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# Cohort profile: The multi-site prospective Liver Disease and Reproductive Aging (LIVRA) study in U.S. women living with and without HIV

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**Abbreviations:**

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| Ab | antibody |
| BMI | body mass index |
| CAP | controlled attenuation parameter |
| CI | confidence interval |
| dB/m | decibels/meter |
| HCV | hepatitis C virus |
| HIV | human immunodeficiency virus |
| HOMA-IR | homeostatic model assessment of insulin resistance |
| IQR | interquartile range |
| kPa | kilopascals |
| LIVRA | Liver Disease and Reproductive Aging |
| LS | liver stiffness |
| MWCCS | MACS-WIHS Combined Cohort Study |
| PLWH | persons living with HIV |
| QA | quality assurance |
| US | United States |
| VCTE | vibration controlled transient elastography |
| WIHS | Women's Interagency HIV Study |

**Conflicts of interest:**

AA has received consulting fees from Merck, Viiv Healthcare, and Gilead Sciences; Merck and Gilead Sciences have provided her institution with funding for her research.

JCP has received consulting fees from Theratechnologies; Gilead Sciences and Merck have provided her institution with funding for her research.

PCT: Merck has provided her institution with funding for her research; Gilead and Lilly have also provided her institution with funding for her to conduct industry-sponsored clinical trials.

MHK reports consulting for Sanofi.

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**Data sharing statement:** Access to individual-level data from the MACS/WIHS Combined Cohort Study Data (MWCCS) may be obtained upon review and approval of a MWCCS concept sheet. Links and instructions for online concept sheet submission are on the study website (http://mwccs.org/).

## ABSTRACT

**Purpose:** The Liver Disease and Reproductive Aging (LIVRA) study leverages the infrastructure of the decades-long multi-center prospective Women's Interagency HIV Study (WIHS) to examine the contributions of HIV, HCV, and aging to liver disease progression in women.

**Participants:** From 2013-2018, LIVRA enrolled 1576 participants (77 HCV-seropositive only, 248 HIV/HCV-seropositive, 868 HIV-seropositive only and 383 HIV/HCV-seronegative) who underwent vibration controlled transient elastography (VCTE). A VCTE Quality Assurance program was established to ensure consistency and accuracy for longitudinal assessment of steatosis (fatty liver) via the controlled attenuation parameter (CAP) and fibrosis via liver stiffness (LS). Demographic, lifestyle factors, anthropometry, clinical and medication history, host genetics, immune markers and hormone levels were collected as part of the WIHS.

**Findings to date:** Prevalence of CAP-measured steatosis (CAP≥248dB/m) and LS-measured significant fibrosis (LS≥7.1kPa) was 48% and 24%, respectively. Waist circumference (WC) and insulin resistance, but not HIV or HCV seropositivity, were independently associated with higher CAP, whereas older age, WC, CAP, and HCV seropositivity (with and without HIV) were associated with higher LS. Excluding scans that were invalid based on traditional criteria for unreliability did not affect the results.

**Future plans:** Enrolled women undergo VCTE at three-year intervals unless LS is ≥9.5 kPa, indicating advanced fibrosis, in which case VCTE is performed annually. Participants also undergo VCTE every 6 months until 18 months after HCV treatment initiation. Analysis of the data collected will provide insights into the impact of aging/ovarian function, host genetics, immune function, and contemporary HIV and HCV treatments on liver disease progression.

## ARTICLE SUMMARY

### Strengths and limitations of this study

- The Liver Disease and Reproductive Aging (LIVRA) study is designed to examine the contributions of HIV, hepatitis C virus (HCV), aging and ovarian function, immune function, host genomics, and contemporary antiviral treatments on hepatic steatosis (or fatty liver) and fibrosis progression measured via longitudinal vibration controlled transient elastography (VCTE).
- The study leverages the infrastructure of the decades-long multi-center prospective Women's Interagency HIV Study (WIHS), which collects biological specimens, socio-demographic data, lifestyle (substance use, food insecurity, and physical activity) factors, anthropometry, clinical and medication history, host genetics, immune markers and hormone levels.
- A rigorous VCTE Quality Assurance program was developed to ensure consistency and accuracy of hepatic steatosis and fibrosis assessments.
- Though limited to women, the LIVRA VCTE protocol is currently being implemented in men enrolled into the newly integrated MACS-WIHS Combined Cohort Study.
- The LIVRA study will allow investigation into the natural history of and potentially modifiable risk factors for hepatic steatosis and fibrosis in women with and without HIV.

## INTRODUCTION

Liver disease is a leading cause of morbidity and mortality among persons living with HIV (PLWH)[1]. However, with the advent of direct acting antiviral agents to treat hepatitis C virus (HCV), the landscape of liver disease in PLWH is broadening with hepatic steatosis (fatty liver) being frequently observed among PLWH[2]. The determinants of hepatic steatosis and fibrosis progression among PLWH are not well known, especially in the modern era of HIV and HCV treatment. Seminal cohort studies of people with hepatic steatosis have excluded PLWH and those with cleared HCV[3,4], and studies of hepatic steatosis in PLWH are often small in sample size.

Although women represent over 50% of all PLWH worldwide[5] and close to 25% of all PLWH in the United States (US)[6], the vast majority of studies of hepatic steatosis among PLWH have been conducted in men. In HIV-seronegative populations, women have a lower overall risk of steatosis than men but a higher risk of advanced fibrosis once steatosis is established, particularly after age 50[7]. Understanding the factors associated with steatosis and its impact on fibrosis among women in general, and in those living with HIV in particular, is of essential importance. Furthermore, few studies have examined the additional contribution of HCV coinfection in women. The Liver Disease and Reproductive Aging (LIVRA) study was designed as an ancillary study of the Women's Interagency HIV Study (WIHS) to evaluate the contributions of HIV, HCV, and reproductive aging to steatosis and fibrosis progression as measured by longitudinal vibration controlled transient elastography (VCTE) among a large cohort of women living with or without HIV.

## COHORT DESCRIPTION

**Study Population and Design**

The WIHS (now part of the MACS-WIHS Combined Cohort Study[8] [MWCCS]) was a multicenter prospective cohort study established to investigate the course of HIV and associated conditions among women living with and without HIV. The WIHS enrolled 4,982 women (3,678 living with HIV infection and 1,304 living without HIV infection) during four recruitment waves: 1994-1995, 2001-2002, 2011-2012, and 2013-2015 from 10 U.S. cities (Bronx and Brooklyn, NY; Chicago, IL; San Francisco, CA; Los Angeles, CA; Washington D.C.; Atlanta, GA; Chapel Hill, NC; Miami, FL; Jackson, MS; and Birmingham, AL). Full details of recruitment, retention, and demographics have been published previously.[9] Baseline sociodemographic characteristics and HIV risk factors were similar between HIV-seropositive and HIV-seronegative women. At semi-annual research visits, participants completed a physical examination, provided biological specimens (e.g. serum, plasma, peripheral blood mononuclear cells, urine), and completed interviewer-administered questionnaires to obtain information regarding socio-demographics, lifestyle data (e.g. substance use, food security, physical activity), and clinical, psychosocial, and medication history. Host genetic data and genome-wide association studies are also available in the WIHS, as are markers of immune activation and inflammation and hormone levels.

From December 2013 to December 2018, 1576 of the approximately 2100 active WIHS women were enrolled into the LIVRA ancillary study. Eligibility included age 35 to 60 years old for women without chronic HCV. All women with chronic HCV infection were eligible for enrollment, regardless of age, as long as they were not currently receiving anti-HCV therapy. Women with positive hepatitis B surface antigen, hemochromatosis, autoimmune hepatitis or primary biliary cholangitis were excluded, as were women who reported using medications associated with steatosis (i.e. systemic corticosteroids, amiodarone, methotrexate), signs of decompensated cirrhosis, current cancer, or severe renal insufficiency. Women who were pregnant or had an

implantable cardiac device were excluded per the VCTE manufacturer (Fibroscan®; Echosens, Paris, France).

As part of the study protocol, longitudinal VCTE examinations are performed at three-year intervals after their baseline measurement unless liver stiffness (LS) values are ≥9.5 kilopascals (kPa), consistent with advanced fibrosis, in which case VCTE are performed annually. Additionally, participants who report HCV treatment after enrollment undergo VCTE every 6 months until 18 months after HCV treatment initiation. The study was approved by the Institutional Review Boards of all participating sites, and all participants provided written informed consent.

**VCTE Performance and Quality Assurance**

VCTE reliability is operator-dependent[10], and therefore proper operator training and experience is important, especially in multicenter studies involving multiple operators. VCTE operators at each site were first trained by Echosens to: 1) operate the device, 2) obtain at least 10 measurements that the device deemed as valid per scan, and 3) recognize images that the device deemed valid but required manual adjustment of probe placement by the operator due to interference from vessel, breathing, or lung, and not being completely in the liver (e.g. probe placed too low or needing to switch to the XL probe due to subcutaneous fat interference). Operators at each WIHS site were then certified by Echosens after demonstrating proficiency in scanning 3 to 5 volunteers.

A Quality Assurance (QA) program was implemented which required operators to complete at least 50 additional training examinations on subjects with a wide range of body types and within the age range of eligible participants. This benchmark was selected because a large multicenter study indicated that at least 50 examinations were needed before an operator was considered proficient to consistently acquire valid scans[11]. Each of the first 25 examinations was reviewed by the QA team, which was comprised of two clinicians and an experienced VCTE operator (Table 1). Upon completion of the review, a written report was provided with the additional option of individualized verbal feedback or an in-person hands-on training and observation. A similar procedure was followed for the next 25 examinations. After completion of 50 training examinations, the final report indicated whether: 1) the operator was certified; 2) additional examinations were needed and/or 3) a site visit for in-person training was warranted. Twenty-nine operators submitted a median of 55 scans (interquartile range [IQR] 51,62) for review before being certified. To maintain certification, operators are required to perform at least 20 examinations per year.

**Assessment of Hepatic Steatosis and Fibrosis**

Hepatic steatosis was estimated in decibels/meter (dB/m) using the VCTE-controlled attenuation parameter (CAP) software and fibrosis was estimated using LS measurements in kPa. Participants were instructed to fast for at least three hours prior to VCTE. Operators were instructed to manually switch from the M probe to the XL probe if images suggested subcutaneous fat interference in the measurement range of the probe, i.e. >2.5 cm distance from skin to liver capsule[12]. All examinations had at least 10 successful measurements. At the time of analyses, examinations were flagged as having abnormal validity if they had an IQR/median LS ratio >30% and/or success rate <60% as per prior published criteria[13], or poor

image quality. The QA team manually reviewed scan images and data on all VCTE examinations with LS≥9.3 kPa, a random selection of 10% of examinations with LS<9.3 kPa, and those with CAP<150 dB/m. This latter criterion was established after image review suggested that some scans with very low CAP values, including those with the lower bound CAP value of 100 dB/m, may partly capture data from outside of the liver.

Of the 1576 VCTE's performed, 56 had an IQR/median >30% (3.6%), 129 had a success rate <60% (8.2%), and 49 had poor image quality on review (3.1%). These categories were not mutually exclusive; 193 had at least one of these indices (12%). Due to an early technical error with the VCTE device, CAP values were missing for 33 women. Steatosis was defined by CAP≥248 dB/m, which was the optimal cut-off for selecting mild or greater steatosis in a meta-analysis including data from 2735 patients with liver biopsies[14] and was 83% sensitive and 72% specific in detecting mild or greater steatosis in a subset of our cohort when compared to magnetic resonance spectroscopy (MRS)-measured liver fat fraction[15]. The following fibrosis categories were used: significant fibrosis (LS≥7.1 kPa), advanced fibrosis (LS≥9.5 kPa), and cirrhosis (LS≥12.5 kPa)[16].

**Covariates**

Race/ethnicity was self-reported as White, Black, or Hispanic (including White and Black Hispanic). Menopause stage was determined based on the Stages of Reproductive Aging Workshop criteria[17]. Self-reported alcohol consumption was categorized as: none; light (>0-7 drinks/week); moderate (7-12 drinks/week); or heavy (>12 drinks/week). Additional demographic and behavioral covariates were obtained through self-report. Body mass index (BMI) was calculated in kg/m$^2$, waist circumference was measured in cm, and the homeostatic model

assessment of insulin resistance (HOMA-IR) was calculated using 8-hour fasting insulin and glucose values.

HIV and HCV seropositivity were defined by documentation of a reactive HIV enzyme immunoassay and reactive second or third-generation HCV enzyme immunoassay, respectively). At WIHS study entry, women underwent testing for HCV antibody (Ab) with HCV RNA if positive. If negative, HCV Ab testing was repeated at last WIHS study visit. For those who became HCV Ab positive, additional retrospective HCV Ab testing was performed to determine when HCV incidence occurred, and then HCV RNA testing was performed at least 2 years later to determine whether they had spontaneously cleared or had chronic infection. For all chronic infections, HCV RNA testing was performed at later visits to determine which had cleared following treatment. HCV RNA was performed using either the COBAS Amplicor Monitor 2.0 or the COBAS Taqman assay, as previously described (both from Roche Diagnostics, Branchburg, New Jersey, USA)[18]. HCV genotyping was conducted on a subset of HCV viremic women using the NC TRUGENE HCV 5′ NC Genotyping Kit (Bayer HealthCare LLC, Tarrytown, NY), as previously described[19].

**Statistical Analysis**

For the purposes of this analysis, HIV and HCV infection categories were defined by serostatus. We compared participant characteristics by infection category using ANOVA or Kruskal-Wallis tests for continuous variables and Chi-square or Fisher's exact tests for categorical variables, as appropriate. We used unadjusted and multivariable adjusted linear regression models to

examine associations with CAP and LS. LS had a right-skewed distribution and therefore was log-transformed to normalize its distribution, and the regression coefficients and their confidence intervals (CI) were exponentiated to calculate percentage differences attributable to each covariate. These analyses were performed for all women with successful VCTE's, and sensitivity analyses were performed excluding the scans with IQR/median >30%, success rate <60%, poor image quality on QA review, or any one of these criteria. In linear regression models where there were missing covariate information, we used the full information maximum likelihood approach in the setting of path analysis instead of the multiple imputation approach for its efficiency[20]. All analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC).

**Baseline characteristics**

Among the 1576 WIHS women enrolled in LIVRA, 77 had HCV monoinfection, 248 HIV/HCV coinfection, 868 HIV infection alone, and 383 neither infection (Table 2). About three quarters were Black, and median age was 51 years (IQR 44,56), with 31% premenopausal, 13% perimenopausal, and 56% postmenopausal. Nearly half reported an annual household income of ≤$12,000, and one-third of the cohort completed less than a high school education. Most women with HIV were taking ART and had undetectable HIV RNA.

**FINDINGS TO DATE**

Women with neither HIV nor HCV had higher median CAP values than women with HCV monoinfection (247 dB/m [IQR 204, 281] vs 232 dB/m [IQR 191, 285]), and women with HIV monoinfection had higher values than women with HIV/HCV coinfection (246 dB/m [IQR 208,

290] vs 237 dB/m [IQR 200, 272]). Similarly, the prevalence of hepatic steatosis based on CAP ≥248 dB/m was highest in women with neither infection (50%), followed by those with HIV monoinfection (49%), HIV/HCV coinfection (43%), and HCV monoinfection (38%) (Figure 1a). The overall prevalence of steatosis was 48%, which is slightly higher than the 43% prevalence observed among women in the 2017-2018 NHANES study[21], which used a CAP cut-off of 263 dB/m, and higher than the 39% reported in a retrospective study of 1511 PLWH from Canada and Italy, which used the same 248 dB/m CAP cut-off as our study[22]. The latter study included mostly White men, whereas our study included mostly Black women, a group that has traditionally been found to have lower risk of hepatic steatosis.

On multivariable analysis, greater waist circumference (a marker of visceral obesity) and greater HOMA-IR were significantly associated with higher CAP values (Table 3). Our findings are consistent with other studies that have demonstrated visceral adiposity and insulin resistance to be key mediators in the pathogenesis of fatty liver disease[23,24]. However, HIV infection was not associated with CAP, suggesting that the clinical phenotype of fatty liver disease in PLWH is predominantly caused by metabolic dysregulation that appears to be independent of HIV infection. By contrast, HCV seropositivity was associated with significantly lower CAP values in analyses adjusted for alcohol use, smoking status, and menopause stage. Further research on the potential mechanisms underlying this observation as well as the impact of HCV clearance on fatty liver disease are warranted.

As expected, women with HCV had higher median LS values compared to women without HCV, with the highest LS observed among those with HCV monoinfection (6.9 kPa [IQR 5.0, 10.7]), followed by those with HIV/HCV coinfection (6.2 kPa [IQR 4.6, 9.1]), HIV monoinfection (5.0 kPa

[IQR 3.9, 6.7]) and those with neither infection (4.9 kPa [IQR 3.9, 6.2]). Prevalence of significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa), and cirrhosis (LS ≥12.5 kPa) was also higher among women with HCV compared to those without HCV (Figure 1b). In multivariable analysis, HIV/HCV coinfection, HCV monoinfection, older age, greater waist circumference, and greater CAP values were each independently associated with higher LS (Table 3). These findings have important implications for women as visceral adiposity and metabolic abnormalities increase during the menopausal transition, independent of chronologic age[25]. Estrogen depletion occurs during the late phase of perimenopause and is associated with increased levels of inflammatory cytokines[26] and mucosal barrier dysfunctions[27]. These changes may result in increased gut microbial translocation and inflammation[28], factors that have also been linked with steatosis and fibrosis[29,30]. Studies are underway evaluating the impact of ovarian follicular reserve on steatosis and fibrosis among LIVRA participants.

Finally, a notable finding was that the factors associated with steatosis and fibrosis were similar after excluding VCTE examinations with IQR/median >30%, success rate <60%, poor image quality, or any of these three criteria (Supplemental Tables 1 and 2).

**Strengths and limitations**

To our knowledge, this is the most comprehensive cohort study evaluating liver disease among US women using VCTE and the largest study of VCTE in women living with HIV. However, our cohort has limitations. First, there are demographic differences by HIV and HCV serostatus, and thus unmeasured confounders may remain despite adjustment for multiple variables. Second, we rely on VCTE to assess liver disease, rather than liver biopsy. However, liver biopsy is infeasible in large cohorts such as ours. Finally, our findings may not be generalizable to men,

but our VCTE protocol is currently being implemented in men enrolled in the newly integrated MWCCS allowing us to address sex differences.

A major strength of the LIVRA study is that it is conducted in a large, nationally representative cohort of women living with HIV in the US[31] where racial and ethnic minorities, people living below the poverty line, and those with less than a high school education are at higher risk for liver disease[32]. Second, LIVRA has detailed longitudinal HIV and HCV data allowing the study of liver steatosis and fibrosis trajectories in women with active HCV viremia, spontaneous HCV clearance, and treated HCV clearance. Third, the inclusion of women with and without HIV and HCV infections will allow us to evaluate the association of viral factors with liver disease utilizing seronegative controls. Finally, due to our rigorous QA program, a low proportion of VTCE scans were invalid based on traditional criteria for unreliability and excluding these scans did not impact our results. With longitudinal assessments, the LIVRA study will enable evaluation of hepatic steatosis and fibrosis progression in women and support investigation of mechanistic pathways and potentially modifiable risk factors for liver disease in high-risk women.

## PATIENT INVOLVEMENT

Participants of the LIVRA Study were not involved in the development of the study question or the liver disease outcomes. However, the national community advisory board of the WIHS (now MWCCS) approved the study of liver disease progression using VCTE as a high scientific priority.

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**Table 1. LIVRA Study Quality Assurance Program for VCTE Operator Training Certification**

| Exams | Guidelines | Training Goals |
|---|---|---|
| 1-25 | **Subjects**: Varied body types and ages, discrete subjects<br>**Parameter goals to work toward**: IQR<30% of median, >60% of measurements deemed valid by device<br>REQUIRED: 10 valid measurements obtained per participant | • Get comfortable with positioning<br>• Familiarize with Fibroscan® screen and available tools; Observe how age and abdominal fat affect Fibroscan® acquisition<br>• Work on consistently achieving valid assessments |
| Scans are reviewed by QA team and operator receives written feedback report. Check-in call and additional in-person training optional. | | |
| 26-50 | **Subjects**: Varied body types and ages, discrete subjects<br>**Parameters**: REQUIRED: IQR<30% of median, >60% of measurements deemed valid by device, 10 valid measurements per participant | • Focus on achieving valid assessments consistently<br>• Utilize deletion to meet parameter requirements |
| Scans are reviewed by QA team and operator receives written feedback report. Additional examinations and site visit may be required prior to certification. | | |
| Maintenance certification:<br>• Operator must perform at least 20 examinations per year<br>• Ongoing written feedback provided for studies reviewed by QA team | | |

**Table 2.** Characteristics of the study population by HIV and HCV antibody status.

| Median (IQR) or % | HIV-/HCV+ (n=77) | HIV+/HCV+ (n=248) | HIV+/HCV- (n=868) | HIV-/HCV- (n=383) | p-value |
|---|---|---|---|---|---|
| **Sociodemographic** | | | | | |
| Age (years) | 56 (49, 60) | 56 (53, 60) | 49 (43, 54) | 47 (41, 54) | <0.001 |
| Race/Ethnicity | | | | | 0.001 |
| Black | 64% | 67% | 76% | 76% | |
| White | 7% | 15% | 9% | 7% | |
| Hispanic | 22% | 17% | 11% | 12% | |
| Other | 7% | 2% | 4% | 5% | |
| Menopause stage | | | | | <0.001 |
| Premenopause | 16% | 7% | 33% | 44% | |
| Perimenopause | 13% | 8% | 14% | 14% | |
| Postmenopause | 71% | 85% | 53% | 43% | |
| Household income | | | | | |
| <$6,000 | 29% | 16% | 12% | 19% | <0.001 |
| $6,000-$12,000 | 44% | 49% | 35% | 26% | |
| $12,001-$36,000 | 7% | 13% | 15% | 13% | |
| >$36,000 | 9% | 9% | 17% | 23% | |
| Education | | | | | |
| Less than high school | 43% | 39% | 31% | 32% | 0.02 |
| High school | 35% | 29% | 34% | 28% | |
| More than high school | 22% | 32% | 35% | 41% | |
| **Lifestyle** | | | | | |
| Alcohol use | | | | | <0.001 |
| None | 51% | 63% | 51% | 42% | |
| Light | 27% | 25% | 40% | 42% | |
| Moderate | 4% | 3% | 3% | 4% | |
| Heavy | 18% | 8% | 6% | 12% | |
| Current smoking | 71% | 54% | 35% | 42% | <0.001 |
| Current marijuana use | 30% | 26% | 20% | 27% | 0.05 |
| Ever injection drug use | 36% | 23% | 1% | 4% | <0.001 |
| **Metabolic** | | | | | |
| BMI (kg/m$^2$) | 30 (25, 36) | 28 (23, 32) | 31 (26, 36) | 32 (27, 38) | <0.001 |
| Waist Circumference (cm) | 99 (88, 115) | 97 (86, 108) | 99 (89, 111) | 100 (88, 113) | 0.12 |
| HOMA-IR | 3.2 (1.6, 6.8) | 2.48 (1.3, 4.9) | 2.1 (1.3, 3.8) | 1.8 (1.1, 3.3) | <0.001 |
| **Liver-related** | | | | | |
| AST (U/L) | 26 (19, 37) | 24 (18, 35) | 19 (15, 23) | 17 (14, 20) | <0.001 |
| ALT (U/L) | 23 (15, 34) | 18 (12, 30) | 15 (12, 21) | 14 (11, 18) | <0.001 |
| **HIV-related** | | | | | |
| Undetectable HIV RNA | | 69% | 72% | | 0.60 |

| | | | |
|---|---|---|---|
| CD4 current (cells/mm$^3$) | 616 (428, 869) | 656 (435, 870) | 0.35 |
| CD4 nadir (cells/mm$^3$) | 215 (107, 320) | 222 (103, 361) | 0.95 |
| History of clinical AIDS | 42% | 28% | <0.001 |
| Current ART | 89% | 89% | 0.81 |

**Figure 1a.** Prevalence of VCTE-estimated hepatic steatosis (CAP≥248 dB/m) by HIV and HCV antibody status. **1b.** Prevalence of VCTE-estimated significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa), and cirrhosis (LS ≥12.5 kPa), as estimated by VCTE-measured liver stiffness, by HIV and HCV antibody status.

**Table 3. Independent associations of clinical and demographic characteristics with CAP and LS ***

| | CAP difference (dB/m) (CI)** | p-value | Liver stiffness % change (CI)*** | p-value |
|---|---|---|---|---|
| Infection Status (ref=HIV-/HCV-) | | | | |
| HIV+/HCV- | 1.1 (-5.3, 7.5) | 0.74 | 4.4% (-1.1%, 10.3%) | 0.12 |
| HIV+/HCV+ | -9.2 (-18.2, -0.3) | 0.04 | 33.4% (23.5%, 44.1%) | <0.001 |
| HIV-/HCV+ | -10.4 (-23.8, 3.1) | 0.13 | 43.2% (27.8%, 60.4%) | <0.001 |
| Age (per 10 years) | 2.4 (-2.0, 6.9) | 0.29 | 4.4% (0.4%, 8.4%) | 0.03 |
| Race/Ethnicity (ref=white non-Hispanic) | | | | |
| Black | -6.1 (-14.2, 2.1) | 0.14 | 2.8% (-4.0%, 10.1%) | 0.43 |
| Hispanic | -3.1 (-12.6, 6.4) | 0.52 | 3.1% (-5.0%, 11.7%) | 0.47 |
| Other | 2.9 (-8.2, 14.0) | 0.61 | 2.5% (-6.7%, 12.7%) | 0.60 |
| Waist circumference (per 10 cm) | 17.8 (16.2, 19.4) | <0.001 | 5.0% (3.3%, 6.6%) | <0.001 |
| HOMA-IR (per doubling) | 1.2 (0.8, 1.6) | <0.001 | 0.3% (-0.1%, 0.7%) | 0.11 |
| CAP (per 10 dB/m) | | | 0.6% (0.1%, 1.0%) | 0.01 |

*All variables shown in the table are included in a single multiple linear regression model (one model for CAP and one for LS). Also adjusted for alcohol use, smoking status, and menopause stage (parameter estimates not shown).
**N=1543
***N=157

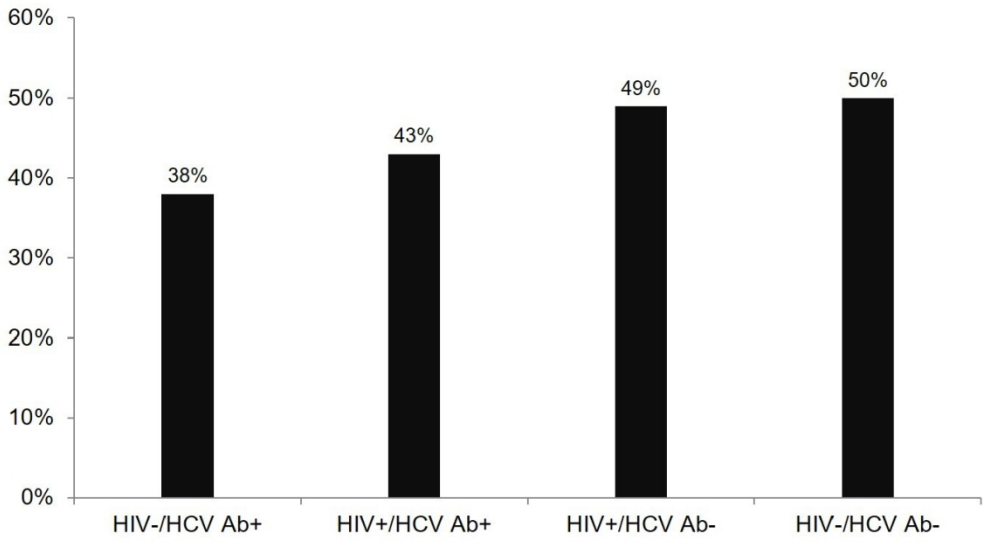


Figure 1a. Prevalence of VCTE-estimated hepatic steatosis (CAP≥248 dB/m) by HIV and HCV antibody status.

279x216mm (123 x 123 DPI)

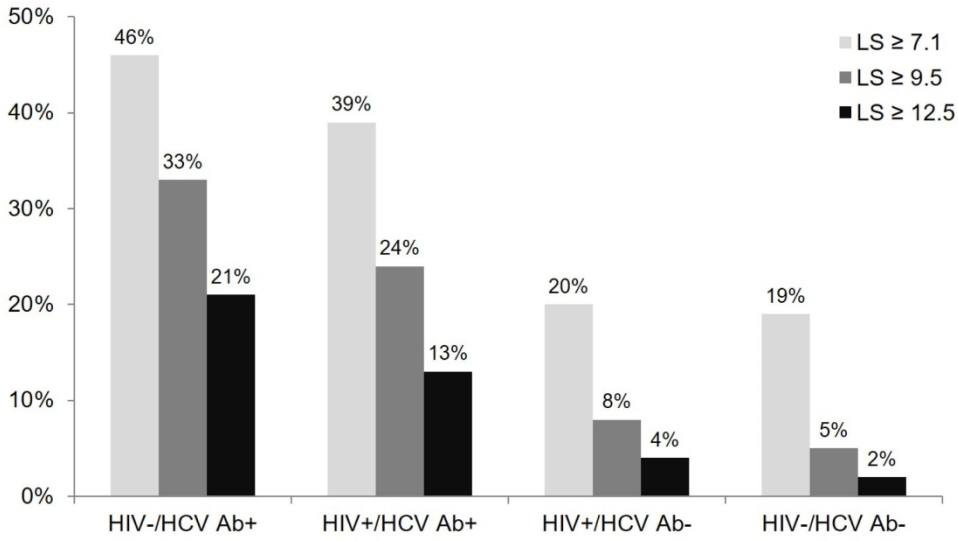


Figure 1b. Prevalence of VCTE-estimated significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa), and cirrhosis (LS ≥12.5 kPa), as estimated by VCTE-measured liver stiffness, by HIV and HCV antibody status.

266x184mm (300 x 300 DPI)

**Supplemental Table 1. Factors associated with CAP in multivariable analysis*, excluding VCTE scans with abnormal validity criteria.**

| | Excluding if IQR/Med >30% (N=1520) | | Excluding success rate <60% (N=1447) | | Excluding poor image quality (N=1527) | | Excluding any abnormal validity criteria (N=1383) | |
|---|---|---|---|---|---|---|---|---|
| | CAP difference (dB/m) | p-value | CAP difference (dB/m) | p-value | CAP difference (dB/m) | p-value | CAP difference (dB/m) | p-value |
| Infection Status (ref=HIV-/HCV-) | | | | | | | | |
| HIV+/HCV- | 0.3 (-6.2, 6.8) | 0.92 | -1.1 (-7.7, 5.5) | 0.74 | 1.4 (-4.8, 7.6) | 0.65 | -0.2 (-6.7, 6.3) | 0.95 |
| HIV-/HCV+ | -11.7 (-25.3, 2) | 0.09 | -16.6 (-30.5, -2.7) | 0.02 | -11.2 (-24, 1.7) | 0.09 | -17.8 (-31.5, -4.1) | 0.01 |
| HIV+/HCV+ | -8.4 (-17.5, 0.7) | 0.07 | -12.1 (-21.2, -3) | 0.01 | -8.5 (-17.2, 0.2) | 0.06 | -9.5 (-18.5, -0.5) | 0.04 |
| Age (per 10 years) | 2.4 (-2.2, 6.9) | 0.31 | 1.6 (-3, 6.2) | 0.50 | 1.3 (-3.1, 5.6) | 0.56 | 0.9 (-3.7, 5.5) | 0.70 |
| Race/Ethnicity (ref=white non-Hispanic) | | | | | | | | |
| Black | -5.6 (-13.9, 2.7) | 0.18 | -8.2 (-16.5, 0) | 0.05 | -5.7 (-13.5, 2.2) | 0.16 | -7.3 (-15.5, 0.9) | 0.08 |
| Hispanic | -4.2 (-13.8, 5.4) | 0.40 | -4.4 (-13.9, 5) | 0.36 | -3.6 (-12.7, 5.6) | 0.45 | -4.9 (-14.2, 4.5) | 0.31 |
| Other | 4.4 (-6.8, 15.6) | 0.44 | 3.6 (-7.5, 14.7) | 0.53 | 2.9 (-7.8, 13.5) | 0.60 | 4.6 (-6.3, 15.6) | 0.40 |
| WC (per 10 cm) | 17.8 (16.2, 19.5) | <0.001 | 17.5 (15.9, 19.2) | <0.001 | 17.8 (16.3, 19.4) | <0.001 | 17.6 (15.9, 19.2) | <0.001 |
| HOMA-IR (per doubling) | 1.2 (0.8, 1.6) | <0.001 | 1.5 (1, 2) | <0.001 | 1.2 (0.8, 1.5) | <0.001 | 1.5 (1, 2) | <0.001 |

*Also adjusted for alcohol use, smoking status, and menopause stage

**Supplemental Table 2. Factors associated with liver stiffness in multivariable analysis, excluding VCTE scans with abnormal validity criteria.**

| | Excluding if IQR/Med >30% (N=1520) | | Excluding success rate <60% (N=1447) | | Excluding poor image quality (N=1527) | | Excluding any abnormal validity criteria (N=1383) | |
|---|---|---|---|---|---|---|---|---|
| | LS % change | p-value | LS % change | p-value | LS % change | p-value | LS % change | p-value |
| Infection Status (ref=HIV-/HCV-) | | | | | | | | |
| HIV+/HCV- | 4.4% (-1.2%, 10.3%) | 0.12 | 4.5% (-1.2%, 10.5%) | 0.12 | 4.1% (-1.4%, 9.9%) | 0.14 | 5.2% (-0.6%, 11.3%) | 0.08 |
| HIV-/HCV+ | 44.2% (28.6%, 61.6%) | <0.001 | 45.1% (29%, 63.1%) | <0.001 | 44.5% (29.3%, 61.5%) | <0.001 | 46.8% (30.5%, 65.2%) | <0.001 |
| HIV+/HCV+ | 34.4% (24.3%, 45.2%) | <0.001 | 35.2% (25%, 46.2%) | <0.001 | 31.1% (21.5%, 41.6%) | <0.001 | 33.6% (23.4%, 44.6%) | <0.001 |
| Age (per 10 years) | 4.6% (0.6%, 8.7%) | 0.02 | 3.4% (-0.6%, 7.5%) | 0.10 | 3.3% (-0.6%, 7.3%) | 0.10 | 3.1% (-0.9%, 7.3%) | 0.13 |
| Race/Ethnicity (ref=white non-Hispanic) | | | | | | | | |
| Black | 2.2% (-4.7%, 9.6%) | 0.54 | 2.3% (-4.6%, 9.7%) | 0.52 | 1% (-5.7%, 8.1%) | 0.78 | 0.2% (-6.6%, 7.6%) | 0.95 |
| Hispanic | 3.7% (-4.5%, 12.4%) | 0.39 | 2.2% (-5.8%, 10.8%) | 0.60 | 1% (-6.8%, 9.4%) | 0.82 | 0.5% (-7.4%, 9.1%) | 0.90 |
| Other | 1.9% (-7.4%, 12%) | 0.70 | 2.8% (-6.5%, 13%) | 0.57 | 1.3% (-7.7%, 11.2%) | 0.79 | 1.5% (-7.7%, 11.6%) | 0.76 |
| WC (per 10 cm) | 4.4% (2.7%, 6.1%) | <0.001 | 3.8% (2.1%, 5.5%) | <0.001 | 4.4% (2.7%, 6.1%) | <0.001 | 3.4% (1.7%, 5.2%) | <0.001 |
| log2HOMA-IR | 0.3% (0%, 0.7%) | 0.08 | 0.1% (-0.4%, 0.5%) | 0.80 | 0.3% (-0.1%, 0.6%) | 0.14 | 0.1% (-0.4%, 0.5%) | 0.79 |
| CAP (per 10 dB/m) | 0.6% (0.1%, 1%) | 0.01 | 0.7% (0.2%, 1.2%) | 0.004 | 0.6% (0.1%, 1.1%) | 0.01 | 0.7% (0.2%, 1.2%) | 0.01 |

*Also adjusted for alcohol use, smoking status, and menopause stage

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 5 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 7 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 7 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 8 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 8 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 9-11 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 9-10 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 9 |
| Study size | 10 | Explain how the study size was arrived at | 8 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 12 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 11 |
| | | (*c*) Explain how missing data were addressed | 11 |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | |
| | | (*e*) Describe any sensitivity analyses | |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 13 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| | | (c) Summarise follow-up time (eg, average and total amount) | |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 13 |

| | | | |
|---|---|---|---|
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included<br>(*b*) Report category boundaries when continuous variables were categorized<br>(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 14<br><br><br><br>14 |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 13 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 15 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 3 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

# Cohort profile: The multi-site prospective Liver Disease and Reproductive Aging (LIVRA) study in U.S. women living with and without HIV

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# Cohort profile: The multi-site prospective Liver Disease and Reproductive Aging (LIVRA) study in U.S. women living with and without HIV

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**Abbreviations:**

| | |
|---|---|
| Ab | antibody |
| BMI | body mass index |
| CAP | controlled attenuation parameter |
| CI | confidence interval |
| dB/m | decibels/meter |
| HCV | hepatitis C virus |
| HIV | human immunodeficiency virus |
| HOMA-IR | homeostatic model assessment of insulin resistance |
| IQR | interquartile range |
| kPa | kilopascals |
| LIVRA | Liver Disease and Reproductive Aging |
| LS | liver stiffness |
| MWCCS | MACS-WIHS Combined Cohort Study |
| PLWH | persons living with HIV |
| QA | quality assurance |
| US | United States |
| VCTE | vibration controlled transient elastography |
| WIHS | Women's Interagency HIV Study |

**Conflicts of interest:**

AA has received consulting fees from Merck, Viiv Healthcare, and Gilead Sciences; Merck and Gilead Sciences have provided her institution with funding for her research.

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**Data availability statement:** Access to individual-level data from the MACS/WIHS Combined Cohort Study Data (MWCCS) may be obtained upon review and approval of a MWCCS concept sheet. Links and instructions for online concept sheet submission are on the study website (http://mwccs.org/).

## ABSTRACT

**Purpose:** The Liver Disease and Reproductive Aging (LIVRA) study leverages the infrastructure of the decades-long multi-center prospective Women's Interagency HIV Study (WIHS) to examine the contributions of HIV, HCV, and aging to liver disease progression in women.

**Participants:** From 2013-2018, LIVRA enrolled 1576 participants (77 HCV-seropositive only, 248 HIV/HCV-seropositive, 868 HIV-seropositive only and 383 HIV/HCV-seronegative) who underwent vibration controlled transient elastography (VCTE). A VCTE Quality Assurance program was established to ensure consistency and accuracy for longitudinal assessment of steatosis (fatty liver) via the controlled attenuation parameter (CAP) and fibrosis via liver stiffness (LS). Demographic, lifestyle factors, anthropometry, clinical and medication history, host genetics, immune markers and hormone levels were collected as part of the WIHS.

**Findings to date:** At baseline, 737 of 1543 women with CAP measurements had steatosis (CAP≥248dB/m) and 375 of 1576 women with LS measurements had significant fibrosis (LS≥7.1kPa), yielding a prevalence of 48% and 24%, respectively. On multivariable analysis, waist circumference (WC) and insulin resistance were independently associated with higher CAP (17.8dB/m per 10 cm [95%CI:16.2,19.5] and 1.2dB/m per doubling [95%CI:0.8,1.6], respectively). By contrast, HIV/HCV seropositivity and HCV seropositivity alone were associated with less steatosis compared to HIV/HCV seronegative women, although the latter did not reach statistical significance (-9.2dB/m [95%CI:-18.2,-0.3] and -10.4dB/m [95%CI:-23.8,3.1], respectively). Factors independently associated with higher LS were age (4.4% per 10 years [95%CI:0.4%,8.4%], WC (5.0% per 10cm [95%CI:3.3%,6.6%], CAP-steatosis (0.6% per 10dB/m [95%CI:0.1%,1.0%]), HIV/HCV seropositivity (33% [95%CI:24%,44%]), and HCV seropositivity

alone (43% [95%CI:28%,60%]). Excluding scans that were invalid based on traditional criteria for unreliability did not affect the results.

**Future plans:** Enrolled women undergo VCTE at three-year intervals unless LS is ≥9.5 kPa, indicating advanced fibrosis, in which case VCTE is performed annually. Participants also undergo VCTE every 6 months until 18 months after HCV treatment initiation. Analysis of the data collected will provide insights into the impact of aging/ovarian function, host genetics, immune function, and contemporary HIV and HCV treatments on liver disease progression.

## ARTICLE SUMMARY

### Strengths and limitations of this study

- The Liver Disease and Reproductive Aging (LIVRA) study is designed to examine the contributions of HIV, hepatitis C virus (HCV), aging and ovarian function, immune function, host genomics, and contemporary antiviral treatments on hepatic steatosis (or fatty liver) and fibrosis progression measured via longitudinal vibration controlled transient elastography (VCTE).
- The study leverages the infrastructure of the decades-long multi-center prospective Women's Interagency HIV Study (WIHS), which collects biological specimens, socio-demographic data, lifestyle (substance use, food insecurity, and physical activity) factors, anthropometry, clinical and medication history, host genetics, immune markers and hormone levels.
- A rigorous VCTE Quality Assurance program was developed to ensure consistency and accuracy of hepatic steatosis and fibrosis assessments.
- Though limited to women, the LIVRA VCTE protocol is currently being implemented in men enrolled into the newly integrated MACS-WIHS Combined Cohort Study.
- The LIVRA study will allow investigation into the natural history of and potentially modifiable risk factors for hepatic steatosis and fibrosis in women with and without HIV.

## INTRODUCTION

Liver disease is a leading cause of morbidity and mortality among persons living with HIV (PLWH)[1]. However, the determinants of hepatic steatosis (fatty liver) and fibrosis progression among women living with HIV in the modern era of HIV and hepatitis C virus (HCV) treatment are not well known. Seminal fatty liver disease cohort studies have excluded PLWH and those with cleared HCV[2,3], and studies of hepatic steatosis in PLWH are often small in sample size. Moreover, the vast majority of studies of hepatic steatosis among PLWH have been conducted in men even though women represent over 50% of all PLWH worldwide[4] and close to 25% of all PLWH in the United States (US)[5].

In HIV-seronegative populations, women have a lower overall risk of steatosis than men but a higher risk of advanced fibrosis once steatosis is established, particularly after age 50[6]. Understanding the factors associated with steatosis and its impact on fibrosis among women in general, and especially in those living with HIV, is of essential importance. The Liver Disease and Reproductive Aging (LIVRA) study was designed as an ancillary study of the Women's Interagency HIV Study (WIHS) to evaluate the contributions of HIV, HCV, and reproductive aging to steatosis and fibrosis progression as measured by longitudinal vibration controlled transient elastography (VCTE) among a large cohort of women living with or without HIV.

## COHORT DESCRIPTION

### Study Population and Design

The WIHS (now part of the MACS-WIHS Combined Cohort Study[7] [MWCCS]) was a multicenter prospective cohort study established to investigate the course of HIV and associated conditions

among women living with and without HIV. The WIHS enrolled 4,982 women (3,678 living with HIV infection and 1,304 living without HIV infection) during four recruitment waves: 1994-1995, 2001-2002, 2011-2012, and 2013-2015 from 10 U.S. cities (Bronx and Brooklyn, NY; Chicago, IL; San Francisco, CA; Los Angeles, CA; Washington D.C.; Atlanta, GA; Chapel Hill, NC; Miami, FL; Jackson, MS; and Birmingham, AL). Full details of recruitment, retention, and demographics have been published previously.[8] Eligibility criteria varied with each wave of enrollment mainly to recruit women with earlier HIV disease stage and who had never taken older antiretroviral drugs associated with increased metabolic perturbations during later waves (i.e., didanosine and stavudine). Eligible women had documentation of reactive anti-HIV serology and if positive, a confirmatory test or if they were HIV-seronegative had risk factor(s) for HIV exposure. Baseline sociodemographic characteristics and HIV risk factors were similar between HIV-seropositive and HIV-seronegative women. At semi-annual research visits, participants completed a physical examination, provided biological specimens (e.g. serum, plasma, peripheral blood mononuclear cells, urine), and completed interviewer-administered questionnaires to obtain information regarding socio-demographics, lifestyle data (e.g. substance use, food security, physical activity), and clinical, psychosocial, and medication history. Host genetic data and genome-wide association studies are also available in the WIHS, as are markers of immune activation and inflammation and hormone levels.

From December 2013 to December 2018, 1576 of the approximately 2100 active WIHS women were enrolled into the LIVRA ancillary study. Eligibility included age 35 to 60 years old for women without chronic HCV. All women with chronic HCV infection were eligible for enrollment, regardless of age, as long as they were not currently receiving anti-HCV therapy. Women with positive hepatitis B surface antigen, hemochromatosis, autoimmune hepatitis or primary biliary cholangitis were excluded, as were women who reported using medications associated with

steatosis (i.e. systemic corticosteroids, amiodarone, methotrexate), signs of decompensated cirrhosis, current cancer, or severe renal insufficiency. Women who were pregnant or had an implantable cardiac device were excluded per the VCTE manufacturer (Fibroscan®; Echosens, Paris, France).

As part of the study protocol, longitudinal VCTE examinations are performed at three-year intervals after their baseline measurement unless liver stiffness (LS) values are ≥9.5 kilopascals (kPa), consistent with advanced fibrosis, in which case VCTE are performed annually. Additionally, participants who report HCV treatment after enrollment undergo VCTE every 6 months until 18 months after HCV treatment initiation. The study was approved by the Institutional Review Boards (IRB) of all participating sites, and all participants provided written informed consent to undergo longitudinal VCTE exam during the WIHS study visit. Each participating institution's IRB has been formally designated to review and monitor biomedical research involving human subjects, with the primary responsibility being the protection of subjects from undue risk and from deprivation of personal rights and dignity, which are the touchstones of ethical research.

**VCTE Performance and Quality Assurance**

VCTE reliability is operator-dependent[9], and therefore proper operator training and experience is important, especially in multicenter studies involving multiple operators. VCTE operators at each site were first trained by Echosens to: 1) operate the device, 2) obtain at least 10 measurements that the device deemed as valid per scan, and 3) recognize images that the

device deemed valid but required manual adjustment of probe placement by the operator due to interference from vessel, breathing, or lung, and not being completely in the liver (e.g. probe placed too low or needing to switch to the XL probe due to subcutaneous fat interference). Operators at each WIHS site were then certified by Echosens after demonstrating proficiency in scanning 3 to 5 volunteers.

A Quality Assurance (QA) program was implemented which required operators to complete at least 50 additional training examinations on subjects with a wide range of body types and within the age range of eligible participants. This benchmark was selected because a large multicenter study indicated that at least 50 examinations were needed before an operator was considered proficient to consistently acquire valid scans[10]. Each of the first 25 examinations was reviewed by the QA team, which was comprised of two clinicians and an experienced VCTE operator (Table 1). Upon completion of the review, a written report was provided with the additional option of individualized verbal feedback or an in-person hands-on training and observation. A similar procedure was followed for the next 25 examinations. After completion of 50 training examinations, the final report indicated whether: 1) the operator was certified; 2) additional examinations were needed and/or 3) a site visit for in-person training was warranted. Twenty-nine operators submitted a median of 55 scans (interquartile range [IQR] 51,62) for review before being certified. To maintain certification, operators are required to perform at least 20 examinations per year.

**Assessment of Hepatic Steatosis and Fibrosis**

Hepatic steatosis was estimated in decibels/meter (dB/m) using the VCTE-controlled attenuation parameter (CAP) software and fibrosis was estimated using LS measurements in

kPa. Participants were instructed to fast for at least three hours prior to VCTE. Operators were instructed to manually switch from the M probe to the XL probe if images suggested subcutaneous fat interference in the measurement range of the probe, i.e. >2.5 cm distance from skin to liver capsule[11]. All examinations had at least 10 successful measurements. At the time of analyses, examinations were flagged as having abnormal validity if they had an IQR/median LS ratio >30% and/or success rate <60% as per prior published criteria[12], or poor image quality. The QA team manually reviewed scan images and data on all VCTE examinations with LS≥9.3 kPa, a random selection of 10% of examinations with LS<9.3 kPa, and those with CAP<150 dB/m. This latter criterion was established after image review suggested that some scans with very low CAP values, including those with the lower bound CAP value of 100 dB/m, may partly capture data from outside of the liver.

Of the 1576 VCTE's performed, 56 had an IQR/median >30% (3.6%), 129 had a success rate <60% (8.2%), and 49 had poor image quality on review (3.1%). These categories were not mutually exclusive; 193 had at least one of these indices (12%). Due to an early technical error with the VCTE device, CAP values were missing for 33 women. Steatosis was defined by CAP≥248 dB/m, which was the optimal cut-off for selecting mild or greater steatosis in a meta-analysis including data from 2735 patients with liver biopsies[13] and was 83% sensitive and 72% specific in detecting mild or greater steatosis in a subset of our cohort when compared to magnetic resonance spectroscopy (MRS)-measured liver fat fraction[14]. The following fibrosis categories were used: significant fibrosis (LS≥7.1 kPa), advanced fibrosis (LS≥9.5 kPa), and cirrhosis (LS≥12.5 kPa)[15].

**Covariates**

Race/ethnicity was self-reported as White, Black, or Hispanic (including White and Black Hispanic). Menopause stage was determined based on the Stages of Reproductive Aging Workshop criteria[16]. Self-reported alcohol consumption was categorized as: none; light (>0-7 drinks/week); moderate (7-12 drinks/week); or heavy (>12 drinks/week). Additional demographic and behavioral covariates were obtained through self-report. Body mass index (BMI) was calculated in $kg/m^2$, waist circumference was measured in cm, and the homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using 8-hour fasting insulin and glucose values.

HIV and HCV seropositivity were defined by documentation of a reactive HIV enzyme immunoassay and reactive second or third-generation HCV enzyme immunoassay, respectively). At WIHS study entry, women underwent testing for HCV antibody (Ab) with HCV RNA if positive. If negative, HCV Ab testing was repeated at last WIHS study visit. For those who became HCV Ab positive, additional retrospective HCV Ab testing was performed to determine when HCV incidence occurred, and then HCV RNA testing was performed at least 2 years later to determine whether they had spontaneously cleared or had chronic infection. For all chronic infections, HCV RNA testing was performed at later visits to determine which had cleared following treatment. HCV RNA was performed using either the COBAS Amplicor Monitor 2.0 or the COBAS Taqman assay, as previously described (both from Roche Diagnostics, Branchburg, New Jersey, USA)[17]. HCV genotyping was conducted on a subset of HCV viremic women using the NC TRUGENE HCV 5′ NC Genotyping Kit (Bayer HealthCare LLC, Tarrytown, NY), as previously described[18].

## Statistical Analysis

For the purposes of this analysis, HIV and HCV infection categories were defined by serostatus. We compared participant characteristics by infection category using ANOVA or Kruskal-Wallis tests for continuous variables and Chi-square or Fisher's exact tests for categorical variables, as appropriate. We used unadjusted and multivariable adjusted linear regression models to examine associations with CAP and LS. LS had a right-skewed distribution and therefore was log-transformed to normalize its distribution, and the regression coefficients and their confidence intervals (CI) were exponentiated to calculate percentage differences attributable to each covariate. These analyses were performed for all women with successful VCTE's, and sensitivity analyses were performed excluding the scans with IQR/median >30%, success rate <60%, poor image quality on QA review, or any one of these criteria. In linear regression models where there were missing covariate information, we used the full information maximum likelihood approach in the setting of path analysis instead of the multiple imputation approach for its efficiency[19]. All analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC).



## Baseline characteristics

Among the 1576 WIHS women enrolled in LIVRA, 77 had HCV monoinfection, 248 HIV/HCV coinfection, 868 HIV infection alone, and 383 neither infection (Table 2). About three quarters were Black, and median age was 51 years (IQR 44,56), with 31% premenopausal, 13% perimenopausal, and 56% postmenopausal. Nearly half reported an annual household income of ≤$12,000, and one-third of the cohort completed less than a high school education. Most women with HIV were taking ART and had undetectable HIV RNA. Among the 325 women with

HCV seropositivity, 136 were HCV viremic at the baseline LIVRA visit (42%), 98 had cleared HCV with treatment (30%), and 91 had cleared HCV spontaneously (28%).

## PATIENT AND PUBLIC INVOLVEMENT

Participants of the LIVRA Study were not involved in the development of the study question or the liver disease outcomes. However, the national community advisory board of the WIHS (now MWCCS) approved the study of liver disease progression using VCTE as a high scientific priority.

## FINDINGS TO DATE

At baseline, women with neither HIV nor HCV had higher median CAP values than women with HCV monoinfection (247 dB/m [IQR 204, 281] vs 232 dB/m [IQR 191, 285]), and women with HIV monoinfection had higher values than women with HIV/HCV coinfection (246 dB/m [IQR 208, 290] vs 237 dB/m [IQR 200, 272]). Similarly, the prevalence of hepatic steatosis based on CAP ≥248 dB/m was highest in women with neither infection (50%), followed by those with HIV monoinfection (49%), HIV/HCV coinfection (43%), and HCV monoinfection (38%) (Figure 1a). The overall prevalence of steatosis was 48%, which is slightly higher than the 43% prevalence observed among women in the 2017-2018 NHANES study[20], which used a CAP cut-off of 263 dB/m, and higher than the 39% reported in a retrospective study of 1511 PLWH from Canada and Italy, which used the same 248 dB/m CAP cut-off as our study[21]. The latter study included mostly White men, whereas our study included mostly Black women, a group that has traditionally been found to have lower risk of hepatic steatosis.

On multivariable analysis, greater waist circumference (a marker of visceral obesity) and greater HOMA-IR were significantly associated with higher CAP values (Table 3). Our findings are consistent with other studies that have demonstrated visceral adiposity and insulin resistance to be key mediators in the pathogenesis of fatty liver disease[22,23]. However, HIV infection was not associated with CAP, suggesting that the clinical phenotype of fatty liver disease in PLWH is predominantly caused by metabolic dysregulation that appears to be independent of HIV infection. By contrast, HCV seropositivity was associated with significantly lower CAP values in analyses adjusted for alcohol use, smoking status, and menopause stage. This is consistent with our prior finding that non-genotype 3 HCV infection was associated with significantly lower magnetic resonance spectroscopy-measured liver fat fraction among 356 men and women with or without HIV and HCV[24]. Further research on the potential mechanisms underlying this observation as well as the impact of HCV clearance on fatty liver disease are warranted.

As expected, women with HCV had higher median LS values compared to women without HCV, with the highest LS observed among those with HCV monoinfection (6.9 kPa [IQR 5.0, 10.7]), followed by those with HIV/HCV coinfection (6.2 kPa [IQR 4.6, 9.1]), HIV monoinfection (5.0 kPa [IQR 3.9, 6.7]) and those with neither infection (4.9 kPa [IQR 3.9, 6.2]). Prevalence of significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa), and cirrhosis (LS ≥12.5 kPa) was also higher among women with HCV compared to those without HCV (Figure 1b). In multivariable analysis, HIV/HCV coinfection, HCV monoinfection, older age, greater waist circumference, and greater CAP values were each independently associated with higher LS (Table 3). These findings have important implications for women as visceral adiposity and metabolic abnormalities increase during the menopausal transition, independent of chronologic age[25]. Estrogen depletion occurs during the late phase of perimenopause and is associated with increased levels of inflammatory cytokines[26] and mucosal barrier dysfunctions[27]. These changes

may result in increased gut microbial translocation and inflammation[28], factors that have also been linked with steatosis and fibrosis[29,30]. Studies are underway evaluating the impact of ovarian follicular reserve on steatosis and fibrosis among LIVRA participants.

In analysis restricted to women living with HIV, HIV related factors such as CD4 count, HIV RNA, and ART regimen, were not significantly associated with CAP or LS on cross-sectional analysis. However, the potential impact of ART class, including integrase strand transfer inhibitors, on incident steatosis and fibrosis will be an important area of research within the cohort prospectively. Finally, a notable finding was that the factors associated with steatosis and fibrosis were similar after excluding VCTE examinations with IQR/median >30%, success rate <60%, poor image quality, or any of these three criteria (Supplemental Tables 1 and 2). In the setting of a large multicenter epidemiologic study, these findings provide assurance about the rigor of our data collection approach. In clinical practice, currently accepted VCTE validity criteria should be adhered to.

## STRENGTHS AND LIMITATIONS

To our knowledge, this is the most comprehensive cohort study evaluating liver disease among US women using VCTE and the largest study of VCTE in women living with HIV. However, our cohort has limitations. First, there are demographic differences by HIV and HCV serostatus, and thus unmeasured confounders may remain despite adjustment for multiple variables. Second, we rely on VCTE to assess liver disease, rather than liver biopsy. However, liver biopsy is infeasible in large cohorts such as ours. Finally, our findings may not be generalizable to men, but our VCTE protocol is currently being implemented in men enrolled in the newly integrated MWCCS allowing us to address sex differences.

A major strength of the LIVRA study is that it is conducted in a large, nationally representative cohort of women living with HIV in the US[31] where racial and ethnic minorities, people living below the poverty line, and those with less than a high school education are at higher risk for liver disease[32]. Second, LIVRA has detailed longitudinal HIV and HCV data allowing the study of liver steatosis and fibrosis trajectories in women with active HCV viremia, spontaneous HCV clearance, and treated HCV clearance. Third, the inclusion of women with and without HIV and HCV infections will allow us to evaluate the association of viral factors with liver disease utilizing seronegative controls. Finally, due to our rigorous QA program, a low proportion of VTCE scans were invalid based on traditional criteria for unreliability and excluding these scans did not impact our results. With longitudinal assessments, the LIVRA study will enable evaluation of hepatic steatosis and fibrosis progression in women and support investigation of mechanistic pathways and potentially modifiable risk factors for liver disease in high-risk women.

## COLLABORATION

The datasets generated and analyzed during the current study are not publicly available but are available from the principal investigator of the LIVRA Study on reasonable request and upon approval by the Executive Committee of the MACS-WIHS Combined Cohort Study. Please see https://statepi.jhsph.edu/mwccs/work-with-us/ for further information on how to work with our cohort.

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**Table 1. LIVRA Study Quality Assurance Program for VCTE Operator Training Certification**

| Exams | Guidelines | Training Goals |
|---|---|---|
| 1-25 | **Subjects**: Varied body types and ages, discrete subjects<br>**Parameter goals to work toward**: IQR<30% of median, >60% of measurements deemed valid by device<br>REQUIRED: 10 valid measurements obtained per participant | • Get comfortable with positioning<br>• Familiarize with Fibroscan® screen and available tools; Observe how age and abdominal fat affect Fibroscan® acquisition<br>• Work on consistently achieving valid assessments |
| Scans are reviewed by QA team and operator receives written feedback report. Check-in call and additional in-person training optional. | | |
| 26-50 | **Subjects**: Varied body types and ages, discrete subjects<br>**Parameters**: REQUIRED: IQR<30% of median, >60% of measurements deemed valid by device, 10 valid measurements per participant | • Focus on achieving valid assessments consistently<br>• Utilize deletion to meet parameter requirements |
| Scans are reviewed by QA team and operator receives written feedback report. Additional examinations and site visit may be required prior to certification. | | |
| Maintenance certification:<br>• Operator must perform at least 20 examinations per year<br>• Ongoing written feedback provided for studies reviewed by QA team | | |

**Table 2.** Characteristics of the study population by HIV and HCV antibody status.

| Median (IQR) or % | HIV-/HCV+ (n=77) | HIV+/HCV+ (n=248) | HIV+/HCV- (n=868) | HIV-/HCV- (n=383) | p-value |
|---|---|---|---|---|---|
| **Sociodemographic** | | | | | |
| Age (years) | 56 (49, 60) | 56 (53, 60) | 49 (43, 54) | 47 (41, 54) | <0.001 |
| Race/Ethnicity | | | | | 0.001 |
| Black | 64% | 67% | 76% | 76% | |
| White | 7% | 15% | 9% | 7% | |
| Hispanic | 22% | 17% | 11% | 12% | |
| Other | 7% | 2% | 4% | 5% | |
| Menopause stage | | | | | <0.001 |
| Premenopause | 16% | 7% | 33% | 44% | |
| Perimenopause | 13% | 8% | 14% | 14% | |
| Postmenopause | 71% | 85% | 53% | 43% | |
| Household income | | | | | |
| <$6,000 | 29% | 16% | 12% | 19% | <0.001 |
| $6,000-$12,000 | 44% | 49% | 35% | 26% | |
| $12,001-$36,000 | 7% | 13% | 15% | 13% | |
| >$36,000 | 9% | 9% | 17% | 23% | |
| Education | | | | | |
| Less than high school | 43% | 39% | 31% | 32% | 0.02 |
| High school | 35% | 29% | 34% | 28% | |
| More than high school | 22% | 32% | 35% | 41% | |
| **Lifestyle** | | | | | |
| Alcohol use | | | | | <0.001 |
| None | 51% | 63% | 51% | 42% | |
| Light | 27% | 25% | 40% | 42% | |
| Moderate | 4% | 3% | 3% | 4% | |
| Heavy | 18% | 8% | 6% | 12% | |
| Current smoking | 71% | 54% | 35% | 42% | <0.001 |
| Current marijuana use | 30% | 26% | 20% | 27% | 0.05 |
| Ever injection drug use | 36% | 23% | 1% | 4% | <0.001 |
| **Metabolic** | | | | | |
| BMI (kg/m$^2$) | 30 (25, 36) | 28 (23, 32) | 31 (26, 36) | 32 (27, 38) | <0.001 |
| Waist Circumference (cm) | 99 (88, 115) | 97 (86, 108) | 99 (89, 111) | 100 (88, 113) | 0.12 |
| HOMA-IR | 3.2 (1.6, 6.8) | 2.48 (1.3, 4.9) | 2.1 (1.3, 3.8) | 1.8 (1.1, 3.3) | <0.001 |
| **Liver-related** | | | | | |
| AST (U/L) | 26 (19, 37) | 24 (18, 35) | 19 (15, 23) | 17 (14, 20) | <0.001 |
| ALT (U/L) | 23 (15, 34) | 18 (12, 30) | 15 (12, 21) | 14 (11, 18) | <0.001 |
| **HIV-related** | | | | | |
| Undetectable HIV RNA | | 69% | 72% | | 0.60 |

| | | | |
|---|---|---|---|
| CD4 current (cells/mm$^3$) | 616 (428, 869) | 656 (435, 870) | 0.35 |
| CD4 nadir (cells/mm$^3$) | 215 (107, 320) | 222 (103, 361) | 0.95 |
| History of clinical AIDS | 42% | 28% | <0.001 |
| Current ART | 89% | 89% | 0.81 |
| NNRTI | 30% | 29% | 0.84 |
| PI | 26% | 25% | 0.80 |
| INSTI | 44% | 48% | 0.22 |
| EI | 1.6% | 0.8% | 0.26 |

NNRTI= non-nucleoside reverse transcriptase inhibitor; PI= protease inhibitor; INSTI= Integrase strand transfer inhibitor; EI= entry inhibitor

**Figure 1a.** Prevalence of VCTE-estimated hepatic steatosis (CAP≥248 dB/m) by HIV and HCV antibody status. **1b.** Prevalence of VCTE-estimated significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa), and cirrhosis (LS ≥12.5 kPa), as estimated by VCTE-measured liver stiffness, by HIV and HCV antibody status.

**Table 3. Independent associations of clinical and demographic characteristics with CAP and LS ***

| | CAP difference (dB/m) (CI)** | p-value | Liver stiffness % change (CI)*** | p-value |
|---|---|---|---|---|
| Infection Status (ref=HIV-/HCV-) | | | | |
| HIV+/HCV- | 1.1 (-5.3, 7.5) | 0.74 | 4.4% (-1.1%, 10.3%) | 0.12 |
| HIV+/HCV+ | -9.2 (-18.2, -0.3) | 0.04 | 33.4% (23.5%, 44.1%) | <0.001 |
| HIV-/HCV+ | -10.4 (-23.8, 3.1) | 0.13 | 43.2% (27.8%, 60.4%) | <0.001 |
| Age (per 10 years) | 2.4 (-2.0, 6.9) | 0.29 | 4.4% (0.4%, 8.4%) | 0.03 |
| Race/Ethnicity (ref=white non-Hispanic) | | | | |
| Black | -6.1 (-14.2, 2.1) | 0.14 | 2.8% (-4.0%, 10.1%) | 0.43 |
| Hispanic | -3.1 (-12.6, 6.4) | 0.52 | 3.1% (-5.0%, 11.7%) | 0.47 |
| Other | 2.9 (-8.2, 14.0) | 0.61 | 2.5% (-6.7%, 12.7%) | 0.60 |
| Waist circumference (per 10 cm) | 17.8 (16.2, 19.4) | <0.001 | 5.0% (3.3%, 6.6%) | <0.001 |
| HOMA-IR (per doubling) | 1.2 (0.8, 1.6) | <0.001 | 0.3% (-0.1%, 0.7%) | 0.11 |
| CAP (per 10 dB/m) | | | 0.6% (0.1%, 1.0%) | 0.01 |

*All variables shown in the table are included in a single multiple linear regression model (one model for CAP and one for LS). Also adjusted for alcohol use, smoking status, and menopause stage (parameter estimates not shown).
**N=1543
***N=157

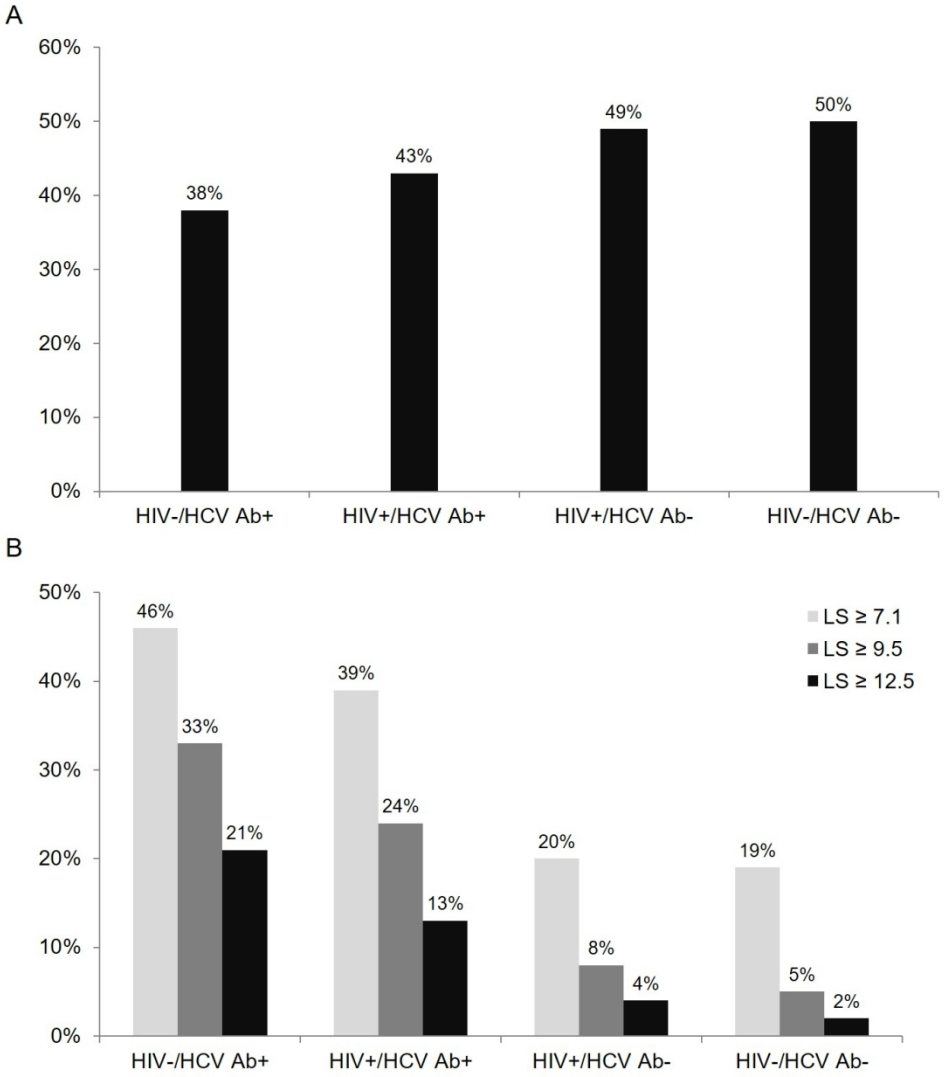


Figure 1A Prevalence of VCTE-estimated hepatic steatosis (CAP≥248 dB/m) by HIV and HCV antibody status. 1B Prevalence of VCTE-estimated significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa), and cirrhosis (LS ≥12.5 kPa), as estimated by VCTE-measured liver stiffness, by HIV and HCV antibody status.

247x269mm (150 x 150 DPI)

**Supplemental Table 1. Factors associated with CAP in multivariable analysis*, excluding VCTE scans with abnormal validity criteria.**

| | Excluding if IQR/Med >30% (N=1520) | | Excluding success rate <60% (N=1447) | | Excluding poor image quality (N=1527) | | Excluding any abnormal validity criteria (N=1383) | |
|---|---|---|---|---|---|---|---|---|
| | CAP difference (dB/m) | p-value | CAP difference (dB/m) | p-value | CAP difference (dB/m) | p-value | CAP difference (dB/m) | p-value |
| Infection Status (ref=HIV-/HCV-) | | | | | | | | |
| HIV+/HCV- | 0.3 (-6.2, 6.8) | 0.92 | -1.1 (-7.7, 5.5) | 0.74 | 1.4 (-4.8, 7.6) | 0.65 | -0.2 (-6.7, 6.3) | 0.95 |
| HIV-/HCV+ | -11.7 (-25.3, 2) | 0.09 | -16.6 (-30.5, -2.7) | 0.02 | -11.2 (-24, 1.7) | 0.09 | -17.8 (-31.5, -4.1) | 0.01 |
| HIV+/HCV+ | -8.4 (-17.5, 0.7) | 0.07 | -12.1 (-21.2, -3) | 0.01 | -8.5 (-17.2, 0.2) | 0.06 | -9.5 (-18.5, -0.5) | 0.04 |
| Age (per 10 years) | 2.4 (-2.2, 6.9) | 0.31 | 1.6 (-3, 6.2) | 0.50 | 1.3 (-3.1, 5.6) | 0.56 | 0.9 (-3.7, 5.5) | 0.70 |
| Race/Ethnicity (ref=white non-Hispanic) | | | | | | | | |
| Black | -5.6 (-13.9, 2.7) | 0.18 | -8.2 (-16.5, 0) | 0.05 | -5.7 (-13.5, 2.2) | 0.16 | -7.3 (-15.5, 0.9) | 0.08 |
| Hispanic | -4.2 (-13.8, 5.4) | 0.40 | -4.4 (-13.9, 5) | 0.36 | -3.6 (-12.7, 5.6) | 0.45 | -4.9 (-14.2, 4.5) | 0.31 |
| Other | 4.4 (-6.8, 15.6) | 0.44 | 3.6 (-7.5, 14.7) | 0.53 | 2.9 (-7.8, 13.5) | 0.60 | 4.6 (-6.3, 15.6) | 0.40 |
| WC (per 10 cm) | 17.8 (16.2, 19.5) | <0.001 | 17.5 (15.9, 19.2) | <0.001 | 17.8 (16.3, 19.4) | <0.001 | 17.6 (15.9, 19.2) | <0.001 |
| HOMA-IR (per doubling) | 1.2 (0.8, 1.6) | <0.001 | 1.5 (1, 2) | <0.001 | 1.2 (0.8, 1.5) | <0.001 | 1.5 (1, 2) | <0.001 |

*Also adjusted for alcohol use, smoking status, and menopause stage

**Supplemental Table 2. Factors associated with liver stiffness in multivariable analysis, excluding VCTE scans with abnormal validity criteria.**

| | Excluding if IQR/Med >30% (N=1520) | | Excluding success rate <60% (N=1447) | | Excluding poor image quality (N=1527) | | Excluding any abnormal validity criteria (N=1383) | |
|---|---|---|---|---|---|---|---|---|
| | LS % change | p-value | LS % change | p-value | LS % change | p-value | LS % change | p-value |
| Infection Status (ref=HIV-/HCV-) | | | | | | | | |
| HIV+/HCV- | 4.4% (-1.2%, 10.3%) | 0.12 | 4.5% (-1.2%, 10.5%) | 0.12 | 4.1% (-1.4%, 9.9%) | 0.14 | 5.2% (-0.6%, 11.3%) | 0.08 |
| HIV-/HCV+ | 44.2% (28.6%, 61.6%) | <0.001 | 45.1% (29%, 63.1%) | <0.001 | 44.5% (29.3%, 61.5%) | <0.001 | 46.8% (30.5%, 65.2%) | <0.001 |
| HIV+/HCV+ | 34.4% (24.3%, 45.2%) | <0.001 | 35.2% (25%, 46.2%) | <0.001 | 31.1% (21.5%, 41.6%) | <0.001 | 33.6% (23.4%, 44.6%) | <0.001 |
| Age (per 10 years) | 4.6% (0.6%, 8.7%) | 0.02 | 3.4% (-0.6%, 7.5%) | 0.10 | 3.3% (-0.6%, 7.3%) | 0.10 | 3.1% (-0.9%, 7.3%) | 0.13 |
| Race/Ethnicity (ref=white non-Hispanic) | | | | | | | | |
| Black | 2.2% (-4.7%, 9.6%) | 0.54 | 2.3% (-4.6%, 9.7%) | 0.52 | 1% (-5.7%, 8.1%) | 0.78 | 0.2% (-6.6%, 7.6%) | 0.95 |
| Hispanic | 3.7% (-4.5%, 12.4%) | 0.39 | 2.2% (-5.8%, 10.8%) | 0.60 | 1% (-6.8%, 9.4%) | 0.82 | 0.5% (-7.4%, 9.1%) | 0.90 |
| Other | 1.9% (-7.4%, 12%) | 0.70 | 2.8% (-6.5%, 13%) | 0.57 | 1.3% (-7.7%, 11.2%) | 0.79 | 1.5% (-7.7%, 11.6%) | 0.76 |
| WC (per 10 cm) | 4.4% (2.7%, 6.1%) | <0.001 | 3.8% (2.1%, 5.5%) | <0.001 | 4.4% (2.7%, 6.1%) | <0.001 | 3.4% (1.7%, 5.2%) | <0.001 |
| log2HOMA-IR | 0.3% (0%, 0.7%) | 0.08 | 0.1% (-0.4%, 0.5%) | 0.80 | 0.3% (-0.1%, 0.6%) | 0.14 | 0.1% (-0.4%, 0.5%) | 0.79 |
| CAP (per 10 dB/m) | 0.6% (0.1%, 1%) | 0.01 | 0.7% (0.2%, 1.2%) | 0.004 | 0.6% (0.1%, 1.1%) | 0.01 | 0.7% (0.2%, 1.2%) | 0.01 |

*Also adjusted for alcohol use, smoking status, and menopause stage

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 5 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 7 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 7 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 8 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 8 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 9-11 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 9-10 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 9 |
| Study size | 10 | Explain how the study size was arrived at | 8 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 12 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 11 |
| | | (*c*) Explain how missing data were addressed | 11 |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | |
| | | (*e*) Describe any sensitivity analyses | |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 13 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| | | (c) Summarise follow-up time (eg, average and total amount) | |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 13 |

| | | | |
|---|---|---|---|
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included<br>(*b*) Report category boundaries when continuous variables were categorized<br>(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 14<br><br><br><br>14 |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 13 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 15 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 3 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.