

Risk Factors Associated With Chronic Liver Enzyme Elevation in Persons With HIV Without Hepatitis B or C Coinfection in the Combination Antiretroviral Therapy Era

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Background. As morbidity due to viral coinfections declines among HIV-infected persons, changes in liver-related morbidity are anticipated. We examined data from the US Military HIV Natural History Study (NHS), a cohort of military beneficiaries, to evaluate incidence and risk factors associated with chronic liver enzyme elevation (cLEE) in HIV-monoinfected patients in the combination antiretroviral therapy (cART) era.

Methods. Participants who were hepatitis B virus and hepatitis C virus seronegative with follow-up after 1996 were included. We defined chronic liver enzyme elevation (cLEE) as alanine aminotransferase elevations ≥ 1.25 times the upper limit of normal on at least 2 visits, for a duration of ≥ 6 months within 2 years. We used multivariate Cox proportional hazards models to examine risk factors for cLEE.

Results. Of 2779 participants, 309 (11%) met criteria for cLEE for an incidence of 1.28/100 PYFU (1.28–1.29/100 PYFU). In an adjusted model, cLEE was associated with Hispanic/other ethnicity (reference Caucasian: hazard ratio [HR], 1.744; 95% CI, 1.270–2.395), non-nucleoside reverse transcriptase inhibitor-based cART (reference boosted protease inhibitors: HR, 2.232; 95% CI, 1.378–3.616), being cART naïve (HR, 6.046; 95% CI, 3.686–9.915), or having cART interruptions (HR, 8.671; 95% CI, 4.651–16.164). African American race (HR, 0.669; 95% CI, 0.510–0.877) and integrase strand transfer inhibitor (INSTI)-based cART (HR, 0.222; 95% CI, 0.104–0.474) were protective.

Conclusions. Our findings demonstrate that initiation and continued use of cART are protective against cLEE and support the hypothesis that HIV infection directly impacts the liver. INSTI-based regimens were protective and could be considered in persons with cLEE.

Keywords. ALT; ART; chronic liver enzyme elevation; hepatitis; HIV.

Combination antiretroviral therapy (cART) has transformed the lives of people with HIV (PWH), with life expectancy nearing that of the general population [1]. However, morbidity and mortality due to non-AIDS-related conditions continue [2, 3], with liver disease remaining a major contributor [2, 4, 5].

Even in the absence of hepatitis B (HBV) or hepatitis C (HCV) coinfection, abnormal liver-associated enzymes (LAEs)

are common in PWH, occurring in 40%–60% of persons on ART [6]. Viral hepatitis, excessive alcohol use, ART toxicity, and nonalcoholic fatty liver disease (NAFLD) can contribute to LAE abnormalities in PWH [7]. However, in clinical practice, the underlying cause of LAE elevations and the clinical significance are often difficult to discern, especially when elevations are mild [8] and hepatitis coinfections are absent.

While occasional mild to moderate LAE elevations may not alarm most practitioners, these abnormalities may be relevant. Alanine aminotransferase (ALT) abnormalities in HIV-monoinfected subjects are infrequently investigated (23% in 1 study, with only 6% undergoing assessment for hepatic fibrosis) [9]. However, high rates of histologic abnormalities, largely non-alcoholic steatohepatitis (NASH), were found in PWH with unexplained chronic transaminase elevations [10]. Furthermore, ALT has been associated with death from liver disease in persons without HBV and HCV [11].

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Historically, research on LAE elevations in PWH has focused on coinfection with HBV/HCV or extreme LAE elevations [6]. Few studies have examined the significance of modest elevations in LAEs in persons without viral hepatitis coinfections in the era of modern cART.

The goals of our study were to evaluate trends in ALT abnormalities, determine the incidence of chronic liver enzyme elevation (cLEE), and identify risk factors associated with cLEE in a large cohort of PWH without HBV or HCV coinfection. We also evaluated the association between cLEE and long-term outcomes.

METHODS

Study Population

The US Military HIV Natural History Study (NHS) is an ongoing, longitudinal, prospective observational cohort started in 1986 and comprised of US active duty military and Department of Defense (DoD) beneficiaries with HIV [12]. Study visits occur about once every 6 months. Participants undergo physical examination and coordinator interview, answer questionnaires, have medical records abstracted for clinical diagnosis, and have blood drawn for clinically relevant parameters including liver function tests.

For this analysis, participants were included if they contributed at least 6 months of follow-up between 1996 and 2017, did not have active HBV and/or HCV, and had normal alanine aminotransferase (ALT) at baseline (ie, at HIV diagnosis).

Patient Consent Statement

All participants provided written informed consent. This study was approved by the Institutional Review Board (IRB) of the Uniformed Services University of the Health Sciences (USU) and participating study sites.

Definitions

Serum ALT is a marker of potential liver injury and was used in our analysis as a surrogate marker of liver disease [7, 11]. Abnormal ALT was defined as $\geq 1.25\times$ the upper limit of normal (Grade 1 or higher), in accordance with NIH-NIAI guidelines [13]. Chronic liver enzyme elevation (cLEE) was defined as an abnormal ALT recorded on at least 2 visits, with intervals between elevations totaling at least 6 months within 2 years. This allowed for intermittent normal values between intervals of elevation. We grouped cART regimens based on the following anchor classes: unboosted protease inhibitor (PI), boosted PI, non-nucleoside reverse transcriptase inhibitor (NNRTI), integrase strand transfer inhibitor (INSTI), and other combinations. We defined treatment interruption as discontinuing treatment for ≥ 6 months.

We defined HBV infection as a positive hepatitis B surface antigen or detectable HBV DNA at any point. We did not exclude subjects with isolated positive hepatitis B core antibody

or resolved infection (ie, core and surface antibody positive). We required a positive HCV antibody or detectable HCV RNA at any point for a diagnosis of hepatitis C. Participants with resolved hepatitis C were not included.

For this study, trained clinical research coordinators perform chart reviews to capture and record protocol-specified diagnoses in the NHS database. We categorized participants as having hyperlipidemia if they had a diagnosis recorded in the NHS database or a low-density lipoprotein cholesterol (LDL) value ≥ 190 mg/dL. Hypertension and cirrhosis were based on the capture of a clinical diagnosis. We defined diabetes mellitus (DM) based on a recorded diagnosis, the use of antidiabetic medications, or laboratory findings suggestive of DM (ie, fasting blood glucose levels >126 mg/dL, random blood glucose >200 mg/dL or hgbA1C ≥ 6.5 , on 2 or more determinations). At-risk drinking was defined as the consumption of at least 3 or 4 drinks per day or 7 and 14 drinks per week among women and men, respectively, using criteria established by the National Institute for Alcohol Abuse and Alcoholism (NIAAA) [14].

Two indirect markers of liver fibrosis were utilized in our analyses, the aspartate transaminase (AST) to platelet ratio index (APRI) and the fibrosis-4 index (FIB-4). While traditionally used for predicting fibrosis in patients with HCV or HIV/HCV coinfection, the APRI may also be useful for predicting NAFLD fibrosis [15]. The FIB-4 index was developed to stage liver disease in persons with HIV/HCV coinfection, although it has been found to be superior to other noninvasive markers in the setting of NALFD [16, 17].

Statistical Analyses

Incidence of cLEE was defined as the number of patients with cLEE divided by the total number of person-years of follow-up time (PYFU). Characteristics between patients with and without cLEE were compared using the chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous variables. Medians and interquartile ranges (IQRs) are presented unless stated otherwise. We censored participants with cLEE at the time they first met criteria for cLEE and censored those without cLEE at their last visit. We analyzed the time to first event.

Risk factors for cLEE included demographics (eg, age, race) and HIV-specific factors (eg, CD4 count, viral load, and cART regimens). Age, CD4 count, and viral load were considered time-dependent covariates. Unadjusted and adjusted hazards ratios are reported with 95% CIs and *P* values. Risk factors significant in the univariate logistic model (listed in Table 1) were evaluated in unadjusted Cox models. Factors significant at a *P* value $<.05$ in the unadjusted Cox model were selected for evaluation in an adjusted Cox model. We took collinearity into consideration in model construction.

As older versions of the study had not required it, body mass index (BMI), hypertension, DM, and hyperlipidemia were not consistently captured until 2006; hence we conducted sensitivity analyses restricted to participants with BMI at HIV diagnosis and participants diagnosed with HIV after 2006 to examine the effects of these risk factors.

Additionally, due to the established hepatotoxicity associated with older antiretrovirals [4, 8, 18], we also performed a sensitivity analysis grouping participants into the following treatment categories: treatment naïve; treatment containing stavudine (D4T), didanosine (ddI), or zalcitabine (ddC), hereafter referred to as “D-drugs”; and all other treatments. As treatment is recommended universally, to better define the role of

cART use and cLEE, we also analyzed data beginning at cART initiation and treated cART as a time-updated variable. All statistical analyses were performed using SAS, version 9.3 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Subject Characteristics

Overall, 2779 participants without HBV or HCV coinfection who had normal baseline ALT were included (Figure 1). The study population largely consisted of younger men (93%; 27.7 [23.7–33.7] years) and was racially diverse, with African American [AA] and Hispanic/other comprising 45% and 15% of the population, respectively. Most participants were diagnosed with HIV

Table 1. Pertinent Participant Characteristics Categorized by the Presence or Absence of Chronic Liver Enzyme Elevation

Variable	All Participants n = 2779	Chronic Liver Enzyme Elevation		P
		No n = 2470	Yes n = 309	
Sex				.3342
Male	2580 (92.8)	2289 (92.7)	291 (94.2)	
Ethnicity/race				.0005
Caucasian	1092 (39.3)	961 (38.9)	131 (42.4)	
African American	1260 (45.3)	1148 (46.5)	112 (36.2)	
Hispanic/other	427 (15.4)	361 (14.6)	66 (21.4)	
Age at HIV diagnosis, y	27.7 [23.7–33.7]	27.6 [23.5–33.5]	29.2 [25.0–35.3]	.0012
Year of HIV diagnosis				<.0001
Before 1996	907 (32.6)	755 (30.6)	152 (49.2)	
1996–2000	350 (12.6)	306 (12.4)	44 (14.2)	
2001–current	1522 (54.8)	1409 (57.0)	113 (36.6)	
Age at cART initiation, y ^a	31.5 [26.2–38.1]	31.0 [25.8–37.8]	34.2 [29.0–40.0]	<.0001
Year of cART initiation ^a				<.0001
Before 1996	8 (0.3)	5 (0.2)	3 (1.0)	
1996–2000	895 (38.0)	730 (35.4)	165 (56.9)	
2001–current	1459 (62.0)	1334 (64.6)	125 (43.1)	
Time from HIV diagnosis to cART initiation, y ^a	1.1 [0.2–4.8]	1.0 [0.2–4.5]	3.4 [0.5–7.0]	<.0001
Hepatitis B status				<.0001
No HBV	1927 (69.34)	1749 (70.81)	178 (57.61)	
Resolved HBV	797 (28.68)	670 (27.13)	127 (41.10)	
Isolated hep B core	55 (1.98)	51 (2.06)	4 (1.29)	
CD4 count at HIV diagnosis, cells/μL ^b	483.0 [350.0–635.0]	488.0 [354.0–638.0]	440.0 [327.0–594.0]	.0114
CD4 count at cART initiation, cells/μL ^{a,c}	366.0 [257.0 – 495.0]	371.0 [264.0 – 509.0]	319.0 [193.0 – 435.0]	<.0001
Nadir CD4 count, cells/μL	302.0 [199.0–420.0]	311.0 [211.0–430.0]	236.0 [130.0–329.0]	<.0001
AIDS diagnosis before cART ^a	119 (5.0)	92 (4.4)	27 (9.2)	.0005
HIV RNA level at cART initiation, log ₁₀ copies/mL ^{a,d}	4.5 [3.9–5.0]	4.5 [3.9–5.0]	4.5 [3.9–5.0]	.8067
Peak HIV RNA level, log ₁₀ copies/mL	4.8 [4.3–5.2]	4.8 [4.3–5.1]	4.9 [4.5–5.4]	<.0001
First ART treatment				<.0001
ART naïve	264 (9.5)	260 (10.5)	4 (1.3)	
ART-containing D-drugs	233 (8.4)	199 (8.1)	34 (11.0)	
All other ART	2282 (82.1)	2011 (81.4)	271 (87.7)	
First cART ^a				<.0001
Unboosted PI	812 (34.4)	664 (32.1)	148 (50.5)	
Boosted PI	198 (8.4)	183 (8.8)	15 (5.1)	
NNRTI	970 (41.1)	858 (41.5)	112 (38.2)	
INSTI	325 (13.8)	318 (15.4)	7 (2.4)	

Table 1. Continued

Variable	All Participants n = 2779	Chronic Liver Enzyme Elevation		P
		No n = 2470	Yes n = 309	
All other cART	57 (2.4)	46 (2.2)	11 (3.8)	
Never on treatment at last follow-up ⁱ	264 (9.5)	260 (10.5)	4 (1.3)	<.0001
Never on ART (received ARV only) at last follow-up ⁱ	153 (5.5)	141 (5.7)	12 (3.9)	
Self-reported alcohol use ^{e,f,g}	1815 (65.3)	1575 (63.8)	240 (77.7)	.3348
Alcohol abuse ^{f,g}	207 (7.4)	175 (7.1)	32 (10.4)	.0390
Self-reported nonprescription drug abuse ^{f,g}	135 (4.9)	113 (4.6)	22 (7.1)	.0498
Hypertension ^{f,g}	711 (25.6)	581 (23.5)	130 (42.1)	<.0001
Hyperlipidemia ^{f,g}	1128 (40.6)	913 (37.0)	215 (69.6)	<.0001
Diabetes mellitus ⁱ	265 (9.5)	178 (7.2)	87 (28.2)	<.0001
Statin use ^{f,g,h}	534 (19.2)	411 (16.6)	123 (39.8)	<.0001
Time from HIV diagnosis to cLEE, y ^j	6.4 [3.1–11.8]	6.8 [3.2–12.4]	4.8 [2.3–7.7]	<.0001
Time from HIV diagnosis to last visit, y	7.5 [3.5–14.0]	6.8 [3.2–12.4]	15.8 [9.2–22.8]	<.0001
Frequency of ALT measurements, before cLEE/last visit, mo	5.23 [3.33–7.93]	5.67 [3.87–8.28]	1.61 [0.76–3.03]	<.0001

Bold text indicates a P value less than 0.05. No. (%) for categorical variables, median [Q1–Q3] for continuous variables. For categorical variables, the “missing” category was not considered for the percentage and P value calculations.

Abbreviations: ALT, alanine aminotransferase; ART, antiretroviral therapy; ARV, antiretroviral; BMI, body mass index; cART, combination antiretroviral therapy; cLEE, chronic liver enzyme elevation; D-drugs: stavudine (D4T), didanosine (ddI), or zalcitabine (ddC); HBV, hepatitis B virus; INSTI, integrase strand transfer inhibitor; NNRT, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

^aIncludes only participants who received cART (n = 2362).

^bMissing data, n = 2528.

^cMissing data, n = 2567.

^dMissing data, n = 2549.

^eMissing data, n = 1916.

^fAt any point in time during the study period.

^gVariable not routinely collected before 2006.

^hMissing data, n = 2747.

ⁱData for participants without cLEE were obtained from the last visit.

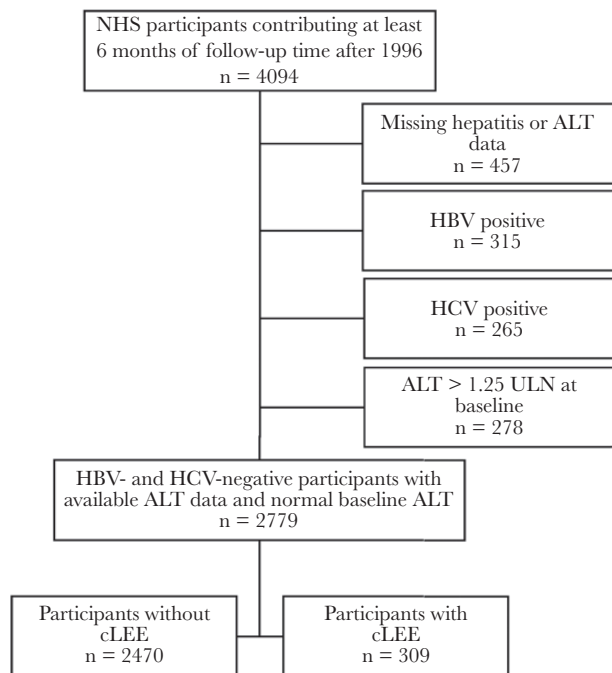


Figure 1. Flowchart of included participants. Abbreviations: ALT, alanine aminotransferase; cLEE, chronic liver enzyme elevation; HBV, hepatitis B virus; HCV, hepatitis C virus; NHS, US Military HIV Natural History Study.

and initiated cART after the year 2000: 55% and 62%, respectively. The median CD4 count at HIV diagnosis (IQR) was 483 (350–635) cells/ μ L, and the median nadir CD4 count (IQR) was 302 (199–420) cells/ μ L. The median time from HIV diagnosis to initiation of cART (IQR) was 1.1 (0.2–4.8) years. Most participants initiated treatments without “D-drugs” (82%), with NNRTI-based regimens being the most common cART (41%). Efavirenz was the most common anchor NNRTI drug. Of the 138 participants with cLEE and exposure to an NNRTI before the onset of cLEE, 119 (86%) had received efavirenz, 19 (14%) nevirapine, and 9 (6.5%) rilpivirine. The prevalence of hypertension, hyperlipidemia, and DM during the study period was 26%, 41%, and 10%, respectively, with 19% of the population ever on a statin. Most participants (65%) reported some alcohol use, with 7% meeting criteria for alcohol abuse. Nonprescription drug abuse was reported by 5% of participants (Table 1).

Comparisons of Participants With and Without cLEE

Participants with cLEE differed from those without cLEE in several aspects, notably ethnicity/race and age (Table 1). Participants with cLEE were more likely to be Caucasian (42.4% vs 38.9%) or Hispanic/other (21.4% vs 14.6%) and were older at both HIV diagnosis (29.2 vs 27.6 years) and

cART initiation (34.2 vs 31.0 years) compared with participants without cLEE. They were more often diagnosed with HIV before the year 2000 (63.4% vs 43%) and, in keeping with this, had a longer time to cART initiation from HIV diagnosis (3.4 vs 1.0 years) and were more likely to initiate treatment with “D-drugs” (11% vs 8.1%) or unboosted PIs (50.5% vs 32.1%). Hypertension (42% vs 24%), hyperlipidemia (70% vs 37%), statin use (40% vs 17%), DM (28% vs 7%), resolved hepatitis B infection (41% vs 27%), and alcohol abuse (10.4% vs 7.1%) were more common in participants with cLEE.

Participants with cLEE were overall less likely to be antiretroviral naïve (1.3% vs 10.5%) and cART naïve (5.2% vs 16.2%). However, 16.8% of participants with cLEE did not initiate any antiretroviral until after meeting criteria for cLEE. Similarly, of the 293 participants with cLEE who received cART, 109 (37%) did not initiate cART until after meeting criteria for cLEE. For this analysis, those with and without cLEE were followed for a median (IQR) of 4.8 (2.3–7.7) years and 6.8 (3.2–12.4) years, respectively.

Incidence of cLEE and ALT Trends

A total of 65 867 ALT measurements were analyzed, and 1180 participants (42%) had at least 1 abnormal ALT value during follow-up, for an incidence of 6.88 per 100 PY (95% CI, 6.87–6.89), and 309 (11%) met the definition of cLEE, with an incidence of 1.28 per 100 PY (95% CI, 1.28–1.29). The median number of months between ALT measures was ~5 months and was different between those with and without cLEE at 1.6 and 5.6 months, respectively.

In general, transient elevations in ALT were common (42.4%, $n = 1180$) and grade 1 ALT abnormalities predominated (66%); however, among those with cLEE ($n = 309$), the majority had ALT abnormalities that were grade ≥ 2 at some point in time (56%, $n = 172$) (Figure 2). While 18% of the participants ($n = 137$) with grade 1 ALT elevations at some point ($n = 730$) met criteria for cLEE, this proportion was 43% ($n = 173$) in those with grade 2 elevations ($n = 400$) (Figure 3). ALT fluctuations were common, and a majority with cLEE ($n = 276$, 89%) reverted to normal. Most participants reverting to normal (57%, $n = 158$) were on the same cART regimen both at cLEE diagnosis and when they reverted. Only 12% ($n = 32$) who were cART naïve at cLEE diagnosis had initiated cART when they reverted. The remaining switched regimens between the 2 time points, either the anchor drug or the nucleoside backbone.

Risk Factors Associated With cLEE

In the adjusted model, risk factors for cLEE included Hispanic/other ethnicity (reference Caucasian: HR, 1.744; 95% CI, 1.270–2.395), NNRTI-based cART (HR, 2.232; 95% CI, 1.378–3.616), cART-naïve status (HR, 6.046; 95% CI, 3.686–9.915), and cART interruption (HR, 8.671; 95% CI, 4.651–16.164). AA race (reference Caucasian: HR, 0.669; 95% CI, 0.510–0.877) and INSTI-based cART were protective (HR, 0.222; 95% CI, 0.104–0.474; $P = .0001$) (Table 2).

In a sensitivity analysis evaluating 1353 participants with BMI values at HIV diagnosis, higher BMI was a risk factor for cLEE, although the sample size for the analysis was nearly halved due to missing values (Supplementary Tables 1). BMI at cART did not show this association (data not shown). As anticipated,

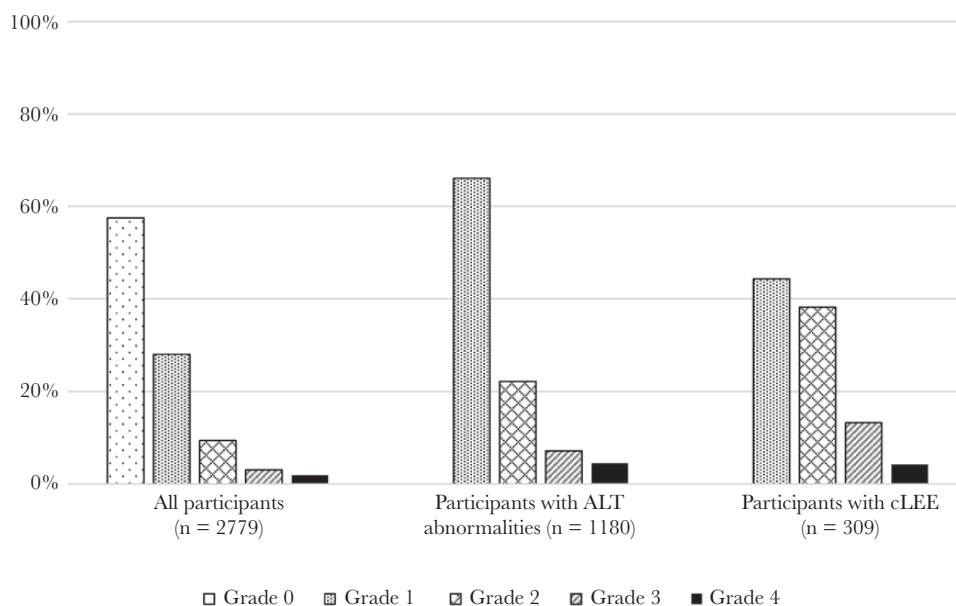


Figure 2. Distribution of highest ALT by grade for all participants, participants with ALT abnormalities, and participants with cLEE. Abbreviations: ALT, alanine aminotransferase; cLEE, chronic liver enzyme elevation.

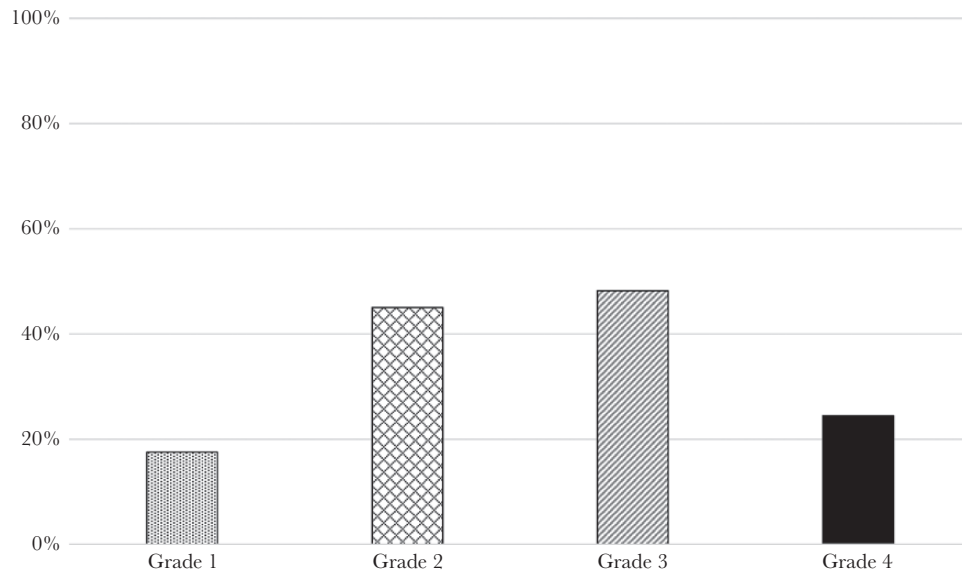


Figure 3. Proportion of participants with abnormal ALT (n = 1180) who met criteria for cLEE based on their highest ALT elevation by grade. Abbreviations: ALT, alanine aminotransferase; cLEE, chronic liver enzyme elevation.

Table 2. Factors Associated With Chronic Liver Enzyme Elevation

Variable	Unadjusted Hazard Ratios		Adjusted Hazard Ratios	
	HR (95% CI)	PValue	HR (95% CI)	PValue
Ethnicity/race				
Caucasian	Reference		Reference	
African American	0.696 (0.532–0.910)	.0081	0.669 (0.510–0.877)	.0036
Hispanic/other	1.652 (1.204–2.266)	.0019	1.744 (1.270–2.395)	.0006
Age at time T (per 10 y) ^a	0.938 (0.802–1.098)	.4250		
Year of HIV diagnosis				
Before 1996	Reference			
1996–2000	1.281 (0.904–1.814)	.1633		
2000–current	1.148 (0.876–1.506)	.3176		
CD4 count at time T (per 100 cells/ μ L) ^a	0.959 (0.918–1.003)	.0680		
HIV RNA viral load at time T (log ₁₀ copies/mL) ^a	1.036 (0.941–1.140)	.4696		
Year of cART initiation				
1996–2000	Reference			
2000–current	0.897 (0.699–1.151)	.3922		
Rx at cLEE^b				
Boosted PI	Reference		Reference	
Unboosted PI	1.568 (0.859–2.862)	.1433	1.651 (0.904–3.017)	.1030
NNRTI	2.253 (1.391–3.650)	.0010	2.232 (1.378–3.616)	.0011
INSTI	0.221 (0.103–0.472)	<.0001	0.222 (0.104–0.474)	.0001
Other cART	0.572 (0.273–1.196)	.1374	0.569 (0.272–1.191)	.1345
cART naïve ^c	5.550 (3.391–9.082)	<.0001	6.046 (3.686–9.915)	<.0001
cART interruption	9.745 (5.238–18.129)	<.0001	8.671 (4.651–16.164)	<.0001
APRI \geq 0.7 before cLEE	1.258 (0.989–1.601)	.0615		

n = 2730, with 273 participants with cLEE.

Abbreviations: APRI, aspartate transaminase to platelet ratio index; cART, combination antiretroviral therapy; cLEE, chronic liver enzyme elevation; HR, hazard ratio; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; Rx, treatment.

^aTime-updated variable.

^bData for participants without cLEE were obtained from the last visit.

^cIncludes participants who did not initiate cART until after cLEE/last visit.

Table 3. Long-term Clinical Outcomes and Indirect Markers of Liver Fibrosis of All Participants by Presence or Absence of Chronic Liver Enzyme Elevation

Variable	All Participant (n = 2779)	Chronic Liver Enzyme Elevation		P Value
		No (n = 2470)	Yes (n = 309)	
Cirrhosis	9 (0.3)	4 (0.6)	5 (1.6)	<.0001
Cardiovascular disease	14 (0.5)	11 (0.4)	3 (1.0)	.2187
Myocardial infarction	36 (1.3)	29 (1.2)	7 (2.3)	.1098
Coronary artery disease without MI	67 (2.4)	49 (2.0)	18 (5.8)	<.0001
Cardiomyopathy	22 (0.8)	19 (0.8)	3 (1.0)	.7062
Congestive heart failure	12 (0.4)	12 (0.4)	0 (0.0)	.2196
Peripheral arterial disease	14 (0.5)	12 (0.5)	2 (0.6)	.7056
FIB-4 > 1.45	1158 (41.7)	962 (38.9)	196 (63.4)	<.0001
APRI ≥ 0.7	1116 (40.2)	880 (35.6)	236 (76.4)	<.0001
FIB-4 > 3.25	331 (11.9)	252 (10.2)	79 (25.6)	<.0001
APRI > 1.5	444 (16.0)	324 (13.1)	120 (38.8)	<.0001

No. (%) for categorical variables. Variables not routinely collected before 2006.

Abbreviations: APRI, aspartate transaminase to platelet ratio index; FIB-4; fibrosis-4 index; MI, myocardial infarction.

“D-drug” use was associated with cLEE (Supplementary Table 2). A protective association with INSTI use was also observed in analyses restricting the data to time points after cART initiation and treating cART use as a time updated variable (Supplementary Table 3) as well as among participants diagnosed with HIV after 2006 (Supplementary Table 4).

Outcomes

Cirrhosis occurred in only 9 participants (0.3%). Rates of documented cardiovascular disease, cardiomyopathy, congestive heart failure, and peripheral arterial disease were similarly low, occurring in <1% of the population. Participants with cLEE were more likely to develop cirrhosis (1.6% vs 0.2%) and have a diagnosis of coronary artery disease without MI (5.8% vs 2.0%) than those without cLEE.

Elevations of indirect markers of liver fibrosis were common among NHS participants. APRI calculations ≥0.7 and FIB-4 >1.45 were observed in 40.2% and 41.7% of participants, respectively. Participants with cLEE were more likely to have APRI ≥0.7 (76.4% vs 35.6%) and FIB-4 >1.45 (63.4% vs 38.9%) (Table 3). About a quarter of the patients with cLEE had a FIB4 >3.25, and ~40% had an APRI >1.5, indicative of progression to advanced fibrosis (Table 3).

DISCUSSION

Even in the absence of hepatitis coinfections, ALT abnormalities were frequent in this cohort, with nearly half (42%) the participants exhibiting at least 1 abnormal ALT. However, these elevations were often transient and did not persist; only 1 in 10 participants met the criteria for cLEE, for an overall incidence of 1.2 per 100 PYFU. Most ALT abnormalities in our cohort were grade 1 or 2, with only 5% demonstrating grade ≥3 elevations. While lower-grade ALT elevations typically garner less attention, our results indicate that nearly 1 in 5 participants with transient grade 1 ALT elevations will progress to cLEE,

and this fraction is significantly higher in those with grade 2 elevations (42%). Future studies should examine if PWH with transient grade ≥2 ALT elevations will benefit from noninvasive monitoring and risk stratification to determine the extent of hepatic fibrosis in addition to serial monitoring of LAEs.

The incidence of cLEE observed in the NHS is lower than reported in other studies, which may be a reflection of a younger cohort with a lower proportion of treatment-naïve participants. Higher incidences of cLEE have been reported in cohorts of older participants, such as the Swiss HIV Cohort study (incidence of 3.9 cases/100 PYFU, median age at HIV diagnosis of 38 years, compared with 28 years in the NHS) [8]. Another retrospective series reported an incidence of 5.8 per 100 PYFU of cLEE, and again participants were older (median age, 40 years) and had a lower nadir CD4 count (147 cells/μL vs 302 cells/μL) [19]. Furthermore, this study’s definition of cLEE was less stringent when compared with ours (ALT greater than the upper limit of normal at 2 consecutive visits 3–12 months apart) [19], likely contributing to the higher incidence reported.

The association between HIV-specific parameters and LAE elevations remains unclear. Several studies have identified associations between elevated HIV RNA levels in HIV-monoinfected participants and markers of liver damage [8, 20] or indirect markers of liver fibrosis [21–23], supporting the hypothesis that HIV infection directly influences liver injury. In our study, CD4 count and HIV RNA levels were not significant; however, the absence of treatment and treatment interruptions had the largest effect sizes for predicting cLEE, supporting the hypothesis that HIV infection may be directly impacting LAE abnormalities.

The only identified risk factor for cLEE not related to treatment was an individual’s race/ethnicity. While Hispanic/other ethnicity was associated with cLEE, AA race was protective. The Swiss HIV Cohort study similarly identified African origin as inversely related to cLEE [8]. Overall, we hypothesize

that the differences in cLEE risk according to race may reflect differences in propensity for NAFLD or hepatic steatosis. In the general population, Hispanics are more likely and AA less likely to have hepatic steatosis compared with Caucasians [24]. Similarly, studies in PWH have demonstrated that AAs are less likely to have NAFLD and severe fibrosis compared with Caucasians [25, 26].

As previously noted, the most significant risk factors for cLEE were lack of cART, either because participants were cART naïve or had interrupted treatment, underscoring the importance of initiating and remaining on cART to prevent cLEE. The hepatotoxic potential of D-drugs [4, 8, 18] and NNRTIs has been previously established and was also observed in this study [27–29]. The effects of the NNRTIs may not be a class effect and could be dictated by the specific drug used; in fact, the hepatotoxic potential of nevirapine is very well recognized [29]. In our study, the majority of participants with cLEE were receiving efavirenz; hence, we did not have adequate power to study the effect of the newer members of this class, such as rilpivirine.

Of particular interest was the protective association observed with INSTI-based cART. This protective association was observed in both the overall analysis where cART use at the time of cLEE was examined and in a sensitivity analysis in which cART use was treated as a time-updated variable beginning at cART initiation. Taramasso et al. similarly found protective associations with INSTI regimens in a cohort containing both HIV/hepatitis-coinfected and HIV-monoinfected participants [30]. Overall, these findings suggest that modern cART may be protective against liver injury. These findings also suggest that INSTI-based regimens could be considered in persons with LAE elevations. However, these medications are relatively new, and long-term outcome data as they relate to LAE abnormalities are limited. Additionally, weight gain has been associated with use of dolutegravir [31], and weight gain in adults is a significant risk factor for NAFLD [32]. Ongoing investigation into the long-term impact of INSTI-based regimens on liver function is warranted.

Features of metabolic syndrome, including hypertension, hyperlipidemia, and DM, were more common in those with cLEE, despite these data not being reliably available for participants diagnosed before 2006. In the subgroup analysis evaluating only patients diagnosed with HIV after 2006, hyperlipidemia was more common in participants with cLEE; however, it was not significant in the multivariate model, likely a reflection of the low numbers of events. As in other retrospective studies, establishing a causal link between conditions associated with metabolic syndrome and cLEE is challenging [33].

Clinically diagnosed cirrhosis was uncommon; nevertheless, elevations in APRI and/or FIB-4 were common overall (about 1 in 3) and occurred more frequently in participants

with cLEE (3 in 4). The clinical significance of this finding is unclear; however, an elevated FIB-4 score has been independently associated with liver fibrosis in HIV-monoinfected persons [34]. In fact, nearly half the participants with cLEE had evidence of advanced fibrosis (based on an APRI >1.5 or FIB-4 >3.25), which suggests that this group should be the focus of interventions and monitoring strategies designed to impede and reverse fibrosis.

Our study has several limitations. First, ALT levels were used as a surrogate marker of liver disease rather than more definitive methods such as imaging, but we did observe an association with cLEE and advanced fibrosis based on noninvasive measures. Alcohol use is common in this population [35], but the impact of alcohol or statins on cLEE could not be completely assessed, as data were not routinely solicited before 2006. We tried to overcome some of these limitations by performing an analysis restricted to those diagnosed with HIV after 2006, but the small number of outcomes limited our ability to detect any associations with alcohol or statin use. Similarly, we could not specifically assess the role of metabolic syndrome on cLEE, as abdominal circumference was not captured. Neither could we assess the impact of nonalcoholic fatty liver disease and steatohepatitis on cLEE. Finally, there could be an ascertainment bias in play, as those with cLEE were more likely to have frequent ALT measurements.

While our cohort was large and diverse, it was comprised solely of DoD beneficiaries who have open access to care and medications. Additionally, active duty personnel are required to undergo biannual HIV testing, often aiding in the early diagnosis and initiation of treatment. As a consequence, the findings in this study may not necessarily reflect those of the general population.

The strengths of this study are the presence of long-term follow-up, the lack of significant confounders provided all participants have access to care and there is limited drug use, and the availability of granular ART and serial ALT information, allowing evaluation of the role of ART, especially modern cART, in cLEE.

In conclusion, transient and persistent ALT abnormalities are common in PWH, and cLEE was observed in 10% of this early diagnosed and treated population with open access to care and medications. The lack of ART and treatment interruptions had the largest effect size. Hence, consistent with clinical guidelines, ART should be initiated earlier rather than later, and INSTI-based cART may offer protective benefit against HIV-associated liver injury.

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Author contributions. We certify that all individuals who qualify as authors have been listed; each has participated in the conception and design of this work, the analysis of data (when applicable), the writing of the document, and the approval of the submission of this version; that the document represents valid work; that if we used information derived from another source, we obtained all necessary approvals to use it and made appropriate acknowledgements in the document; and that each takes public responsibility for it. Some authors on this paper are military service members and/or employees of the US Government. As such, this work was prepared as part of official duties. Title 17 U.S.C. 105 provides that "Copyright protection under this title is not available for any work of the United States Government." Title 17 U.S.C. 101 defines a United States Government work as a work prepared by a military service member or employee of the United States Government as part of that person's official duties.

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