to the desired location for scan acquisition, plug it into an electrical outlet. For safety, it is advised to lock the wheels during the duration of the scan by pushing down the grey wheel levers.

- Turn on the machine by pressing the power button below the screen on the left-hand side of the machine.

- After several seconds the start-up screen will appear, touch the **Operator** icon (at the back of the circle – it defaults to the Fibroscan Admin password which will not work with your password).

MD

- Press the up arrow button to enter caps lock mode (see right), and enter "MD" as the password, then press the OK to continue.
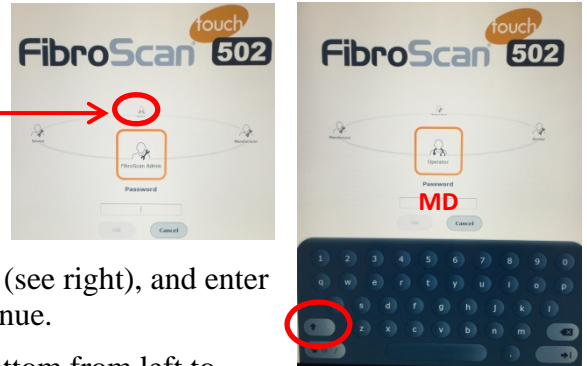
You will next see the following Home screen:

The icons on the bottom from left to right indicate menus: Power, Settings, Archives, and Acquisition. Select the triangular Acquisition icon on the right to go to the data entry screen to set up the scan acquisition.

G.   ENTERING PARTICIPANT DATA INTO FIBROSCAN

Use care when entering participant data. Once a participant file is created, it cannot be edited within the machine, only deleted. Please double-check each entry. It is also important to keep scan data free of PHI and PII as much as possible. Therefore, the following protocol for entering participant data has been created. After selecting the Acquisition icon on the home screen the screen on the right will appear.

The following procedures are for entering the first training scan and the Baseline scan using the FIBROSCAN® 502 Touch machine:

New patient information

Past scan information

Caps-Lock ON

Next arrow
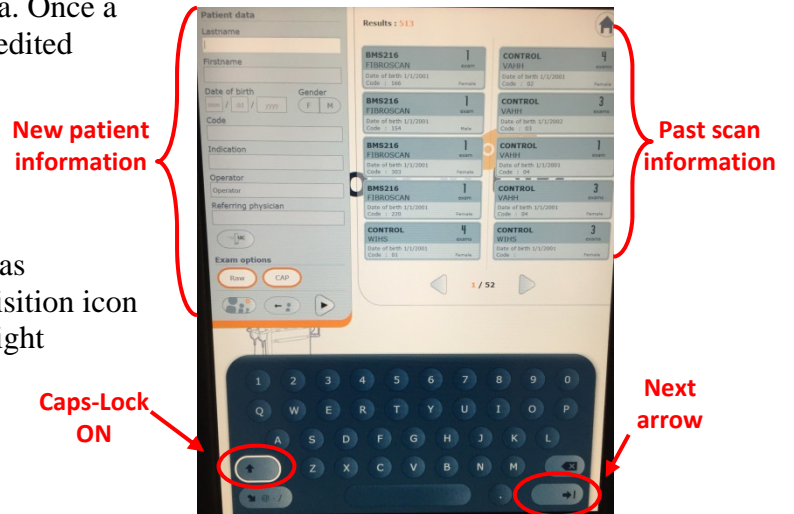
- **Lastname:** For the <u>training scan</u> enter "TRAINING"; For the 1st/Baseline scan enter "WIHS."

- **Firstname:** Should remain blank.

- **Birthdate:** Enter "01" for month, "01" for day, enter the *participant's* year of birth.

- **Gender:** Should remain blank.

- **Code:** Should remain blank for <u>training scan</u>; enter the 8-digit WIHSID code without breaks or dashes for the 1st/Baseline scan.

- **Admitting diagnosis:** Should be left blank.

- **Operator:** Enter the Fibroscan® Operator 's initials. The field defaults to "Operator", which needs to be deleted, then add your initials.

- **CAP & Raw:** Should be circled with orange. If it states, "CAP disabled," tap on the CAP button to enable.

Training Scan: *1st/ Baseline Scan pt*

TRAINING   **WIHS**

1960   **196**

555555

Delete "Operator" and add your initials → **HSF**   **HSF**
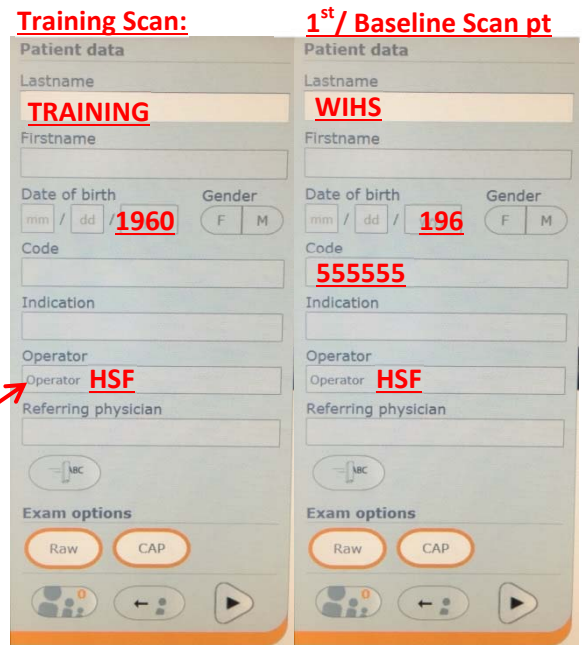
- Advance to the next open box by touching the next data box.

- Double check that all data are entered correctly, especially the year of birth and the WIHSID, as they cannot be edited later. Then press the Next (▷) icon.

The following procedures are for entering <u>follow up</u> scans for WIHS participants who have been scanned at baseline:

To enter data for follow-up scans for WIHS participants who have had previous scans, scroll thru the right side of the screen to find the previously entered data for that participant, tap the correct patient's information and her data will then auto fill on the left side of the screen. Tap on the correct participant's previous scan (it will highlight with orange square) to autofill the rest of the participants data. Change the Operator initials as needed.

WIHS

2001
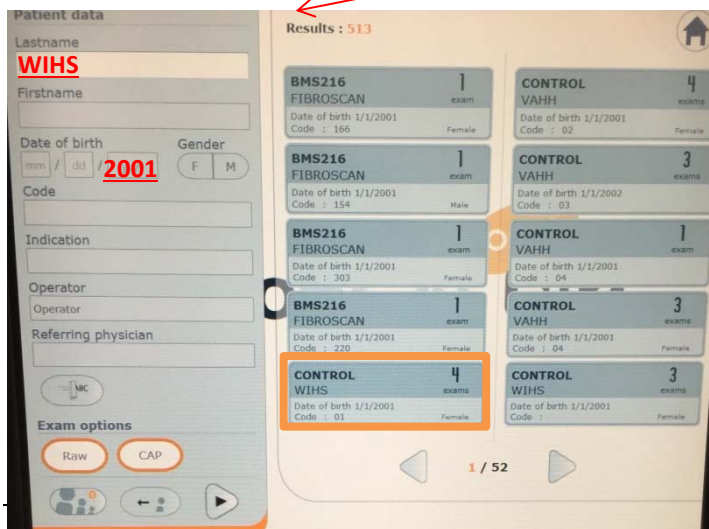
NOTE: After the first WIHS participant has been entered into the machine we recommend that you use their scan information as a template for subsequent participants using the procedure above. **Verify this is the correct participant by checking the WIHSID number and birth year, and enter the correct operator initials**.

H. PARTICIPANT POSITIONING

General positioning

Before the participant lies supine on the exam table, the participant should move clothing so the operator can access the right side of the participant's torso. Once the participant is positioned on the exam table, ask her to raise her right arm over her head in order to widen the intercostal space.

The Fibroscan® machine and the operator will be positioned on the participant's right side. The operator may need to adjust seat height so that one elbow can rest on the exam table for stabilization during the scan.

Modifying positioning

For some participants, holding their right arm above their head may be uncomfortable, especially if the scan lasts more than several minutes. An alternative may be crossing their right arm across their chest and over the left shoulder. Allow for regular breaks from this positioning if necessary.

If the intercostal space still cannot be comfortably identified, have the participant cross her right ankle over her left and point her feet away from the operator, while keeping her torso and hips in the same position. If possible, ask her to stretch her right side further by also pointing her head and shoulders away from the operator. This "C"- or banana-shaped positioning ("banana curl") should reduce interference with the adjacent ribs and thus prevent a rib echo.

Probe placement

Locate the xiphoid process (see Figure A) by feeling for the springy area at the tip of the sternum, where the lowest ribs meet. Then move your finger laterally to the right of the xiphoid process until you find the nearest intercostal space (see Figure B). Then apply ultrasound gel on this spot to mark for initial placement.
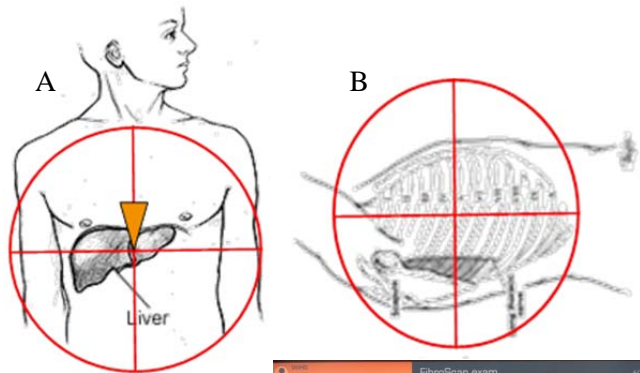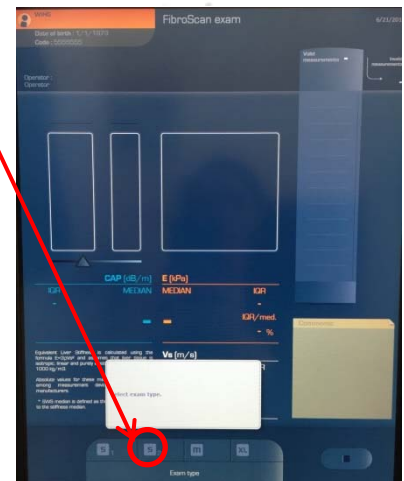
SCAN ACQUISITION

Preparing to take a valid scan

Once the participant is properly positioned and the intercostal space is identified, then turn to the Fibroscan® screen. The operator will be prompted regarding which probe to use. Always start with the M probe.

Next, position the M probe **perpendicular** to the skin over the intercostal space that should be coated with ultrasound gel. Hold the probe stably with both hands. Watch the Fibroscan® screen for several key items indicated below from a successful scan:


Look for green bars

In the ultrasound box, look for striated tissue, noting any bright white areas at the top which could indicate excess fat and a need to switch to the XL probe

Look for straight line; If curvy add more gel

*Not pictured in this screen shot, but will show probe pressure gauge. Needs to be green (blue light activated on probe) to take reading

The most helpful of these indicators will be the ultrasound box, where you are looking for thin, alternating bands of dark and light areas that indicate striated tissue of the liver. As in the example above, despite there being many bands of light and dark areas, the overall appearance is fairly homogeneous. Below are examples of ultrasound inputs:


A   B   C   D   E
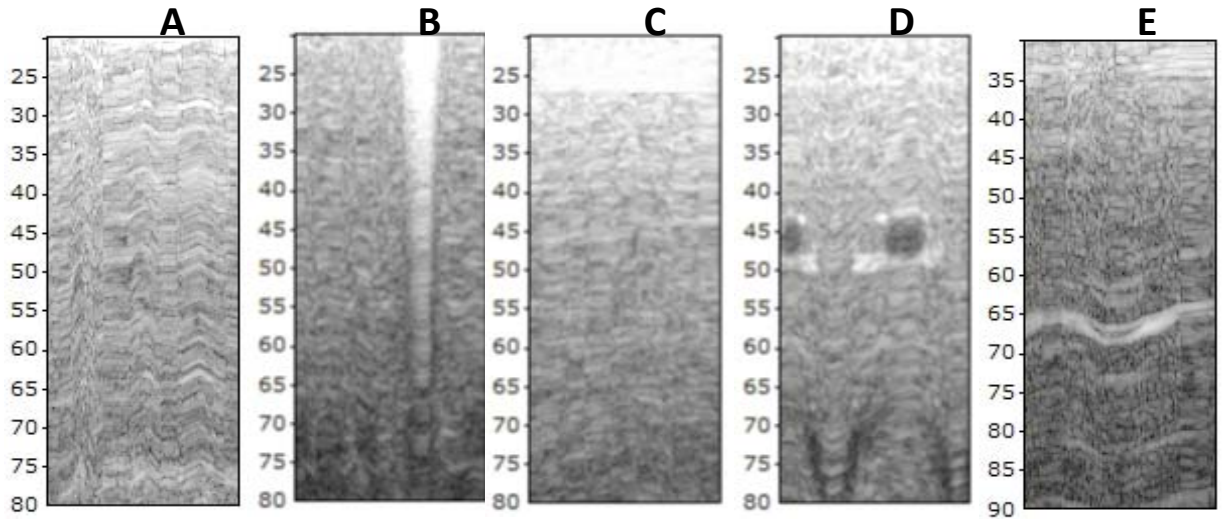
- A is an example of high quality striated liver tissue. This should be accompanied by green bars above the ultrasound box, and indicates good placement.

- B is an example of lung tissue, where the wave-like pattern shows alternating depths of white space. If this pattern is observed, reposition the probe to the next intercostal space below, until only liver tissue is observed.

- C is an example of excess subcutaneous fat where the white area at the top end of the ultrasound box does not fluctuate with the breath. Having this white band may not interfere with acquisition of valid scans; however, if the white band dips below the 25mm line on the y-axis as shown in Figure C above, then switch to the XL probe by

pressing the XL icon on the bottom of the screen and select OK (See *Tips for a Successful Scan*).

- If there is a thicker band of bright white or darker grey, as seen in D and E, this may indicate a blood vessel or other type of vasculature. If this pattern is observed, simply shift the probe position slightly within the same intercostal space, but ensure that the probe remains perpendicular to the skin until the vasculature no longer appears on the ultrasound. Patterns indicative of vasculature are more likely to lead to an invalid measurement reading or interference band on the wave graph.

- Occasionally the ultrasound wave will not look like striated liver tissue. In these cases, always reposition the probe either along the same intercostal space or in an adjacent intercostal space, before attempting to acquire a scan. Always ensure that the probe is perpendicular to the skin.

Acquiring the scan

- Once the visual checks have been made, inform the participant that they will feel a small "thump" as the scan is acquired, and, optionally, count down before depressing the button. If the reading is deemed valid by the machine, there will be a graph of the wave with a white dotted line tracing the left-most slope of black on the graph. If invalid, there will be no values listed, no trend line superimposed over the wave, and there will be a red banner, and you will need to try a slightly different spot or acquire the scan at a different spot in the participant's breathing cycle.
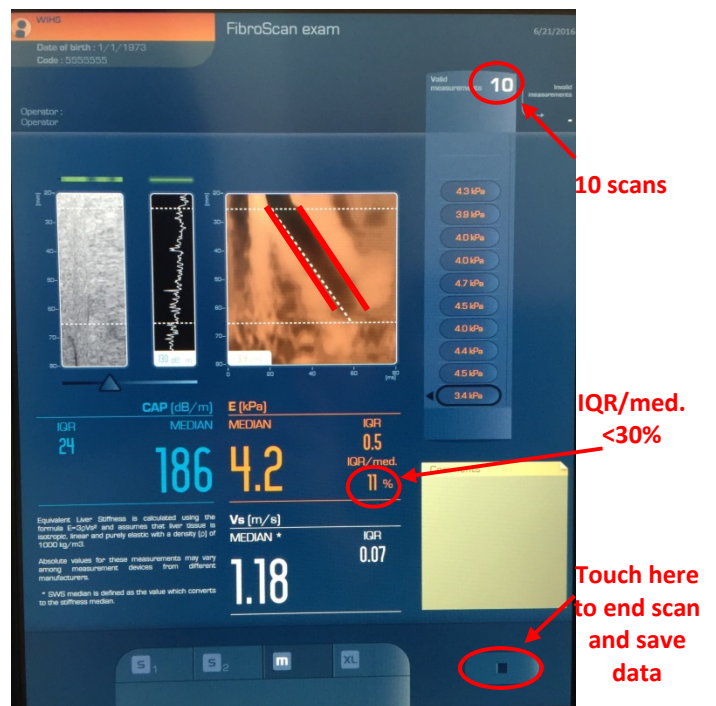
- Note the parallel edges of the wave graph to the right. Non-parallel edges of the wave graph, whether deemed valid or invalid by the machine, may indicate incorrect placing (see *Section: Deleting scans*).

- If all valid scans have parallel edges, continue until you have taken **10 valid scans**, with **>60% success rate** and **<30% IQR/med.** Note: *The software no longer calculates this so you will need to hand calculate the Success Rate (# Valid measurements divided by # Total measurements*. Then stop and finish the scan by touching the square button on the bottom right corner of the screen.

- SKIP the next screen (weight, height, fasting conditions, etc.) by hitting OK at the bottom of the screen.
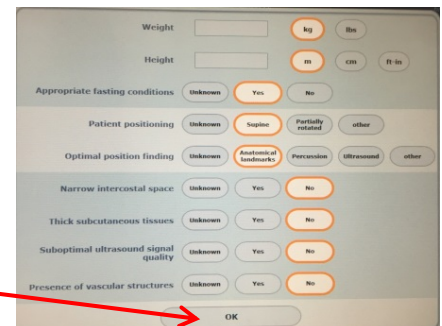
Deleting scans

During the process of performing 10 consecutive scans, there are situations where you may want to delete scans:

Scenario 1: You note probable interference from anatomical structures such as the probe being too close to a rib, causing a deflection of the wave, and non-parallel edges of the wave graph (See Figure to the right). This scan value should be deleted, even though the machine did not indicate that it was an invalid scan read. Operators must pay close attention to the wave graphs. The TE QC team will review PDF images from a random sample of scans performed to ensure that wave graphs are appropriate.

Scenario 2: Operator repositions the probe because the participant moves significantly before all 10 scans are acquired or the probe slips from its location. All prior scans acquired should be deleted to ensure that all values were taken from the same location.

The Fibroscan® will not allow you to delete an individual scan. For example, if a non-parallel edge was noted when doing the 9th out of a set of 10 valid scans or after you performed all 10 scans, then click on the scan value you wish to delete, the device will ask you if you want to delete all scans from the indicated scan and before. When you press OK, the scan values will be permanently deleted, with only the later scan values remaining. The IQR/med% will be recalculated

Tips for a successful scan

- Acquire the scan right before the participant inhales (or after exhalation), so that the lungs are less likely to interfere with acquisition of liver images. Sometimes the probe will be correctly placed in the intercostal space, but scan acquisition will need to be timed with the patient's breathing to obtain valid scans.

- Be sure that the probe is correctly positioned and maintained **perpendicular** to the skin while obtaining the 10 valid scans. Otherwise, an inaccurate measure may be acquired even though the device indicates that they are valid readings.
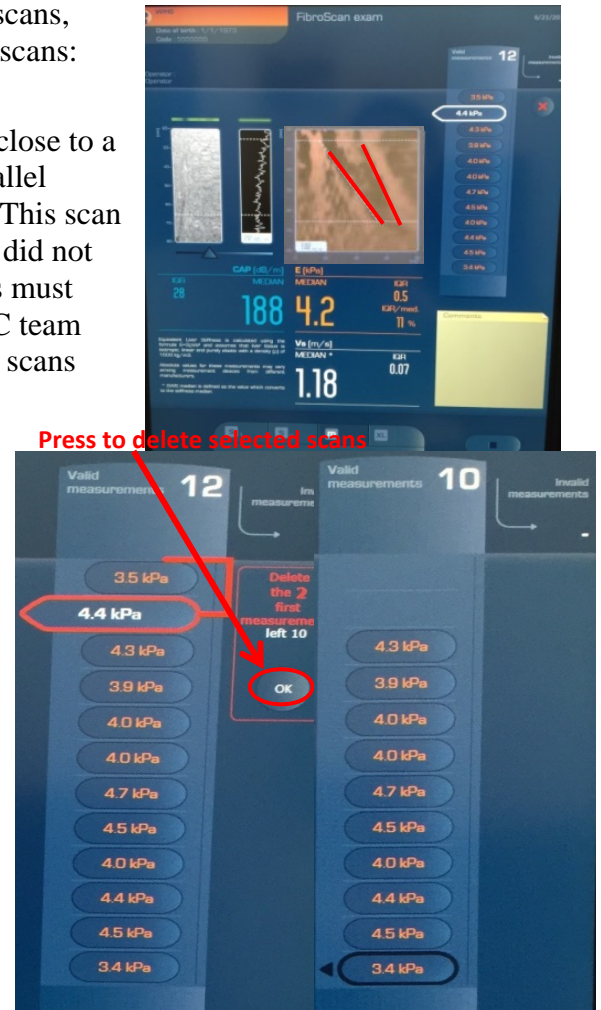
- When the first scan read indicates severe fibrosis (>9.3kPa), it is a good idea to slightly reposition the probe to make sure that the high number is not simply the result of a rib echo or other placement issue

- While the Fibroscan® device should provide a probe recommendation (i.e., switch to XL probe), sometimes the device will not indicate this. The operator should always double check the top of the ultrasound wave graph to make sure that a white layer does not dip below

  the 25mm dotted white line. If this occurs, switch to the XL probe by pressing the XL icon on the bottom of the screen and select OK.
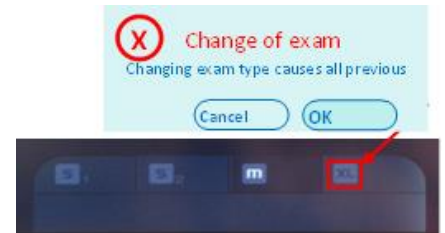

- If you are having difficulty keeping the success rate over 60%, the entire scan data can be reset by clicking on the most recent scan performed, which will also delete all prior scans (see Figure to the right).

- If there are several invalid scans followed by valid scans, deleting the first valid scan will greatly increase the success rate by also deleting the invalid scans from the record. It is good practice to obtain 11 valid scans once proper positioning is found, so that any initial invalid scans will be deleted when you delete the first valid scan.

- One irregular reading (from hitting a rib or simply a much higher or lower reading than the others) will often not skew the median stiffness or IQR too much, but after two irregular readings it is advised to delete all the scans and start over.
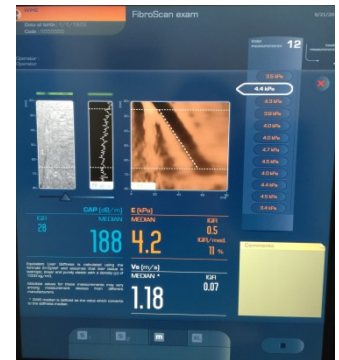
I. **UPLOADING FIBROSCAN DATA**

Once a month, all scans performed (and those that have not yet been transferred to a computer) should be transferred via password-protected flash drive to a computer.

1) From home screen, select Archives:
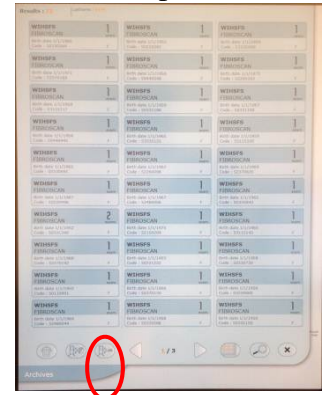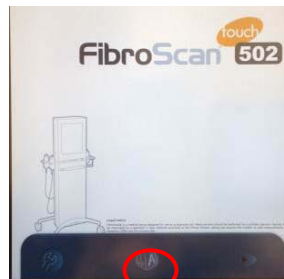
2) First import Fibroscan® data as an Excel file. Select the 3rd icon from the left on the bottom row of the screen with the term ".xls". Make sure you have a password-protected flash drive inserted and unlocked in a Fibroscan® USB port.

3) The machine will ask if you want your data to be anonymous; select "No."
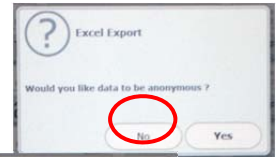
4) Transfer may take several seconds. Wait until this box pops up to indicate the transfer was successful, then click "OK."

5) Next, you will need to export each individual (new) scan in .PDF format. First, select the scan to import:

6) An orange border will appear around the selected scan. You will need to tap on the bottom right corner of the box to further expand the scan description:
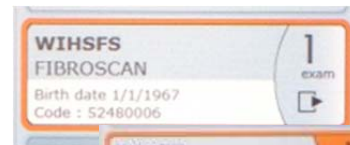
7) Tap on the dark blue box summarizing the results of the scan:

The next screen will look similar to the acquisition screen. Select the icon 2nd to the right at the bottom of the screen with the Adobe logo:

8) Once the icon is selected, it will become outlined in white. After several seconds this message should appear on your screen; select "OK."

9) Now return to the Archives by selecting the back arrow.

10) To prepare to open the next scan, collapse the expanded box by tapping on the bottom right corner of the orange area of the box.

11) Leaving the first uploaded scan highlighted to track your place, repeat Steps 5-11 for every scan from the past week, creating .PDF files for each scan.

12) Once all .PDF files have been copied to your password-protected flash drive, return to the home screen by pressing the "X."
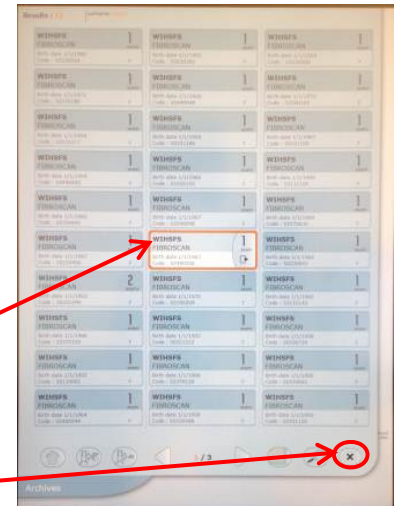
13) Remove the password-protected flash drive, insert the drive into a computer's USB slot, and view files saved on the flash drive. There should be Adobe Acrobat Documents (PDF) for every scan uploaded, as well as Microsoft Excel 97-2003 Worksheets that look something like:

CF60286_20131216_100927.pdf	4/23/2014 10:13 AM	Adobe Acrobat D...	1,033 KB
CF60286_20140106_163042.pdf	4/23/2014 4:36 PM	Adobe Acrobat D...	1,076 KB
CF60286_20140108_094830.pdf	4/23/2014 3:55 PM	Adobe Acrobat D...	998 KB
CF60286_20140310_094213.xls	3/10/2014 9:42 AM	Microsoft Excel 97...	715 KB

14) Each .PDF file is automatically named by Fibroscan® serial number along with the date and time of scan. For filing purposes, rename each file with WIHSID and date of scan. To do this, open up the .PDF, click on "File," then "Save As" "PDF." Direct your path to the correct Fibroscan Data folder and rename by WIHSID and date in the format: "#########.YYYY-MM-DD.PDF"

15) The Excel file with the extension ".xls" also needs to be renamed. Open the file, click "Save As," rename as "FibroscanData.YYYY-MM-DD" with the day the data is uploaded, and change the file extension from ".xml" to "Excel Workbook (*.xlsx)." If you do not resave as an ".xls" or ".xlsx" file it may not be able to be opened on subsequent computers.

J.   QUALITY CONTROL REVIEW AND CERTIFICATION

Training scans will be sent via **UCSF Box** to the TE QC Team for review. In addition to there being <u>exactly</u> 10 valid scans, >60% valid scans, and a <30% IQR/median stiffness value, specific quality checkpoints will be reviewed, including: correct use of M vs XL probe (i.e., fat layer beyond the 25mm depth requires XL probe); avoidance of lung, vascular, or other non-liver tissue; and accurate line-fitting in the wave graph, including

achieving parallel lines not only in the dark compression band, but also in other alternating bands of tan and black expansion/compression bands that "echo" the line slope, making sure to avoid under- or over-estimation of the velocity. For approval, at least 75% of the 50 scans (38/50 and above) must be considered acceptable. Reports from the TE QC team to the operator will include critiques and advice for improvement.

Operators that have 60 to 74% acceptable scans (30/50 to 37/50) will be asked to perform 20 additional scans with PDF files sent to the TE QC Team. If they achieve more than 75% acceptable scans for the combined 70 training scans (53/70 and above), then they will be approved to begin WIHS Fibroscans. Operators that have less than 60% acceptable scans (29/50 and below) will be sent a detailed feedback report, a conference call request and/or a scheduled site visit (See **Section E**: *WIHS Training Requirements* for more details).

K.   SENDING SCANS TO WDMAC

At the end of every semiannual visit, send all WIHS participant scan data (Excel files only) to WDMAC via password-encrypted mechanism. Patient identifiers and non-WIHS data should be removed.   WIHS sites that have a listed LIVRA start date anytime from October 2015 to September 30, 2016 should send their WIHS scan output to WDMAC. Send only the excel data output from the Fibroscan® device for WIHS participants seen from start date for the operator (See Table 2) through September 30, 2016. These scans should be sent to WDMAC by October 7, 2016, so that the Fibroscan® QC team can review scan output.

***Please remember to download WIHS scans monthly from the Fibroscan and store in a password-protected file until ready to send to WDMAC.**

L.   PAYMENT

Fee for Service Agreements will be set-up through NCIRE, the agency associated with the VA that administers NIH grants. Sites will be paid $100 per scan.

M.   REFERENCES

1.   French AL, Gawel SH, Hershow R, et al. Trends in mortality and causes of death among women with HIV in the United States: a 10-year study. *Journal of acquired immune deficiency syndromes (1999).* Aug 1 2009;51(4):399-406.

2.   Salmon-Ceron D, Lewden C, Morlat P, et al. Liver disease as a major cause of death among HIV infected patients: role of hepatitis C and B viruses and alcohol. *Journal of hepatology.* Jun 2005;42(6):799-805.

3.   Weber R, Sabin CA, Friis-Moller N, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. *Archives of internal medicine.* Aug 14-28 2006;166(15):1632-1641.

4.   Bani-Sadr F, Carrat F, Bedossa P, et al. Hepatic steatosis in HIV-HCV coinfected patients: analysis of risk factors. *AIDS (London, England).* Feb 28 2006;20(4):525-531.

5.   Benhamou Y, Bochet M, Di Martino V, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. *Hepatology.* 1999;30(4):1054-1058.

6.   Rodriguez-Guardado A, Maradona JA, Asensi V, Cartón JA, Casado L. Hepatitis C virus in patients with HIV infection and lipodystrophy. *JAIDS Journal of Acquired Immune Deficiency Syndromes.* 2003;32(3):348.

7.   Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *The Lancet.* 1997;349(9055):825-832.

8.   Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. *Journal of hepatology.* 2001;34(5):730-739.

9.   Blackard JT, Welge JA, Taylor LE, et al. HIV mono-infection is associated with FIB-4 - A noninvasive index of liver fibrosis - in women. *Clin Infect Dis.* Mar 1 2011;52(5):674-680.

10.   Price JC, Seaberg EC, Badri S, Witt MD, D'Acunto K, Thio CL. HIV monoinfection is associated with increased aspartate aminotransferase-to-platelet ratio index, a surrogate marker for hepatic fibrosis. *The Journal of infectious diseases.* Mar 15 2012;205(6):1005-1013.

11.   Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endocrine reviews.* Feb 2002;23(1):90-119.

12.   Davis SR, Castelo-Branco C, Chedraui P, et al. Understanding weight gain at menopause. *Climacteric.* Oct;15(5):419-429.

13.   Currier J, Scherzer R, Bacchetti P, et al. Regional adipose tissue and lipid and lipoprotein levels in HIV-infected women. *Journal of acquired immune deficiency syndromes (1999).* May 1 2008;48(1):35-43.

14.   Eastburn A, Scherzer R, Zolopa AR, et al. Association of low level viremia with inflammation and mortality in HIV-infected adults. *PloS one.* 2011;6(11):e26320.

15.   Howard AA, Hoover DR, Anastos K, et al. The effects of opiate use and hepatitis C virus infection on risk of diabetes mellitus in the Women's Interagency HIV Study. *Journal of acquired immune deficiency syndromes (1999).* Jun 2010;54(2):152-159.

16.   Reingold J, Wanke C, Kotler D, et al. Association of HIV infection and HIV/HCV coinfection with C-reactive protein levels: the fat redistribution and metabolic change in HIV infection (FRAM) study. *Journal of acquired immune deficiency syndromes (1999).* Jun 1 2008;48(2):142-148.

17.   Tien PC, Schneider MF, Cole SR, et al. Antiretroviral therapy exposure and incidence of diabetes mellitus in the Women's Interagency HIV Study. *AIDS (London, England).* Aug 20 2007;21(13):1739-1745.

18.   Tien PC, Schneider MF, Cole SR, et al. Antiretroviral therapy exposure and insulin resistance in the Women's Interagency HIV study. *Journal of acquired immune deficiency syndromes (1999).* Dec 1 2008;49(4):369-376.

19.   Tien PC, Bacchetti P, Gripshover B, Overton ET, Rimland D, Kotler D. Association between hepatitis C virus coinfection and regional adipose tissue volume in HIV-infected men and women. *Journal of acquired immune deficiency syndromes (1999).* May 1 2007;45(1):60-65.

20.   Kirk GD, Astemborski J, Mehta SH, et al. Assessment of liver fibrosis by transient elastography in persons with hepatitis C virus infection or HIV-hepatitis C virus coinfection. *Clin Infect Dis.* Apr 1 2009;48(7):963-972.

21.   Sasso M, Beaugrand M, de Ledinghen V, et al. Controlled attenuation parameter (CAP): a novel VCTE guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. *Ultrasound in medicine & biology.* Nov 2010;36(11):1825-1835.

22.   Kettaneh A, Marcellin P, Douvin C, et al. Features associated with success rate and performance of FibroScan measurements for the diagnosis of cirrhosis in HCV patients: a prospective study of 935 patients. *Journal of hepatology.* Apr 2007;46(4):628-634.