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MAJOR ARTICLE

Low Level Viremia Is Associated With Serious non-AIDS Events in People With HIV

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Background. The consequences of low-level viremia in people with HIV are unclear. We used data from the US Military HIV Natural History Study to examine the association of low-level viremia (LLV) and serious non-AIDS events (SNAEs).

Methods. Included participants initiated antiretroviral therapy after 1996 and had ≥3 viral loads (VLs) measured, using an assay with a lower limit of detection of <50 copies/mL, ≥6 months after antiretroviral therapy initiation. VLs were categorized as lower levels of LLV (51–199 copies/mL), higher level of low-level viremia (HLLV; 200–999 copies/mL), and (VF; ≥200 copies/mL on 2 or more successive determinations or a single VL ≥1000 copies/mL), and virologic suppression (VS; ie, VL <50 copies/mL). Viral blips (ie, VLs between 50 and 999 copies/mL that are preceded and succeeded by VL <50 copies/mL) were analyzed in the VS category. Cox proportional hazards models were used to examine the association of LLV and SNAEs, adjusted hazard ratios and 95% confidence intervals are presented.

Results. A total of 439 (17.4%) SNAEs were recorded among the 2528 participants (93% male, 40% Caucasian, 43% African American) followed for a median of 11 years. In 8.5% and 4.6% of the participants, respectively, LLV and HLLV were the highest recorded viremia strata. Compared with VS, SNAEs were associated with LLV (1.3 [1.2–1.4]), HLLV (1.6 [1.5–1.7]), and virologic failure (1.7 [1.7–1.8]).

Conclusions. The results of this study suggest that LLV is associated with the occurrence of SNAEs and needs further study.

Keywords. early treated; HIV; low- level viremia; military cohort; serious non-AIDS events.

Low-level viremia refers to episodes of detectable HIV viremia (ie, >50 copies/mL) that do not meet the criteria for virologic failure (VF) or blips [1]. VL cutoffs used to define low-level viremia vary based on the organization making the recommendation [1, 2]. Current US guidelines define low-level viremia as viral loads (VLs) that are detectable but <200 copies/mL, whereas the World Health Organization uses a threshold of <1000 copies/mL to define this entity [1, 2]. Although the deleterious consequences of higher levels of low-level viremia (HLLV) (ie, VL 200–999 copies/mL) are recognized and accepted, the significance of lower levels of viremia (LLV) (ie, 51–199 copies/mL) is unclear [3–6]. The data on whether episodes of LLV are clinically consequential are mixed. Although older studies have failed to demonstrate an association with LLV and VF, others have demonstrated that persistent LLV is associated with VF [7–10]. Few studies have examined the impact of LLV on clinical events such as AIDS/serious non-AIDS events (SNAEs) and death with inconclusive results, with some suggesting increased risk of deaths/SNAEs and AIDS, yet others have failed to demonstrate an association [5, 6, 11, 12]. During episodes of HLLV, the virus has been known to evolve, even acquiring drug resistance mutations, hence, HLLV episodes are likely due to completed cycles of viral replication; however, the genesis of LLV is not well understood [13]. Some have suggested that LLV episodes are due to the periodic release of replication incompetent proviruses, whereas others have suggested that these episodes might be artefactual and because of the higher sensitivity of VL assays currently in use [14, 15]. Thus, one might be inclined to think of LLV as a random biological phenomenon attributable to the assay in use. On the contrary, if LLV were due to ongoing viral replication in immune privileged sites (eg, the central nervous system), it

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would be meaningful. The question of whether LLV episodes confer an increased risk of SNAEs remains unanswered [15]. We used data from the US Military HIV Natural History Study (NHS), a well-characterized, racially diverse cohort of Department of Defense beneficiaries, to examine the question of whether episodes of LLV are associated with SNAEs [16].

METHODS

Study Population

The NHS is a prospective, multicenter, open cohort. Participants have access to care with minimal barriers to treatment and monitoring. Because of the mandatory HIV screening policies, participants are identified and initiate antiretroviral therapy (ART) early in infection [16]. NHS visits occur about every 6–12 months, participants undergo blood draws, are examined by a physician, and are interviewed by research personnel. Trained research personnel collect clinical diagnoses (recorded by the physicians and any additional corroborating materials, such as radiology/histopathology), ART history, and results of laboratory testing to include creatinine, CD4 counts, and VLs. Before commencing data abstraction, research personnel receive training and undergo a proficiency check. To assist with data abstraction, written guidance (NHS codebook), which provides a listing and detailed descriptions of the diagnosis being collected, is available as is access to study physicians for assistance with coding. Moreover, to ensure data quality, the principal study investigator performed a random 10% adjudication of all SNAEs and found them to be accurate. For this retrospective analysis, we included participants who were enrolled in the NHS before 1 March 2020 and initiated ART after 1 January 1996 and had ≥3 documented VLs (using an assay with a lower limit of quantification of <50 copies/mL) ≥6 months after ART initiation, and while on ART. Follow-up for this report ended on 6 July 2022.

Definitions

We categorized subject VLs into 4 mutually exclusive exposure categories. VF was defined as having a VL of ≥200 copies/mL on 2 consecutive measurements (at least 3 months apart) or a single VL of ≥1000 copies/mL, 6 months after initiating ART and while on ART [1]. In consideration of the World Health Organization definition and other reports, participants with 1 or more nonconsecutive ("unconfirmed") VL measurements between 200 and 999 copies/mL that did not meet the definition of VF or blips were classified as having HLLV, VLs between 51–199 copies/mL were categorized as LLV if they did not meet criteria for blips (Supplementary Figure 1). If all measured VLs were ≤50 copies/mL, then participants were considered virally suppressed (VS). As the phenomenon of viral blips (ie, episodes of transient viremia during which VL values range between 51 and 999 copies/mL, preceded and followed by VLs that are ≤50 copies/mL) is distinct from LLV, blips were analyzed in the VS category [1]. A complete list of SNAEs examined is provided in Table 1, and includes chronic kidney disease (CKD), malignancies excluding nonmelanomatous skin cancers, cardiovascular disease, and cirrhosis. Supplementary Material provides descriptions of the SNAEs and definitions of comorbidities analyzed.

Table 1. Listing of Serious non-AIDS Events (SNAEs) by Gender and Category

SNAE categories	Male n = 395, 90.0% n (%)	Female n = 44, 10.0% n (%)	Total n = 439 n (%)
Cancer			
Anal	21 (5.3)	0 (0.0)	21 (4.8)
Breast	0 (0.0)	5 (11.4)	5 (1.1)
Colon	1 (0.3)	0 (0.0)	1 (0.2)
Leukemia	1 (0.3)	0 (0.0)	1 (0.2)
Lung	3 (0.8)	0 (0.0)	3 (0.7)
Hodgkin lymphoma	7 (1.8)	0 (0.0)	7 (1.6)
Melanoma	12 (3.0)	0 (0.0)	12 (2.7)
Multiple myeloma	1 (0.3)	0 (0.0)	1 (0.2)
Other[a]	17 (4.3)	5 (11.4)	22 (5.0)
Prostate	23 (5.8)	0 (0.0)	23 (5.2)
Heart related			
Cardiomyopathy	9 (2.3)	0 (0.0)	9 (2.1)
Congestive heart failure	3 (0.8)	0 (0.0)	3 (0.7)
Myocarditis	0 (0.0)	0 (0.0)	0 (0.0)
Pericardial effusion	0 (0.0)	0 (0.0)	0 (0.0)
Pericarditis	5 (1.3)	0 (0.0)	5 (1.1)
Vascular			
Cerebrovascular disease	15 (3.8)	2 (4.5)	17 (3.9)
Coronary artery disease without myocardial infarction	47 (11.9)	3 (6.8)	50 (11.4)
Deep vein thrombosis	18 (4.6)	2 (4.5)	20 (4.6)
Myocardial infarction	19 (4.8)	3 (6.8)	22 (5.0)
Peripheral artery disease	5 (1.3)	1 (2.3)	6 (1.4)
Kidney			
Chronic kidney disease stage 3[b]	171 (43.3)	21 (47.8)	192 (43.7)
Liver			
Cirrhosis	17 (4.3)	2 (4.5)	19 (4.3)

[a]Other cancer include renal (3), thyroid (3), oropharyngeal (3), pancreas (2), bladder (2), testicular (1), liver (1), vulvovaginal cancer (1), esophageal (1), unknown primary (1), peritoneal (1), cancer without known primary (3).

[b]Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate of <60 mL/min lasting for a minimum of 90 d. Estimated glomerular filtration rate was calculated using the CKD-EPI creatinine equation.

Statistical Considerations

Descriptive statistics are presented as medians with interquartile ranges for continuous variables and counts with proportions for categorical variables. For group comparisons, Kruskal-Wallis test and chi-square/Fisher exact test was used to calculate 2-sided *P* values for continuous and categorical variables, respectively.

We used an adjusted Cox proportional hazard model to assess the association between viremia categories and the first occurrence of a SNAE. The validity of the proportional hazard assumption was graphically evaluated and using Schonfeld

residuals was met. Study follow-up started 6 months post-ART initiation and concluded either at the occurrence of the first SNAE or date of the last recorded study visit or death (whichever occurred first). In multivariable analysis, time-varying viremia categories were examined, this decision was made a priori and predicated by the assumption that the effects of LLV would be dependent on the time spent in a category. Throughout the follow-up period, with changes in VL strata, participants were reclassified into higher or lower strata and time spent in each stratum was accounted. Variable selection for adjustment was based on recognized association with SNAEs and prior reports, including gender, race, VL at ART initiation, time from HIV diagnosis to ART initiation, and time-updated covariates (eg, age, CD4 count, ART regimen) [4–6]. Missing data were addressed with complete case analysis approach (ie, records without missing values for any variables were incorporated into the models). Adjusted hazard ratios and 95% confidence intervals are presented. All reported *P* values are 2-sided with a $P < .05$ indicating significance. All analyses were conducted using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

We performed several sensitivity analyses. To assess whether LLV carries the same import in the current ART era, we performed an analysis restricted to participants initiating ART after 2006. In this analysis, in addition to the previously described variables, we also adjusted for a measure of adherence (ie, the medication possession ratio [MPR]) [17]. Since 2006, the NHS has captured information on potential confounders (hypertension, diabetes, and obesity). To evaluate the effects of hypertension, diabetes, and obesity on the primary exposure of interest, separate multivariable models were used. We used the CKD EPI creatinine equation to calculate the estimated glomerular filtration rate (eGFR). In our main analysis, we defined CKD as an eGFR of <60 mL/min for 90 or more days [18]. Because the integrase strand inhibitors (INSTIs) bictegravir and dolutegravir and the pharmaco-enhancer cobicistat artefactually elevate creatinine values, the use of eGFR to define CKD may have led to an overestimation of cases of CKD [1]. Thus, we performed a sensitivity analysis in which a stringent definition of CKD was used (ie, an eGFR of 30 mL/min or lower). Liver disease is being increasingly recognized as a significant issue in people with HIV (PWH) [19]. In our primary analysis, cases of subclinical liver disease were not included. The Fibrosis-4 index (FIB-4) is a validated surrogate marker for liver disease [20]. We performed a sensitivity analysis in which we classified participants with a FIB-4 index of >3.25 as having liver disease and was evaluated as a SNAE [20].

RESULTS

Patient Characteristics

A total of 2528 participants met our eligibility criteria and were followed for 34 541.37 person-years from HIV diagnosis and 25 489.69 person-years from ART initiation, Figure 1. Participants were predominantly male (93.4%) and ethnically diverse (42.8% African American, 40.0% Caucasian, 17.2% Hispanic/Other), Table 2. The median age at HIV diagnosis was 28.9 years (24.5–35.2), time from HIV diagnosis to the initiation of ART was 1.1 year (0.2–5.3), and the nadir CD4 count was 311 cells/μL (219–421). The median CD4 count at HIV diagnosis was 459 cells/μL (327–616) and the VL was 31 685 copies/mL (8691–87 997). The median follow-up time from HIV diagnosis and ART initiation were 11 years (5.6–19.1) and 8.2 years (4.4–14.0), respectively. LLV was the highest viremia strata observed in 8.5%, HLLV in 4.6% and VF in 33.9%, whereas 53% were VS at all measured time points. Among 1194 participants initiating ART after 2006, the highest viremia strata observed was LLV in 10.1%, HLLV in 2.9%, and VF in 7.7%.

SNAEs

CKD (192 participants, 7.6%) was the most common and cirrhosis (19 participants, <1%) the least likely SNAE, Table 1. Cardiovascular disease was recorded in 132 (5.2%) and non–AIDS-defining cancers in 96 (3.8%), participants. Approximately 35% had ≥2 SNAE types recorded. The median age at SNAE diagnosis was 51.9 years (46.1–58.5), and most participants had preserved CD4 cells, median 616 cells/μL (381–831) and were VS (78.6%); however, 6.2% had LLV and 3.2% had HLLV. A median of 17.4 years had elapsed from HIV diagnosis to the occurrence of SNAE (11.5–22.5). A total of 81 deaths were recorded, of which 38 (8.7%) amongst those had a SNAE and 17 (45%) were related to the SNAE, Supplementary Table 1.

Comparison of Those With and Without SNAE

There were differences between those with and without SNAEs, Table 2. Those with SNAEs were more likely to be women (10.0% vs 5.8%), Caucasian (49.7% vs 37.9%), older at the time of HIV diagnosis (median 33.3 vs 28.3 years), more likely to have experienced VF (53.3% vs 29.9%), received their HIV diagnosis before 1996 (61.3% vs 25.5%), delayed initiation of ART after HIV diagnosis (median 4.8 years vs 0.7 years), had a lower nadir CD4 count (median 266 cells/μL vs 322 cells/μL), and VLs at ART initiation (median 22 406 copies/mL vs 33 225 copies/mL). Initial ART regimens differed, reflecting differences in ART regimens that were in use at the time of their HIV diagnosis, Table 2. A comparison of baseline characteristics based on highest ever VL strata is presented in Supplementary Table 2. There were differences between the groups including a greater number of VL measurements in those with viremia compared with those who were suppressed.

Risk Factors Associated With SNAEs

In the adjusted Cox regression model, Table 3, in comparison to those who remained VS, participants with ≥1 episodes of

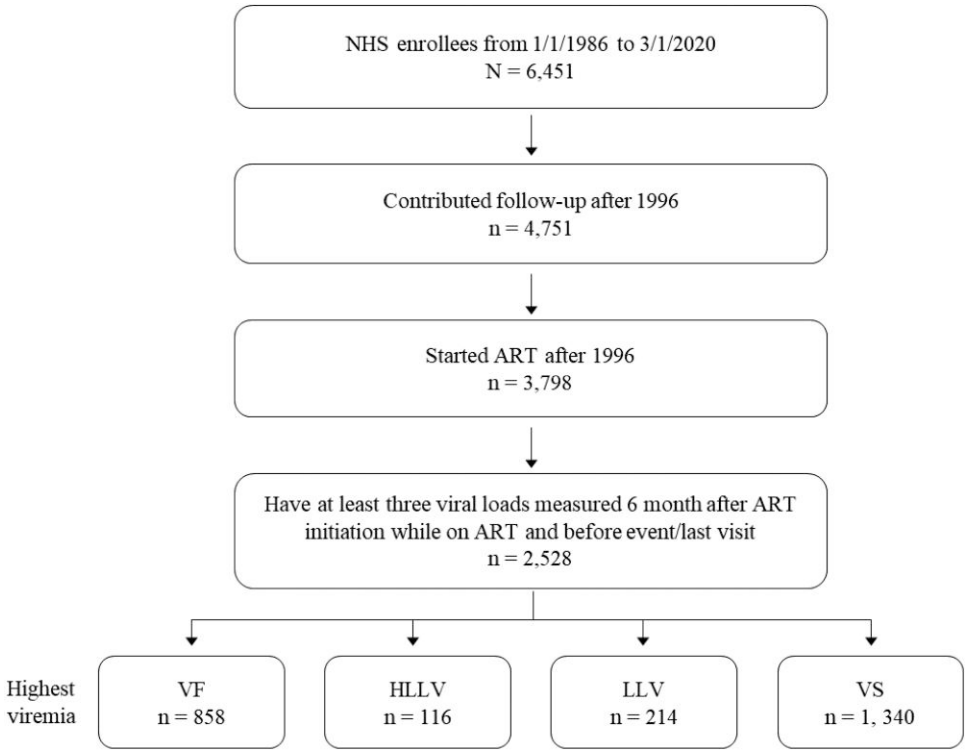

Figure 1. Selection of study population. ART, antiretroviral therapy; HLLV, higher level of low-level viremia; LLV, lower level of low-level viremia; NHS, Natural History Study; VF, virologic failure; VS, viral suppression.

LLV (1.31 [1.22–1.41]), HLLV (1.57 [1.46–1.70]) and VF (1.75, [1.67–1.82]) had a higher hazard of SNAEs. Other factors associated with SNAEs included female gender (1.37 [1.29–1.45]); older age (for every 10-year increase in age; 1.63 [1.60–1.66]); delays in ART initiation (for every year increase; 1.02 [1.01–1.02]), and regimen type. In comparison with INSTI-based regimens, use of boosted protease inhibitors (3.74 [3.39–4.13]), and nonnucleoside reverse transcriptase (2.69 [2.45–2.96]) were associated with SNAEs. A higher CD4 count (for every 100-cell increase; 0.94 [0.93–0.94]) and being African American (0.70 [0.68–0.73]) or Hispanic/Other ethnicity (0.81 [0.77–0.86]) were protective.

Sensitivity Analyses

In an adjusted analysis, restricted to those initiating ART after 2006, the associations of LLV (1.35 [1.02–1.78]), HLLV (2.07 [1.28–3.34]), and VF (3.06 [2.18–4.30]) remained after adjustment for the MPR. As observed in the primary analysis, similar deleterious associations were observed with female gender, Caucasian ethnicity, older age, lower CD4 counts, higher VL at ART initiation, and ART regimen type, Table 4. We also observed an association with MPR >90% and SNAEs. In separate multivariable models examining the comorbidities diabetes, hypertension, and obesity individually, the association with LLV and SNAE remained (Supplementary Tables 3–5).

In another sensitivity analysis in which a creatinine clearance of <30 mL/min was used to define CKD, the previously observed associations with LLV (1.41 [1.30–1.53]), HLLV (1.58 [1.44–1.73]), and VF remained (1.76 [1.67–1.85]), Supplementary Table 6. Finally, in an analysis that used FIB-4 >3.25 as a surrogate for liver disease, we observed an association with LLV (1.30 [1.21–1.39]), HLLV (1.74 [1.62–1.87]), and VF (1.58 [1.51–1.66]), Supplementary Table 7.

DISCUSSION

In keeping with prior reports, in approximately 13% of the participants, LLV was the highest viral strata recorded (8.5% with LLV and 4.6% with HLLV) [4, 21–23]. Rates were similar in those initiating ART after 2006 (LLV in 10.1% and HLLV in 2.9%), underscoring the importance of LLV in the current ART era (ie, since the introduction of fixed-dose combinations). Given the frequency of our observation, we can expect that most clinicians treating PWH will encounter patients with LLV. The phenomenon of LLV can be disconcerting to both patients and clinicians alike, raising questions about its origin, management, and consequences. There are limited data on whether episodes of LLV portend a greater risk of AIDS/SNAEs or death. Data from a Spanish cohort make a distinction between LLV and VLs between 200 and 499 copies/mL,

Table 2. Characteristics of NHS Participant Stratified by the Presence or Absence of Serious non-AIDS Events (SNAEs)

	SNAE—No n = 2089, 82.6% n (%)	SNAE—Yes n = 439, 17.4% n (%)	Overall n = 2528 n (%)	*P*
Demographics				
Gender				.0013
Male	1967 (94.2)	395 (90.0)	2362 (93.4)	
Female	122 (5.8)	44 (10.0)	166 (6.6)	
Race				<.0001
Caucasian	792 (37.9)	218 (49.7)	1010 (40.0)	
African American	920 (44.0)	162 (36.9)	1082 (42.8)	
Hispanic/Other	377 (18.0)	59 (13.4)	436 (17.2)	
Age at HIV diagnosis (y), median [Q1–Q3]	28.3 [24.0–33.9]	33.3 [27.9–40.2]	28.9 [24.5–35.2]	<.0001
Age at AI (y), median [Q1–Q3]	31.4 [26.2–37.6]	39.2 [34.1–45.1]	32.7 [27.3–39.1]	<.0001
Age at SNAE/LV (y), median [Q1–Q3]	40.8 [32.4–50.0]	51.9 [46.1–58.5]	43.1 [34.0–52.5]	<.0001
Viremia status				
Highest viremia strata[a]				<.0001
VS	927 (44.4)	101 (23.0)	1028 (40.7)	
Blips	259 (12.4)	53 (12.1)	312 (12.3)	
LLV	183 (8.8)	31 (7.1)	214 (8.5)	
HLLV	96 (4.6)	20 (4.6)	116 (4.6)	
VF	624 (29.9)	234 (53.3)	858 (33.9)	
Viremia strata at last visit[b]				<.0001
VS	1787 (85.5)	345 (78.6)	2132 (84.3)	
LLV	127 (6.1)	27 (6.2)	154 (6.1)	
HLLV	45 (2.2)	14 (3.2)	59 (2.3)	
VF	130 (6.2)	53 (12.1)	183 (7.2)	
HIV+/ART related				
HIV diagnosis era				<.0001
Before 1996	532 (25.5)	269 (61.3)	801 (31.7)	
1996–2000	255 (12.2)	58 (13.2)	313 (12.4)	
2000–current	1302 (62.3)	112 (25.5)	1414 (55.9)	
Year of AI (calendar y), median [Q1–Q3]	2006 [1998–2012]	1998 [1997–2004]	2004 [1997–2011]	<.0001
ART era				<.0001
1996–2000	655 (31.4)	286 (65.1)	941 (37.2)	
2000+	1434 (68.6)	153 (34.9)	1587 (62.8)	
Time from HIV diagnosis to AI (y), median [Q1–Q3]	0.7 [0.2–4.3]	4.8 [0.9–8.9]	1.1 [0.2–5.3]	<.0001
First ART regimen				<.0001
Unboosted PI	565 (27.0)	251 (57.2)	816 (32.3)	
Boosted PI	178 (8.5)	34 (7.7)	212 (8.4)	
NNRTI	913 (43.7)	122 (27.8)	1035 (40.9)	
PI + NNRTI + NRTI	47 (2.2)	21 (4.8)	68 (2.7)	
INSTI	378 (18.1)	10 (2.3)	388 (15.3)	
Other combinations	8 (0.4)	1 (0.2)	9 (0.4)	
ART at SNAE/LV				<.0001
Unboosted PI	94 (4.5)	33 (7.5)	127 (5.0)	
Boosted PI	237 (11.3)	103 (23.5)	340 (13.4)	
NNRTI	624 (29.9)	125 (28.5)	749 (29.6)	
PI + NNRTI + NRTI	43 (2.1)	18 (4.1)	61 (2.4)	
3 NRTI	1 (0.0)	0 (0.0)	1 (0.0)	
INSTI	962 (46.1)	91 (20.7)	1053 (41.7)	
Other combination	123 (5.9)	50 (11.4)	173 (6.8)	
Treatment interruption	5 (0.2)	19 (4.3)	24 (0.9)	
ARV before AI				<.0001
No	1537 (73.6)	185 (42.1)	1722 (68.1)	
Yes	552 (26.4)	254 (57.9)	806 (31.9)	
Laboratory				
CD4 at HIV diagnosis (cells/μL), median [Q1–Q3][c]	458.0 [328.0–614.0]	465.0 [323.0–627.0]	458.5 [327.0–616.0]	.7621
CD4 at AI (cells/μL), median [Q1–Q3][d]	375.0 [263.0–509.0]	324.0 [198.0–456.0]	369.0 [256.0–498.0]	<.0001

Table 2. Continued

	SNAE—No n = 2089, 82.6% n (%)	SNAE—Yes n = 439, 17.4% n (%)	Overall n = 2528 n (%)	*P*
CD4 at SNAE/LV (cells/μL), median [Q1–Q3]	700.0 [528.0–910.0]	616.0 [381.0–831.0]	684.0 [504.0–899.0]	<.0001
nadir CD4 (cells/μL), median [Q1–Q3]	322.0 [231.0–433.0]	266.0 [168.0–358.0]	310.5 [219.0–421.0]	<.0001
VL at HIV diagnosis (copies/mL), median [Q1–Q3][e]	32 083 [8578.0–87 535]	30 095 [8754.0–95 318]	31 685 [8691.0–87 997]	.7847
VL at AI (copies/mL), median [Q1–Q3][f]	33 225 [7613.0–95 517]	22 406 [3808.0–78 981]	31 363 [6800.0–93 442]	.0024
VL at SNAE/LV (copies/mL), median [Q1–Q3]	20.0 [20.0–50.0]	29.0 [20.0–50.0]	20.0 [20.0–50.0]	<.0001
Other				
HIV dx to SNAE/LV (y), median [Q1–Q3]	9.6 [5.1–17.8]	17.4 [11.5–22.5]	11.0 [5.6–19.1]	<.0001
AI to SNAE/LV (y), median [Q1–Q3]	7.5 [4.1–13.2]	11.0 [6.8–16.0]	8.2 [4.4–14.0]	<.0001

Kruskal-Wallis test and chi-square/Fisher's exact test were used to calculate *P*-value for continuous and categorical variable respectively.

Abbreviations: AI, antiretroviral therapy initiation; ART, antiretroviral therapy; ARV, antiretroviral drug; LV, last visit; HLLV, higher level of low-level viremia; INSTI, integrase strand transfer inhibitor; LLV, lower level of low-level viremia; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SNAE, serious non-AIDS event; VL, viral load; VS, viral suppression.

[a]The highest viremia status achieved 6 mo after ART initiation and before or at the occurrence of SNAE or their last visit.

[b]Last viremia status before the occurrence of SNAE or their last visit.

[c]Missing values, n = 284.

[d]Missing values, n = 229.

[e]Missing values, n = 727.

[f]Missing values, n = 245.

whereas LLV was not associated with mortality, AIDS, or SNAEs, higher VLs (ie, VL of 200–499 copies/mL) were associated with AIDS and SNAEs [5]. In a large study examining data from the ART Cohort Collaboration, which combined data from 18 cohorts in Europe and North America, neither LLV or VLs ranging between 200 and 499 copies were associated with AIDS or deaths; SNAEs were not examined in this study [6]. Two recent studies, which provide contemporary data, have suggested that LLV has deleterious consequences. In a nationwide Swedish study, LLV conferred an increased risk of death but not SNAEs, whereas HLLV was associated with SNAEs [11]. In a smaller study from China, both LLV and HLLV were associated with SNAEs [12]. Our study adds to this growing, but small, body of evidence that suggests LLV may not just be a random biological phenomenon and may have clinically significant consequences.

Our work differs from prior reports in some respects. Earlier stages of renal and liver disease have not been previously examined as outcomes. We defined CKD as stage III or higher and performed a sensitivity analysis using the FIB-4 index as a surrogate for liver disease. Because strategies designed to reverse or alter the progression of liver and kidney disease are likely to work at earlier stages, including these endpoints is a strength and differentiates this work from prior reports. Although prior reports have analyzed viremia as a time-varying covariate [4, 11], once participants reach a higher VL stratum, they were analyzed in that stratum, with the implicit assumption being that the effects of higher level viremia are enduring (even if followed by viral suppression), which might not be the case. We hypothesized that the effects of viremia on the outcome is related to the time spent in the different viral strata. This assumption gives equal weighting to the time spent in a stratum regardless of when the viremia occurred during the disease course, which also has limitations [24]. Larger studies are needed to evaluate whether a threshold exists beyond which time spent in the LLV or HLLV stratum increases the risk of SNAEs and whether the temporality of the event matters (ie, early or late in the disease course) [24].

The optimal management of LLV is unclear. One recent study suggests that, mechanistically, drugs that block virion production/budding, rather than integration, might influence LLV and should be examined [25]. Recommendations for how patients with LLV should be approached vary based on the societies issuing the guidance. Although the US guidelines recommend against ART change in most individuals with LLV, the European AIDS Society recommends resistance testing at VLs >50 copies/mL and maintaining ART only if resistance is not demonstrated and the current regimen contains a drug with a high genetic barrier to resistance [1, 26].

The origins of LLV while on effective ART is much debated and is likely multifaceted and variable. One potential explanation is imperfect adherence to ART (from competing priorities, structural barriers to care, and/or pill fatigue) leading to subtherapeutic levels and detectable viremia [27, 28]. In fact, lower adherence as measured by lower drug levels, unannounced pill counts, and medication monitoring systems have been associated with LLV [28–30]. Several recent studies have suggested that imperfect adherence even in those with an undetectable VL has biological and clinical consequences that include an increased risk of inflammation, cardiovascular disease, and non-cardiovascular mortality [31–33], which in turn might contribute to the risk of SNAEs. Even with perfect adherence,

Table 3. Univariate and Multivariable Cox Proportional Hazard Model Evaluating Risk Factors for Serious non-AIDS Events (SNAE)

	Unadjusted				Adjusted			
	HR	95% CI		*P*	HR	95% CI		*P*
Time updated viremia categories (referent: VS)								
LLV	1.766	1.653	1.886	<.0001	1.308	1.217	1.405	<.0001
HLLV	2.112	1.970	2.265	<.0001	1.571	1.457	1.695	<.0001
VF	2.711	2.618	2.807	<.0001	1.746	1.671	1.824	<.0001

N = 2283. SNAE cases, n = 387. Adjusted for gender (male, female), race (Caucasian, African-American, Hispanic/Other), time updated age (for every 10-year increase), log viral load at ART initiation, time from HIV diagnosis to ART initiation (for every year increase), time updated CD4 counts (for every 100 cell increase), and time updated ART regimen (integrase strand inhibitors, boosted protease inhibitors, nonnucleoside reverse transcriptase inhibitors, unboosted protease inhibitors, and other combination). SNAE categories: cancers including anal cancer (n = 20), breast cancer (n = 5), colon cancer (n = 1), leukemia (n = 1), lung cancer (n = 3), Hodgkin lymphoma (n = 7), melanoma (n = 11), multiple myeloma (n = 1), prostate cancer (n = 20), other cancers (n = 19); cardiovascular events include cardiomyopathy (n = 7), congestive heart failure (n = 1), pericarditis (n = 5), cerebrovascular disease (n = 16), coronary artery disease without myocardial infarction (n = 45), deep vein thrombosis (n = 17), myocardial infarction (n = 17), peripheral artery disease (n = 3); chronic kidney disease (n = 171); and cirrhosis (n = 17).

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HLLV, higher level of low-level viremia; HR, hazard ratio; LLV, low-level viremia; VF, virologic failure; VS, virologic suppression.

Table 4. Univariate and Multivariable Cox Proportional Hazard Ratio Model Evaluation Risk Factors Associated With Serious non-AIDS Events (SNAEs) in Those Initiating ART After 2006

	Unadjusted				Adjusted			
	HR	95% CI		*P*	HR	95% CI		*P*
Time updated viremia categories (Referent: VS)								
LLV	1.548	1.181	2.030	.0016	1.345	1.019	1.775	.0363
HLLV	2.640	1.676	4.158	<.0001	2.069	1.276	3.356	.0032
VF	2.281	1.704	3.054	<.0001	3.061	2.178	4.304	<.0001

N = 1130. SNAE case, n = 83. Adjusted for gender (male, female), race (Caucasian, African-American, Hispanic/Other), time updated age (for every 10-year increase), log viral load at ART initiation, time from HIV diagnosis to ART initiation (for every year increase), time updated CD4 counts (for every 100-cell increase), time updated ART regimen (integrase strand inhibitors, boosted protease inhibitors, nonnucleoside reverse transcriptase inhibitors, unboosted protease inhibitors, and other combination), and medication possession ratio (calculated as an overall average <90% vs >90%). SNAE categories: cancers including anal cancer (n = 3), lung cancer (n = 1), Hodgkin lymphoma (n = 1), melanoma (n = 2), prostate cancer (n = 1), other cancers (n = 3); cardiovascular events include: cardiomyopathy (n = 1), pericarditis (n = 2), cerebrovascular disease (n = 3), coronary artery disease without myocardial infarction (n = 8), deep vein thrombosis (n = 5), myocardial infarction (n = 3), chronic kidney disease including: (n = 49); cirrhosis (n = 1).

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HLLV, higher level of low-level viremia; HR, hazard ratio; LLV, low-level viremia; VF, virologic failure; VS, virologic suppression.

pharmacokinetic considerations, such as insufficient drug concentration at anatomically protected sites and drug–drug interactions/host polymorphisms leading to lower drug levels could potentially explain LLV episodes [15]. Although speculative, imperfect adherence in one domain might transcend into other domains. For example, an individual who fails to take their ART consistently may also have imperfect adherence with other interventions (eg, statins, antihypertensives). In our analysis, in the subset that initiated ART after 2006, despite adjusting for a measure of adherence, the association with LLV/HLLV remained, suggesting other mechanisms may be operational.

Persistent viremia can also arise from large clonal populations of HIV-infected cells in individuals with therapeutic levels of drugs [25, 34, 35]. Even if episodes of LLV are due to replication incompetent defective provirus, such episodes could have clinical consequences. Defective pro-viruses are not inert; they can produce viral proteins and extracellular virus-like particles that may trigger innate and adaptive immune response and contribute to the persistent immune activation and LLV observed in suppressed PWH [36, 37]. Regardless of the etiology of LLV, our results suggest an association of SNAEs with LLV. Hence, those with LLV may benefit from closer follow-up, repeat measurements of VL, adherence assessment, and evaluation of possible drug–drug interactions that could result in lower drug levels. These results also argue for a downward shift in the VL benchmarks used for defining LLV and VF in resource constrained settings from the current value of ≥1000 copies/mL to ≥200 copies/mL [2].

The strengths of this study are that participants had access to care and minimal intravenous drug use, thereby limiting the number of confounders that could affect our results [16]. We were able to comprehensively capture the outcomes (because of the single-payer system and a median follow up of 11 years and 8.2 years after ART initiation) and exposure of interest (a median of 12 VL measures per participant). In addition, multiple sensitivity analyses were conducted, with similar results strengthening our observations. This study also provides contemporary data on the impact of LLV in the era of fixed-dose combination antiretrovirals (ie, those initiating ART after 2006); even in this group, we noted an association with both LLV and HLLV and the subsequent occurrence of SNAEs.

In our retrospective study, VL were measured serially at prespecified time points (about every 6–12 months), hence, we may have not capture intermittent periods of higher levels of viremia that may have contributed to systemic inflammation and the risk of SNAEs [38]. To overcome this limitation,

viremia copy-years, a cumulative measure of exposure to HIV replication, has been proposed and is associated with mortality [39]. However, such a measure would be unable to address the question of whether LLV is associated with SNAEs and hence was not used.

There are limitations to our study, the main among them is our inability to adjust for several potential confounders that are associated with the outcome. For example, hypertension and diabetes were not adjusted for because these exposures have not been captured consistently through the 35+ years of the NHS [40]. Since 2006, the NHS has captured information on diabetes, hypertension, and obesity. In the subgroup that initiated ART after 2006, the associations with LLV and SNAEs remained even after adjusting for these comorbidities. However, the numbers of SNAEs were lower (n = 83), and to avoid model overfitting, we were limited to analysis in which we analyzed each comorbidity individually in separate models. Larger studies are needed to evaluate if the effects of LLV will remain after adjusting for these confounders. Our cohort comprises primarily men, and these results may not be generalizable to women. Of note, despite the low numbers of women participants, female gender was independently associated with SNAEs. These results need confirmation in cohorts with a larger representation of women. The potential for informational bias should be considered; those with LLV/HLLV and VF had more VLs measured than those with VS, indicating more interaction with the health care system representing opportunities for a SNAE to be captured.

In conclusion, this study adds to the growing body of literature that suggests any level of LLV may be associated with adverse clinical outcomes. Cross-cohort collaborations, to increase sample size and power, should be pursued to definitively answer the question of whether LLV confers an increased risk for SNAEs in the era of INSTI-based ART.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Patient consent statement. All individuals who participated in the HIV Natural History Study provided written informed consent. The HIV Natural History Study has been approved by the institutional review board of the Uniformed Services University of the Health Sciences and by the institutional review board of each participating center.

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