MAJOR ARTICLE

Liver Fibrosis by Transient Elastography and Virologic Outcomes After Introduction of Tenofovir in Lamivudine-Experienced Adults With HIV and Hepatitis B Virus Coinfection in Ghana

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Background. Antiretroviral treatment (ART) programs in sub-Saharan Africa have for many years included lamivudine as the sole hepatitis B virus (HBV) inhibitor. Long-term outcomes and the effects of introducing tenofovir as part of ART in these populations have not been characterized.

Methods. The study comprised a cross-sectional analysis of 106 human immunodeficiency virus (HIV)/HBV–coinfected subjects maintained on lamivudine, as well as a prospective analysis of 76 lamivudine-experienced subjects who introduced tenofovir. Patients underwent assessment of liver fibrosis by transient elastography (TE) and testing to characterize HIV type 1 (HIV-1) and HBV replication.

Results. After a median of 45 months of lamivudine treatment, HIV-1 RNA and HBV DNA were detectable in 35 of 106 (33.0%) and 54 of 106 (50.9%) subjects, respectively, with corresponding drug resistance rates of 17 of 106 (16.0%) and 31 of 106 (29.2%), respectively. Median TE values were 5.7 kPa (interquartile range, 4.7–7.2 kPa) and independently associated with HBV DNA load, aspartate aminotransferase levels, and platelet counts; 13 of 106 (12.3%) subjects had TE measurements >9.4 kPa. Twelve months after the first assessment, and a median of 7.8 months after introducing tenofovir, HBV DNA levels declined by a mean of 1.5 $\log_{10}$ IU/mL ($P < .001$). TE values changed by a mean of −0.2 kPa ($P = .097$), and declined significantly in subjects who had pretenofovir HBV DNA levels >2000 IU/mL (mean, −0.8 kPa; $P = .048$) or TE values >7.6 kPa (mean, −1.2 kPa; $P = .021$). HIV-1 RNA detection rates remained unchanged.

Conclusions. A proportion of HIV/HBV-coinfected patients on long-term lamivudine-containing ART had poor HIV and HBV suppression, drug resistance, and TE values indicative of advanced liver fibrosis. Tenofovir improved HBV control and reduced liver stiffness in subjects with high HBV DNA load and TE values.

Keywords. hepatitis B; lamivudine; tenofovir; transient elastography; Africa.

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Hepatitis B virus (HBV) coinfection with human immunodeficiency virus (HIV) is characterized by accelerated progression of liver fibrosis and enhanced risk of liver-related mortality [1]. Treatment guidelines recommend maximal suppression of both HIV and HBV with antiretroviral therapy (ART) regimens typically containing tenofovir plus lamivudine or emtricitabine [2].

In Western cohorts, HIV/HBV-coinfected patients receiving lamivudine as the sole HBV-active antiviral agent showed a high risk of virologic breakthrough with emergence of HBV drug resistance and progression of liver fibrosis [3–6].

In sub-Saharan Africa (SSA), 6%–25% of HIV-infected people are chronically coinfected with HBV [7]. Due to lack of routine HBV screening and limited availability of tenofovir, ART programs have for many years been "HBV-blind" and contained lamivudine as the sole HBV-active agent. Limited data suggest that levels of HBV replication and rates of emergent HBV drug resistance during lamivudine exposure vary geographically across SSA [8–14]. Data on the associated indices of liver disease are scarce. Following revised recommendations from the World Health Organization (WHO) [2], national ART programs in SSA are increasingly adopting tenofovir for first-line therapy, although access remains far from universal. In Western HBV-infected cohorts with and without HIV, therapy with tenofovir has been shown to result in regression of liver fibrosis [15–18], with histologic improvements documented after 1 year [17] and continuing at 5 years [16]. The extent to which tenofovir-containing ART can influence parameters of liver fibrosis in HIV/HBV-coinfected patients with long-term lamivudine exposure in SSA is unknown.

Transient elastography (TE) has been validated in Western cohorts for assessing liver disease of diverse etiologies, including chronic hepatitis B [19], and provides a simple option for the noninvasive evaluation of liver fibrosis in resource-limited settings. Evidence from Burkina Faso [20] and The Gambia [21] indicates that TE has high concordance with histologically determined hepatic fibrosis among patients with HBV infection. Two studies from Nigeria [22] and Uganda [23] also reported that HIV infection, HBV infection, and HBV DNA levels were each predictive of high TE measurements. The first study included 16 HIV/HBV-coinfected patients, whereas the second analyzed 94 HIV/HBV-coinfected patients who were naive to ART.

In this study, we evaluated hepatic fibrosis using TE and correlated the findings with simultaneously measured markers of liver disease and HIV and HBV replication among coinfected patients attending for HIV care in Ghana. The study comprised a cross-sectional analysis of tenofovir-naive, lamivudine-experienced subjects, and a prospective analysis of lamivudine-experienced subjects who were assessed before and after introduction of tenofovir as part of ART.

METHODS

Study Population

HEPIK (Hepatitis B Infection in Kumasi) is a prospective study of HIV/HBV-coinfected adults based at the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. The study received ethics approval from the Kwame Nkrumah University of Science and Technology, Ghana, and started recruitment in October 2010. Participants gave written informed consent.

Transient Elastography and Sampling

TE was performed in July 2011 and July 2012 using portable equipment (Fibroscan, Ecosens, France). Valid TE measurements showed an interquartile range/median ratio (IQR/M) ≤0.30 or IQR/M >0.30 with median readings <7.1 kPa [24]. Interpretative cutoffs for histologically defined Metavir scores were 5.9 kPa (F2, moderate fibrosis), 7.6 kPa (F3, advanced fibrosis), and 9.4 kPa (F4, cirrhosis), as determined for HIV/HBV coinfection [25]. Blood samples were collected at the time of TE. CD4 cell counts, full blood counts, and serum biochemistry were performed in the KATH diagnostic laboratory. Serum and plasma were stored at −80°C and shipped frozen to the United Kingdom for further testing. A random subset of 39 samples already tested for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) at KATH were retested in the United Kingdom; results showed excellent agreement (Pearson correlation coefficient, r^2 0.927 and 0.967, respectively; $P < .001$, analysis of variance). The AST-to-platelet ratio index (APRI) and Fibrosis-4 (FIB-4) predictive scores were calculated from standard equations [26].

HIV Status

Plasma HIV type 1 (HIV-1) RNA was quantified by RealTime HIV-1 assay (Abbott Diagnostics, UK). The lower limit of quantification (LLOQ) was 40 copies/mL. Samples with HIV-1 RNA >200 copies/mL underwent Sanger sequencing of HIV-1 reverse transcriptase (amino acids [aa] 1–323) and protease (aa 1–99) to detect drug resistance–associated mutations (RAMs) affecting the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs), as described [27]. HIV-1 RNA load and drug resistance were assessed retrospectively. Virologic monitoring was not part of routine care at KATH, and ART failure was defined by clinical and immunologic parameters.

Hepatitis B, C, and D Virus Status

Serum hepatitis B surface antigen (HBsAg) was detected by Determine HBsAg (Alere); Determine-negative samples were retested by Murex HBsAg enzyme immunoassay (Abbott Diagnostics). Hepatitis B e antigen (HBeAg) and anti-HBe antibody were measured by Architect (Abbott Diagnostics). Plasma HBV DNA was quantified by real-time polymerase chain reaction (PCR) as described elsewhere (LLOQ, 14 IU/mL) [8]. Samples with HBV DNA >100 IU/mL underwent sequencing of the HBV polymerase gene (aa 1–344) as described previously [8]. Real-time PCR was used to detect hepatitis C virus (HCV) RNA and hepatitis D virus (HDV) RNA (LLOQ, 50 IU/mL and 500 IU/mL, respectively). HDV antibody was measured by HDV total antibody DIA.PRO (Diagnostic Biomarkers, Italy). We did not rely on HCV antibody detection due to poor performance in this population [26].

Statistical Analysis

The correlation between HIV-1 RNA and HBV DNA detection was assessed by Spearman rank correlation coefficient. Characteristics of HBeAg-positive and HBeAg-negative subjects were compared by Fisher exact test, Mann–Whitney–Wilcoxon test, or independent-samples *t* test. Factors associated with TE measurements were analyzed by univariate and multivariable linear regression using stepwise selection. Variables considered were age, sex, body mass index, duration of ART and lamivudine exposure, platelet and CD4 cell counts, HBeAg status, and levels of hemoglobin, ALT, AST, HIV-1 RNA, and HBV DNA. Due to collinearity between some variables (eg, HBeAg status and HBV DNA load), sensitivity analyses were performed to select the best-fitting variable for each collinear pair. One outlier (TE measurement 75 kPa) was excluded due to distortion of the model. Changes in HIV-1 RNA, HBV DNA, and ALT and AST levels were analyzed using Wilcoxon signed-rank test, the sign test, or McNemar test for continuous and categorical variables, as appropriate. Changes in TE values were analyzed using paired *t* test on log-transformed values. Receiver operating characteristic (ROC) curves were used to assess APRI and FIB-4 for the prediction of liver stiffness values >7.6 kPa and >9.4 kPa. Analyses were performed with SPSS software, version 21 (IBM SPSS).

RESULTS

Study Participants

Between October 2010 and July 2012, 1643 consecutive adults underwent HBsAg testing, and 230 (14.0%; [95% confidence

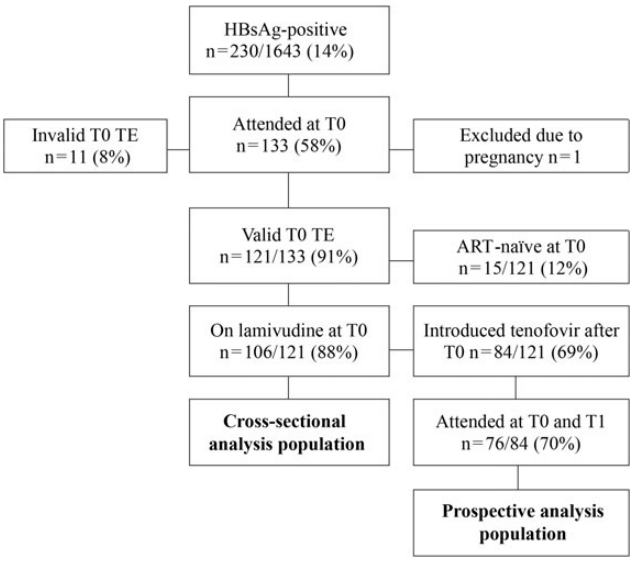

Figure 1. Flow diagram of study and analysis plan. Abbreviations: ART, antiretroviral therapy; HBsAg, hepatitis B surface antigen; T0, July 2011; T1, July 2012; TE, transient elastography.

Table 1. Characteristics of Tenofovir-Naive Human Immunodeficiency Virus/Hepatitis B Virus–Coinfected Subjects at the Time of Their First Transient Elastography

Characteristics	ART-Naive	On Lamivudine
No. (%)	15 (8.6)	106 (60.6)
Female, No. (%)	12 (80.0)	62 (58.5)
Age, y, median (IQR)	34 (29–38)	40 (36–47)
HIV diagnosis duration, mo, median (IQR)	13 (6–46)	51 (33–75)
BMI, kg/m^2, median (IQR)	23 (21–26)	24 (21–27)
Hemoglobin, g/dL, median (IQR)	12.0 (10.0–12.8)	12.6 (11.7–13.7)
Platelet count, $\times 10^9$ cells/L, median (IQR)	265 (213–365)	240 (188–283)
CD4 count, cells/μL, median (IQR)	614 (447–865)	571 (366–766)
ART duration, mo, median (IQR)	. . .	45 (27–64)
NNRTI-based ART, No. (%)	. . .	103 (97.2)
PI-based ART, No. (%)	. . .	3 (2.8)
Lamivudine duration, mo, median (IQR)	. . .	45 (27–64)
Zidovudine duration, mo, median (IQR)	. . .	22 (10–45)
Stavudine duration, mo, median (IQR)	. . .	4 (0–28)
Nevirapine duration, mo, median (IQR)	. . .	0 (0–38)
Efavirenz duration, mo, median (IQR)	. . .	13 (0–51)
HIV-1 RNA, $\log_{10}$ copies/mL, median (IQR)	4.8 (3.5–5.7)	UD (UD–2.2)
HBV DNA, $\log_{10}$ IU/mL, median (IQR)	2.9 (1.5–4.8)	1.2 (UD–6.3)
HBeAg-positive, No. (%)	3 (20.0)	32 (29.1)
Hepatitis C RNA positive, No. (%)	0 (0)	1 (0.9)
Hepatitis D antibody positive, No. (%)	0 (0)	2 (1.9)[a]
ALT, U/L, median (IQR)	28 (17–39)	25 (18–37)
AST, U/L, median (IQR)	30 (22–57)	29 (23–41)
APRI score, median (IQR)	0.3 (0.2–0.8)	0.3 (0.2–0.5)
FIB-4 score, median (IQR)	0.9 (0.7–1.4)	1.0 (0.8–1.7)
Liver stiffness, kPa, median (IQR)	6.9 (4.7–8.8)	5.7 (4.7–7.2)
Liver stiffness, kPa (categorised), No. (%)[b]		
<5.9 (F0/F1)	7 (46.7)	56 (52.8)
5.9–7.5 (F2)	3 (20.0)	26 (24.5)
7.6–9.3 (F3)	2 (13.0)	11 (10.4)
≥9.4 (F4)	3 (20.0)	13 (12.3)

Abbreviations: ALT, alanine aminotransferase; APRI, AST–to-platelet ratio index; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; FIB-4, fibrosis-4; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; UD, undetectable (HIV-1 RNA <40 copies/mL; HBV DNA <14 IU/mL).

[a] Both patients tested hepatitis D virus RNA negative.

[b] Metavir interpretive cutoffs based on Miailhes et al [25].

Table 2. Hepatitis B Virus–Related Parameters by Hepatitis B e Antigen Status at the Time of Transient Elastography Among Tenofovir-Naive Subjects Receiving Lamivudine-Based Antiretroviral Therapy

Characteristic	HBeAg Positive	HBeAg Negative	*P* Value
No.	30	76	. . .
Female, No. (%)	17 (56.7)	45 (59.2)	.83
Age, y, median (IQR)	44 (35–50)	40 (36–45)	.23
BMI, kg/m^2, median (IQR)	21.4 (19.0–26.0)	24.5 (22.0–27.5)	.03
Hemoglobin, g/dL, median (IQR)	12.8 (11.7–13.9)	12.6 (11.7–13.7)	.80
Platelet count, $\times 10^9$ cells/L, median (IQR)	211 (173–265)	253 (210–289)	.06
CD4 count, cells/μL, median (IQR)	516 (379–665)	589 (355–783)	.36
Lamivudine duration, mo, median (IQR)	49 (31–71)	42 (26–62)	.46
HIV-1 RNA, $\log_{10}$ copies/mL, median (IQR)	UD (UD–2.5)	UD (UD–2.0)	.65
HBV DNA, $\log_{10}$ IU/mL, median (IQR)	7.1 (4.5–8.3)	UD (UD–2.2)	<.001
ALT, U/L, median (IQR)	31 (20–52)	24 (16–33)	.03
AST, U/L, median (IQR)	34 (26–54)	28 (22–36)	.03
APRI score, median (IQR)	0.4 (0.3–0.7)	0.3 (0.2–0.4)	.004
FIB-4 score, median (IQR)	1.3 (0.9–2.2)	0.9 (0.7–1.5)	.026
Liver stiffness, kPa, median (IQR)	7.0 (5.5–9.5)	5.4 (4.5–6.9)	.001
Liver stiffness, kPa (categorised), No. (%)[a]			
<5.9 (F0/F1)	10 (33.3)	46 (60.5)	.015
5.9–7.5 (F2)	8 (26.7)	18 (23.7)	
7.6–9.3 (F3)	4 (13.3)	7 (9.2)	
≥9.4 (F4)	8 (26.7)	5 (6.5)	
HBV DNA, No. (%)			
<14 IU/mL	3 (10.0)	49 (65.3)	<.001
14–99 IU/mL	0 (0)	7 (9.3)	. . .
100–1999 IU/mL	1 (3.3)	9 (12.0)	. . .
≥2000 IU/mL	26 (86.7)	11 (14.5)	. . .
HBV sequences, No. (%)[b]	26 (86.7)	18 (23.7)	. . .
HBV RAMs, No. (%)	22 (73.3)	9 (11.8)	<.001
M204V, V173L, L180M	12	5	. . .
M204V, L180M	6	1	. . .
M204I, L80I	1	1	. . .
M204V/I, L180M, L80I/V	2	1	. . .
M204I, A181S	0	1	. . .
M204I, L180M	1	0	. . .

Abbreviations: ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; FIB-4, Fibrosis-4; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; RAM, resistance-associated mutation; UD, undetectable (HIV-1 RNA <40 copies/mL; HBV DNA <14 IU/mL).

[a] Metavir interpretive cutoffs based on Miailhes et al [25].

[b] The HBV drug resistance analysis (HBV DNA >100 IU/mL) comprised 47 subjects, 44 of whom yielded a sequence. The median HBV DNA load was 7.4 (IQR, 6.2–8.4) vs 3.4 (IQR, 2.6–6.2) $\log_{10}$ IU/mL in subjects with vs those without HBV RAMs ($P < .001$). HBV genotypes were E (n = 43) and A1 (n = 1).

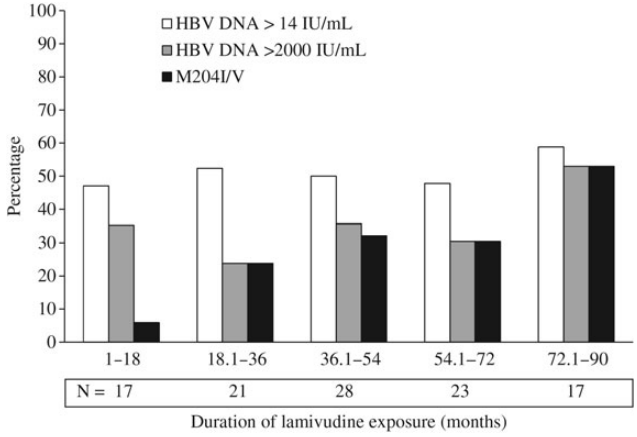

Figure 2. Hepatitis B virus (HBV) DNA levels and prevalence of the HBV lamivudine resistance-associated mutations M204I and M204V according to duration of lamivudine exposure.

interval {CI}, 12.4%–15.8%]) had positive results (220 Determine-positive; 10 Determine-negative/Murex-positive). Among the 230 HBsAg-positive subjects, 5 were also positive for HDV antibody (2.1% [95% CI, .8%–5.1%]), of whom 2 had detectable HDV RNA (0.9% [95% CI, .03%–3.3%]); 2 others had detectable HCV RNA (0.9% [95% CI, .03%–3.3%]). All HBsAg-positive subjects were invited to attend for TE and sampling, and travel expenses were reimbursed. Overall, 133 of 230 (57.8%) subjects attended, and 121 of 133 (91.0%) had a valid TE result (Figure 1). Relative to the 133 subjects who attended, the 97 HBsAg-positive subjects excluded from this analysis were more likely to be female (62/106 [71.1%] vs 69/97 [58.5%]; $P = .078$), with a lower CD4 cell count (median, 402 vs 570 cells/μL; $P < .001$), but with no difference in age (median, 38 vs 40 years; $P = .13$).

Cross-sectional Analysis of Tenofovir-Naive Subjects Receiving Lamivudine

The characteristics of the study population are summarized in Table 1. At the time of TE, 15 of 121 (12.4%) subjects were ART naive, whereas 106 of 121 (87.6%) were receiving lamivudine in combination with zidovudine (89/106 [84.0%]) or stavudine (17/106 [16.0%]), plus efavirenz (60/106 [56.6%]), nevirapine (43/108 [38.9%]), or a PI (3/106 [4.5%]; lopinavir/ritonavir or nelfinavir). After a median of 45 months of ART, plasma HIV-1 RNA was <40 copies/mL in 71 of 106 (67%) subjects and >1000 copies/mL in 21 of 106 (19.8%) subjects. Resistance testing was successful in 23 of 25 subjects with HIV-1 RNA >200 copies/mL. Overall, 17 of 106 (16%) subjects harbored ≥1 HIV-1 RAM, predominantly affecting the NRTIs (n = 15) and the NNRTIs (n = 16); 14 of 106 (13.2%) subjects had dual NRTI and NNRTI resistance. NRTI RAMs comprised M184V in all 15 cases; 3 subjects also showed the thymidine

Table 3. Factors Associated With Liver Stiffness Among Tenofovir-Naive Subjects Receiving Lamivudine-Based Antiretroviral Therapy[a]

	Univariate Analysis			Multivariable Analysis		
Characteristics	Coefficient	95% CI	*P* Value	Coefficient	95% CI	*P* Value
Sex, male	0.44	−1.49 to 2.36	.653			
Age, y	0.10	−.01 to .21	.083			
HIV diagnosis duration, mo	0.03	.00 to .07	.058			
BMI, kg/m^2	−0.04	−.25 to .16	.685			
Hemoglobin, g/dL	−0.84	−.68 to .52	.782			
Platelet count, ×10^9 cells/L	−0.02	−.03 to −.01	.002	−0.01	−.02 to .00	.022
CD4 count, cells/μL	0.00	.00 to .00	.623			
Lamivudine duration, mo	0.04	.00 to .08	.033			
Stavudine duration, mo	0.00	−.04 to .05	.858			
Nevirapine duration, mo	0.01	−.03 to .05	.579			
HIV-1 RNA, log$_{10}$ copies/mL	−0.27	−1.07 to .53	.510			
HBV DNA, log$_{10}$ IU/mL	0.74	.43 to 1.04	<.001	0.54	.23 to .85	.001
HBeAg status, positive	3.14	1.13 to 5.15	.003			
ALT, U/L	0.08	.03 to .14	.004			
AST, U/L	0.09	.05 to .14	<.001	0.05	.01 to .10	.02

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus.

[a] Coefficients describe unit increase or decrease in each variable per 1-kPa increase in liver stiffness. The linear regression equation is given by transient elastography [kPa] = 6.37 + 0.54 (HBV DNA [log$_{10}$ IU/mL]) + 0.05(AST [U/L]) − 0.01(platelet count [10^9/L]).

analogue mutations T215F, T215NSTY, and D67N + K70R + K219Q, respectively. No major PI RAMs were identified.

After a median of 45 months of lamivudine, HBV DNA was <14 IU/mL in 52 of 106 (49.1%) subjects. Among subjects with detectable HBV DNA, 23 of 54 (42.6%) also had detectable HIV-1 RNA (Spearman ρ = 0.21; *P* = .033). Resistance testing was successful in 44 of 47 subjects with HBV DNA >100 IU/mL. Overall, 31 of 106 (29.2%) subjects harbored ≥1 HBV RAM, comprising M204I or M204V in all cases, and commonly accompanied by ≥1 compensatory mutation (L80I, V173L, L180M) (Table 2). The prevalence of M204I/V was 5.9% in 17 subjects with ≤18 months of lamivudine exposure, and 52.9% in 17 subjects with between 72 and 90 months of exposure (Figure 2). HBeAg-positive subjects (30/106 [28.3%]) had higher HBV DNA levels, prevalence of HBV RAMs, ALT and AST levels, and APRI and FIB-4 scores than HBeAg-negative subjects, whereas their body mass index and platelet counts were lower (Table 2).

Median TE values were 5.7 kPa (IQR, 4.7–7.2 kPa) in the total population on lamivudine, 7.0 kPa (IQR, 5.5–9.5 kPa) in HBeAg-positive subjects, and 5.4 kPa (IQR, 4.5–6.9 kPa) in HBeAg-negative subjects (*P* = .001) (Table 2). By univariate analysis, longer duration of lamivudine exposure was associated with higher TE measurements. By multivariable analysis, 3 variables—higher HBV DNA load, higher AST levels, and lower platelet counts—were independently associated with increased liver stiffness (Table 3). The linear regression equation for the TE measurement was given as follows: TE [kPa] = 6.37 + 0.54 (HBV DNA [log$_{10}$ IU/mL]) + 0.05(AST [U/L]) − 0.01(platelet count [10^9/L]). The model adjusted R^2 was 0.280. Including APRI in place of AST and platelets improved the model fit (adjusted R^2 = 0.330). The areas under the ROC curve for TE measurements >7.6 kPa and >9.4 kPa were 0.73 and 0.85, respectively, with APRI, and 0.65 and 0.79, respectively, with FIB-4 (Supplementary Figure 1).

Prospective Analysis of Lamivudine-Experienced Subjects Who Introduced Tenofovir

A median of 4.4 months (IQR, 2.8–7.1 months) after undergoing a valid TE, a subset of 76 subjects introduced tenofovir as part of ART, usually (75/76 [98.7%]) while continuing lamivudine. After an additional median of 7.8 months (IQR, 6.1–9.3 months), the patients underwent a second TE. Changes in TE values and HIV and HBV virologic status between the first (time zero [T0]: July 2011) and the second (T1: July 2012) assessment are shown in Tables 4 and 5. There was no significant change in the proportion of subjects with HIV-1 RNA >40 copies/mL (25% at both T0 and T1) and >1000 copies/mL (16% at T0 vs 14% at T1). HBV DNA levels declined by a mean of −1.5 log$_{10}$ IU/mL (95% CI, −2.1 to −.9; *P* < .001), which reduced HBV DNA detection rates and proportions with HBV DNA >2000 IU/mL (Table 4). HBV DNA levels declined by a mean of −4.3 log$_{10}$ IU/mL in subjects who at T0 were HBeAg positive (95% CI, −5.3 to −3.3; *P* < .001), and by a mean of −4.9 log$_{10}$ IU/

Table 4. Comparison of Subject Characteristics Before (T0) and After (T1) Introduction of Tenofovir[a]

Characteristics	T0	T1	*P* Value
Total No.	76	76	. . .
Lamivudine duration, mo, median (IQR)	48 (26–63)	60 (38–75)	. . .
Tenofovir duration, mo, median (IQR)	0 (0, 0)	7.8 (6.1–9.3)	. . .
BMI, kg/m^2, median (IQR)	24.0 (21.1–27.1)	25.0 (21.8–26.3)	.19
Hemoglobin, g/dL, median (IQR)	12.7 (11.7–13.8)	12.5 (11.9–13.8)	.12
Platelet count ×10^9 cells/μL, median (IQR)	249 (211–303)	254 (217–308)	.28
CD4 count, cells/μL, median (IQR)	586 (355–767)	616 (387–775)	.97
NNRTI-based ART, No. (%)	73 (96.1)	70 (92.1)	.49
PI-based ART, No. (%)	3 (3.9)	6 (7.9)	
HIV-1 RNA, log$_{10}$ copies/mL, median (IQR)	UD (UD–1.6)	UD (UD–1.6)	.85
HBeAg-positive, No. (%)	23 (30)	18 (24)	.06
HBV DNA, log$_{10}$ IU/mL, median (IQR)	UD (UD–6.0)	UD (UD–UD)	<.001
HBV DNA, No. (%)			
<14 IU/mL	44 (57.9)	55 (78.6)	.001
14–99 IU/mL	4 (5.3)	2 (2.9)	. . .
100–1999 IU/mL	4 (5.3)	8 (11.4)	. . .
≥2000 IU/mL	24 (31.6)	5 (7.1)	. . .
HBV sequences, No. (%)[b]	27	12	. . .
HBV RAMs, No. (%)	19 (25)	11 (15)[c]	.04
M204V, V173L, L180M	10	6	. . .
M204V, L180M	5	3	. . .
M204I/V, L80I, L180M	2	0	. . .
M204I, L80I	1	2	. . .
M204I, A181S	1	0	. . .
ALT, U/L, median (IQR)	24 (17–34)	24 (18–33)	.96
AST, U/L, median (IQR)	28 (24–37)	26 (22–36)	.37
Liver stiffness, kPa, median (IQR)	5.5 (4.7–7.1)	5.5 (4.4–6.4)	.06
Liver stiffness ≥9.4 kPa, No. (%)	7 (9)	4 (5)	.38

Abbreviations: ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RAM, resistance-associated mutation; T0, July 2011; T1, July 2012; UD, undetectable (HIV-1 RNA <40 copies/mL; HBV DNA <14 IU/mL).

[a] Seventy-six subjects (59% female) underwent assessment in July 2011 (T0), introduced tenofovir a median of 4 months later, and underwent a second assessment in July 2012 (T1). At T0, subjects had a median age of 40 years (IQR, 36–46 years) and 48 months of ART (IQR, 26–63 months).

[b] The HBV drug resistance analysis (HBV DNA >100 IU/mL) comprised 28 subjects at T0 and 13 subjects at T1, and 27 and 12, respectively, yielded a sequence.

[c] Only 2 of 11 subjects had new HBV RAMs (M204V + L180M) at T1, comprising 1 patient with HBV DNA <14 IU/mL at T0 and 1475 IU/mL at T1, and 1 with HBV DNA 7.6 log$_{10}$ IU/mL and no HBV RAMs at T0 and 2.9 log$_{10}$ IU/mL at T1.

mL in those who had HBV RAMs (95% CI, −5.7 to −4.1; $P < .001$) (Figure 3). Five of 23 (21.7%) HBeAg-positive subjects lost HBeAg, and 1 acquired anti-HBe antibody at T1. There was no significant change in ALT and AST levels either overall or by HBeAg status (Supplementary Table 1). The mean change in TE values was −0.23 kPa (95% CI −.72 to .25; $P = .097$), and this reduced the proportion of subjects with TE scores >9.4 kPa (Figure 4). The largest reductions in TE values were seen in those subjects that pretenofovir were HBeAg positive, had HBV DNA levels >2000 IU/mL, or TE measurements >7.6 kPa (Table 5).

DISCUSSION

This study presents the first analysis of liver fibrosis by TE, and associated markers of liver disease and virologic status, among HIV/HBV-coinfected subjects with long-term lamivudine exposure in SSA, and is the first to analyze prospectively the effect of introducing tenofovir in such populations. At 14.0%, HBsAg seroprevalence in the Kumasi cohort was high, whereas HCV or HDV infection was rare. After nearly 4 years of lamivudine-containing ART, more than half of patients had persistent HBV replication, one-third had HBV DNA levels >2000 IU/mL, nearly one-third had HBV drug resistance, and 1 in 8 had TE measurements consistent with advanced fibrosis. HBV responses to the introduction of tenofovir, while usually continuing lamivudine, were highly encouraging, with marked reductions in HBV DNA levels and reduced TE measurements in those with higher baseline measurements.

The virologic expression of HIV/HBV coinfection varies across SSA, and the underlying determinants are poorly understood. Observational studies suggest mild outcomes in cohorts receiving lamivudine-containing ART without tenofovir in Kenya, Cameroon, and southern Africa, with high rates of HBV DNA suppression and a low risk of HBV drug resistance [9–14]. In contrast, coinfected patients in Malawi show poor HBV DNA suppression and rapid emergence of HBV drug resistance after starting lamivudine [8]. Previous reports analyzed cohorts with 12–24 months of lamivudine exposure. In Kumasi, after a median of 45 months of treatment with lamivudine, HBV DNA suppression rates were 9% in HBeAg-positive subjects and 63% HBeAg-negative subjects, and HBV DNA load, prevalence of HBV drug resistance, and liver stiffness were progressively higher in subjects with longer duration of exposure. The prevalence of HBV drug resistance was 29% overall, increasing from 6% in subjects with ≤18 months of lamivudine exposure to 53% in those treated for between 72 and 90 months. As expected [8], HBeAg status and HBV DNA levels influenced the rates of HBV resistance.

The HBV mutation patterns were those classically associated with prolonged lamivudine exposure, primarily M204I/V plus

Table 5. Results of Transient Elastography (TE) at T0 and T1 According to Hepatitis B e Antigen Status, Hepatitis B Virus DNA Levels, and TE Values at T0

T0 Status	No.	Median kPa (IQR)		Mean Change (95% CI)	*P* Value[a]
		T0	T1		
All patients	76	5.5 (4.7–7.1)	5.4 (4.5–6.4)	−0.2 (−.7 to .3)	.097
HBeAg positive	23	6.2 (5.0–7.9)	5.6 (4.3–6.9)	−0.5 (−1.8 to .7)	.026
HBeAg negative	53	5.4 (4.5–6.8)	5.3 (4.6–6.4)	−0.1 (−.6 to .3)	.56
HBV DNA >2000 IU/mL	24	7.0 (5.4–9.8)	5.9 (4.5–8.9)	−0.8 (−2.2 to .5)	.048
HBV DNA <2000 IU/mL	52	5.3 (4.5–6.3)	5.3 (4.4–6.1)	−0.1 (−.5 to .4)	.50
HBV DNA >20 000 IU/mL	22	7.0 (5.2–9.9)	5.9 (4.4–9.0)	−0.7 (−2.1 to .7)	.028
HBV DNA <20 000 IU/mL	54	5.4 (4.5–6.4)	5.3 (4.5–6.1)	−0.1 (−.5 to .4)	.56
TE at T0 >7.6 kPa	63	9.7 (8.1–15.4)	8.3 (5.7–14.0)	−1.2 (−3.5 to 1.2)	.021
TE at T0 ≤7.6 kPa	13	5.3 (4.5–6.2)	5.3 (4.3–6.1)	−0.0 (−.4 to .3)	.46

Abbreviations: CI, confidence interval; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; IQR, interquartile range; T0, July 2011; T1, July 2012; TE, transient elastography.

[a] Log_{10}-transformed paired-samples *t* test.

compensatory mutations that restore viral fitness, explaining the high HBV DNA load measured in subjects with resistance. The patterns predicted cross-resistance to lamivudine, emtricitabine, telbivudine, and entecavir; 2 subjects showed A181S and A181T, which also confer resistance to adefovir and, to an extent, tenofovir. Thus, the majority of patients were expected to respond virologically to tenofovir. Indeed, after a median of 7.8 months of tenofovir, usually with ongoing lamivudine, both HBV DNA load and liver stiffness were reduced, and marked decreases were seen in those subjects who, pretenofovir, had shown high HBV DNA levels, the presence of HBV RAMs, and high TE measurements. The findings are thus consistent with studies in Western settings that used either liver biopsies or TE to monitor changes in fibrosis in patients with HBV or HIV/HBV coinfection receiving tenofovir [16–18, 28].

TE provides a simple and validated measure of liver fibrosis, with several advantages relative to biopsy including portability, increased volume of liver sampled, reduced diagnostic error in nonhomogenous fibrosis, and avoidance of adverse events [19–21, 28, 29]. We found that HBV DNA load was the factor most strongly associated with TE measurements. This is consistent with findings from ART-naive, HIV/HBV-coinfected subjects in Nigeria [22]. In HBV-infected subjects in Taiwan, HBV DNA load was similarly the strongest predictor of progression to cirrhosis over 11 years of follow-up [30]. AST levels and platelet counts and the APRI score were also associated with liver stiffness. An APRI cutoff score of 0.56 excluded TE measurements ≥9.4 kPa with a negative predictive value of 97%,

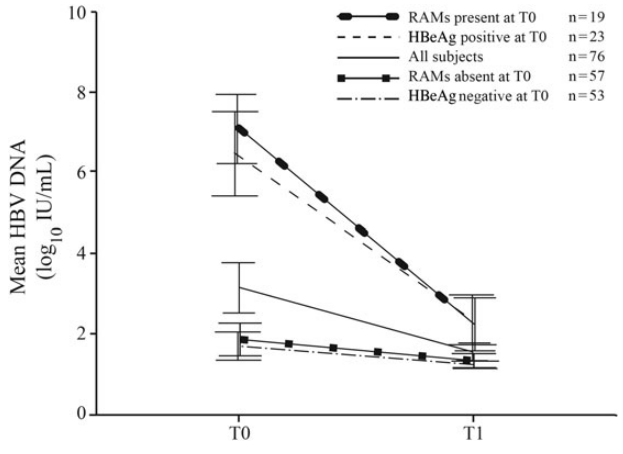

Figure 3. Change in hepatitis B virus (HBV) DNA levels between T0 and T1, stratified by hepatitis B e antigen (HBeAg) status and presence of HBV resistance-associated mutations (RAMs) at T0. Abbreviations: T0, July 2011; T1, July 2012.

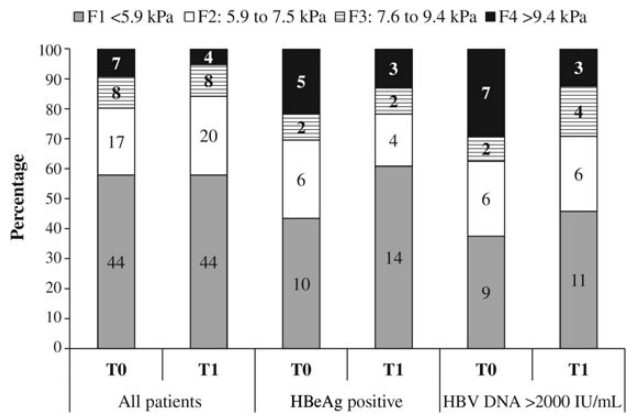

Figure 4. Change in transient elastography interpretative value categories between T0 and T1. Abbreviations: HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; T0, July 2011; T1, July 2012.

which suggests utility as a screening tool. This finding requires confirmation.

In previous studies in Burkina Faso, Cameroon, Cote d'Ivoire, Senegal, and Togo, HIV virologic failure rates ranged from 4% to 26% after 24–36 months of ART [31]. Long-term data are scarce. In the Kumasi cohort, the majority of patients were receiving NNRTI-based ART. After a median of 45 months of therapy, although immunologic responses were good, 21% of patients showed HIV-1 RNA levels >1000 copies/mL, the WHO-defined threshold for virologic failure, and at least 16% had drug resistance. In the absence of virologic monitoring, however, changes to PI-based ART were uncommon in routine practice. There was an association between HIV-1 RNA detection and HBV DNA detection, possibly reflecting inadequate compliance to ART and documenting overall poor therapeutic efficacy.

There are limitations to this study. Loss to follow-up is common in SSA, and our estimates are subject to survivorship and attrition bias; the rate of loss to follow-up is 10% per year in HEPIK. Although we reimbursed travel expenses, debilitated patients may have been unable to travel to clinic for TE. Sanger sequencing might have underestimated the prevalence of HIV and HBV drug resistance: we previously found that after just 6 months of lamivudine-containing ART, virtually all patients with persistent HBV viremia harbored M204I when tested by deep sequencing [8]. In this study, we were unable to use deep sequencing or sequence samples with HIV-1 RNA levels <200 copies/mL due to small volumes. As a consequence of limited local infrastructure, we had no assessment of treatment adherence and were unable to obtain liver biopsies, abdominal ultrasound scans, and more extensive biochemical panels to corroborate the TE findings. TE measurements may be falsely elevated in acute hepatitis, extrahepatic cholestasis, or, marginally, after a recent meal [32]. Only 1 patient had ALT levels more than twice above the upper limit, and excluding this patient did not affect the regression model (data not shown). ALT and AST were not markedly elevated, suggesting that HBV suppression is indeed the driver of improved TE measurements. We obtained bilirubin levels in 43 patients and none had raised values (data not shown). We did not require patients to attend for TE fasted, although scans took place in the mornings prior to lunch. Finally, we tested patients for HCV and HDV, but did not investigate other potential causes of liver disease (eg, schistosomiasis, alcohol abuse). Further studies are planned to ascertain the influence of these co-factors on liver disease in Ghana, and to measure the long-term efficacy of tenofovir.

Chronic viral hepatitis is a leading cause of morbidity and mortality in Western HIV-infected cohorts [33]. With expanded access to ART and reduced HIV-related mortality, there is potential for a high burden of liver disease to emerge in SSA, a risk amplified by high rates of HIV/HBV coinfection and lack of defined strategies for the diagnosis and management of viral hepatitis. Our findings that a substantial proportion of HIV/HBV-coinfected subjects in Kumasi were at risk of progressive liver disease has led to the adoption of routine HBV screening in the HIV clinic, and HBV-coinfected patients have been prioritized for tenofovir use as it becomes more widely available. These developments, together with the early responses to tenofovir documented in the study, offer encouragement that improved control of HBV coinfection is an achievable goal across Africa.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Lacombe K, Rockstroh J. HIV and viral hepatitis coinfections: advances and challenges. Gut **2012**; 61(suppl 1):i47–58.
2. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach. Geneva, Switzerland: WHO, **2013**.
3. Benhamou Y, Bochet M, Thibault V, et al. Long-term incidence of hepatitis B virus resistance to lamivudine in human immunodeficiency virus-infected patients. Hepatology **1999**; 30:1302–6.
4. Gish R, Jia JD, Locarnini S, Zoulim F. Selection of chronic hepatitis B therapy with high barrier to resistance. Lancet Infect Dis **2012**; 12:341–53.
5. Lok AS, Lai CL, Leung N, et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology **2003**; 125: 1714–22.
6. Matthews GV, Bartholomeusz A, Locarnini S, et al. Characteristics of drug resistant HBV in an international collaborative study of HIV-HBV–infected individuals on extended lamivudine therapy. AIDS **2006**; 20:863–70.
7. Matthews PC, Geretti AM, Goulder PJ, Klenerman P. Epidemiology and impact of HIV coinfection with hepatitis B and hepatitis C viruses in sub-Saharan Africa. J Clin Virol **2014**; 61:20–33.

8. Aoudjane S, Chaponda M, Gonzalez Del Castillo AA, et al. Hepatitis B virus sub-genotype A1 infection is characterized by high replication levels and rapid emergence of drug resistance in HIV-positive adults receiving first-line antiretroviral therapy in Malawi. Clin Infect Dis **2014**; 59:1618–26.
9. Day SL, Odem-Davis K, Mandaliya KN, et al. Prevalence, clinical and virologic outcomes of hepatitis B virus co-infection in HIV-1 positive Kenyan women on antiretroviral therapy. PLoS One **2013**; 8:e59346.
10. Hamers RL, Zaaijer HL, Wallis CL, et al. HIV-HBV coinfection in southern Africa and the effect of lamivudine- versus tenofovir-containing cART on HBV outcomes. J Acquir Immune Defic Syndr **2013**; 64:174–82.
11. Honge BL, Jespersen S, Medina C, et al. Hepatitis B and Delta virus are prevalent but often subclinical co-infections among HIV infected patients in Guinea-Bissau, West Africa: a cross-sectional study. PLoS One **2014**; 9:e99971.
12. Ive P, MacLeod W, Mkumla N, et al. Low prevalence of liver disease but regional differences in HBV treatment characteristics mark HIV/HBV co-infection in a South African HIV clinical trial. PLoS One **2013**; 8: e74900.
13. Kim HN, Scott J, Cent A, et al. HBV lamivudine resistance among hepatitis B and HIV coinfected patients starting lamivudine, stavudine and nevirapine in Kenya. J Viral Hepat **2011**; 18:e447–52.
14. Kouanfack C, Aghokeng AF, Mondain AM, et al. Lamivudine-resistant HBV infection in HIV-positive patients receiving antiretroviral therapy in a public routine clinic in Cameroon. Antivir Ther **2012**; 17:321–6.
15. Boyd A, Lasnier E, Molina JM, et al. Liver fibrosis changes in HIV-HBV-coinfected patients: clinical, biochemical and histological effect of long-term tenofovir disoproxil fumarate use. Antivir Ther **2010**; 15:963–74.
16. Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet **2013**; 381:468–75.
17. Marcellin P, Heathcote EJ, Buti M, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med **2008**; 359:2442–55.
18. Martin-Carbonero L, Teixeira T, Poveda E, et al. Clinical and virological outcomes in HIV-infected patients with chronic hepatitis B on long-term nucleos(t)ide analogues. AIDS **2011**; 25:73–9.
19. Chon YE, Choi EH, Song KJ, et al. Performance of transient elastography for the staging of liver fibrosis in patients with chronic hepatitis B: a meta-analysis. PLoS One **2012**; 7:e44930.
20. Bonnard P, Sombie R, Lescure FX, et al. Comparison of elastography, serum marker scores, and histology for the assessment of liver fibrosis in hepatitis B virus (HBV)-infected patients in Burkina Faso. Am J Trop Med Hyg **2010**; 82:454–8.
21. Lemoine M, Shimakawa Y, Goldin R, et al. P1019 validation and comparison of non-invasive markers of liver fibrosis in West-African patients with chronic hepatitis B living in the Gambia. In: International Liver Congress 2014. 49th Annual meeting of the European Association for the Study of the Liver, Vol 60. London, UK: Elsevier, **2014**:S414–5.
22. Hawkins C, Agbaji O, Ugoagwu P, et al. Assessment of liver fibrosis by transient elastography in patients with HIV and hepatitis B virus coinfection in Nigeria. Clin Infect Dis **2013**; 57:e189–92.
23. Stabinski L, Reynolds SJ, Ocama P, et al. High prevalence of liver fibrosis associated with HIV infection: a study in rural Rakai, Uganda. Antivir Ther **2011**; 16:405–11.
24. Boursier J, Zarski JP, de Ledinghen V, et al. Determination of reliability criteria for liver stiffness evaluation by transient elastography. Hepatology **2013**; 57:1182–91.
25. Miailhes P, Pradat P, Chevallier M, et al. Proficiency of transient elastography compared to liver biopsy for the assessment of fibrosis in HIV/HBV-coinfected patients. J Viral Hepat **2011**; 18:61–9.
26. Xu XY, Kong H, Song RX, et al. The effectiveness of noninvasive biomarkers to predict hepatitis B-related significant fibrosis and cirrhosis: a systematic review and meta-analysis of diagnostic test accuracy. PLoS One **2014**; 9:e100182.
27. Doyle T, Smith C, Vitiello P, et al. Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. Clin Infect Dis **2012**; 54:724–32.
28. Tsochatzis EA, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. J Hepatol **2011**; 54:650–9.
29. Singh S, Fujii LL, Murad MH, et al. Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis. Clin Gastroenterol Hepatol **2013**; 11:1573–84 e1–2; quiz e88–9.
30. Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology **2006**; 130:678–86.
31. Aghokeng AF, Monleau M, Eymard-Duvernay S, et al. Extraordinary heterogeneity of virological outcomes in patients receiving highly antiretroviral therapy and monitored with the World Health Organization public health approach in sub-Saharan Africa and southeast Asia. Clin Infect Dis **2014**; 58:99–109.
32. Arena U, Lupsor Platon M, Stasi C, et al. Liver stiffness is influenced by a standardized meal in patients with chronic hepatitis C virus at different stages of fibrotic evolution. Hepatology **2013**; 58:65–72.
33. Lemoine M, Eholie S, Lacombe K. Reducing the neglected burden of viral hepatitis in Africa: strategies for a global approach. J Hepatol **2015**; 62:469–76.