**Liver fibrosis by transient elastography and virologic outcomes after introduction of tenofovir in lamivudine-experienced adults with HIV and hepatitis B virus (HBV) co-infection in Ghana**

**Authors**

Alexander J Stockdale, MBChB, MRCP1; Richard Odame Phillips, FWACP, PhD2,3; Apostolos Beloukas, PhD1; Lambert Tetteh Appiah, MD3; David Chadwick, MRCP, PhD4; Sanjay Bhagani, FRCP5; Laura Bonnett, MMathStat PhD1,6; Fred Stephen Sarfo, MD, PhD2,3; Geoffrey Dusheiko, FRCP7; Anna Maria Geretti, MD, PhD1; - HEPIK Study Group.

1Institute of Infection & Global Health, University of Liverpool, Liverpool, United Kingdom (UK); 2Department of Medicine, Kwame Nkrumah University of Science & Technology and 3Komfo Anokye Teaching Hospital, Kumasi, Ghana; 4Centre for Clinical Infection, James Cook University Hospital, Middlesbrough, UK; 5Department of Infectious Diseases, Royal Free Hospital, London, UK; 6Department of Biostatistics, University of Liverpool, Liverpool, UK; 7Division of Medicine, University College London, London, UK.

**Keywords**: Hepatitis B, lamivudine, resistance, tenofovir, transient elastography, Africa

**Running title:** Outcomes of HIV/HBV co-infection in Ghana

**Word counts: Abstract 250, Article 2999**

**Contact information**

Prof Anna Maria Geretti, MD, PhD

Institute of Infection & Global Health

University of Liverpool, 8 West Derby Street L69 7BE, United Kingdom

+44 151 795 9665; [geretti@liverpool.ac.uk](mailto:geretti@liverpool.ac.uk)

**Alternative contact**

Dr Alexander J Stockdale, MBChB

Institute of Infection & Global Health

University of Liverpool, 8 West Derby Street L69 7BE, United Kingdom

+44 151 795 9665; [A.Stockdale@liverpool.ac.uk](mailto:A.Stockdale@liverpool.ac.uk)

**Summary**

HIV/HBV co-infected patients on long-term lamivudine-containing ART demonstrated suboptimal HIV and HBV suppression, with lamivudine resistance and evidence of liver fibrosis by transient elastography. Both HBV DNA load and liver stiffness measurements declined after introduction of tenofovir.

**Abstract**

**Background**

Antiretroviral treatment (ART) programs in sub-Saharan Africa have for many years included lamivudine as the sole 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 HIV-HBV co-infected subjects maintained on lamivudine, and 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-1 and HBV replication.

**Results**

After median 45 months of lamivudine, HIV-1 RNA and HBV DNA were detectable in 35/106 (33.0%) and 54/106 (50.9%) subjects respectively, with corresponding drug-resistance rates of 17/106 (16.0%) and 31/106 (29.2%). TE values were median 5.7 kPa (IQR 4.7, 7.2) and independently associated with HBV DNA load, AST levels, and platelet counts; 13/106 (12.3%) subjects had TE measurements >9.4 kPa. Twelve months after the first assessment, and median 7.8 months after introducing tenofovir, HBV DNA levels declined by mean 1.5 log10 IU/ml (p<0.001). TE values changed by mean -0.2 kPa (p=0.097), and declined significantly in subjects that pre-tenofovir had HBV DNA levels >2000 IU/ml (mean -0.8 kPa; p=0.048) or TE values >7.6 kPa (mean -1.2 kPa; p=0.021). HIV-1 RNA detection rates remained unchanged.

**Conclusions**

A proportion of HIV/HBV co-infected 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.

**Introduction**

HBV co-infection with 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 ART regimens typically containing tenofovir plus lamivudine or emtricitabine.[2] In Western cohorts, HIV/HBV co-infected 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-positive people are chronically co-infected 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 Organisation (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 histological improvements documented after one year [17] and continuing at five years [16]. The extent to which tenofovir-containing ART can influence parameters of liver fibrosis in HIV/HBV co-infected patients with long-term lamivudine exposure in SSA is unknown.

Transient elastography 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 non-invasive 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 co-infected subjects, whereas the second analyzed 94 HIV/HBV co-infected patients that were naïve 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 co-infected patients attending for HIV care in Ghana. The study comprised a cross-sectional analysis of tenofovir-naïve, 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 co-infected 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 cut-offs 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 co-infection.[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 -80oC and shipped frozen to the United Kingdom (UK) for further testing. A random subset of 39 samples already tested for alanine and aspartate aminotransferases (ALT, AST) at KATH were retested in the UK; results showed excellent agreement (Pearson’s correlation coefficients r2 0.927 and 0.967, respectively; p<0.001, ANOVA). The AST to Platelet Ratio Index (APRI)and Fibrosis-4 (FIB-4)predictive scores were calculated from standard equations. [27]

*HIV status*

Plasma HIV-1 RNA was quantified by RealTime HIV-1 (Abbott Diagnostics, UK). The lower limit of quantification (LLQ) 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), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs), as described.[26]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 immunological parameters.

*HBV, HCV, HDV status*

Serum HBsAg was detected by Determine HBsAg (Alere); Determine-negative samples were retested by Murex HBsAg EIA (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 PCR as described (LLQ 14 IU/ml).[8] Samples with HBV DNA >100 IU/ml underwent sequencing of the HBV polymerase gene (aa1-344) as described.[8]Real-time PCR was used to detect Hepatitis C virus (HCV) RNA and Hepatitis D virus (HDV) RNA (LLQ 50 IU/ml and 500 IU/ml, respectively). HDV antibody was measured by HDV total Ab DIA.PRO (Diagnostic Biomarkers, Italy). We did not rely on HCV antibody detection due to poor performance in this population. [27]

*Statistical analysis*

The correlation between HIV-1 RNA and HBV DNA detection was assessed by Spearman’s Rank Correlation Coefficient. Characteristics of HBeAg-positive and HBeAg-negative subjects were compared by Fisher’s exact test, Mann-Whitney-Wilcoxon, 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, gender, 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 co-linearity between some variables (e.g., 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, ALT and AST levels were analyzed using Wilcoxon-Signed ranks test, the sign test, or McNemar’s 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 >7.6kPa and >9.4kPa. Analyses were performed with SPSS v.21 (IBM, USA).

**RESULTS**

**Study participants**

Between October 2010 and July 2012, 1643 consecutive adults underwent HBsAg testing and 230 (14.0%, 95% confidence interval, CI 12.4-15.8%) were positive (220 Determine-positive; 10 Determine-negative/Murex-positive). Among the 230 HBsAg-positive subjects, five were also positive for HDV antibody (2.1%; 95% CI 0.8-5.1%), of which two had detectable HDV RNA (0.9%; 95% CI 0.03-3.3%); two others had detectable HCV RNA (0.9%; 95% CI 0.03-3.3%). All HBsAg-positive subjects were invited to attend for TE and sampling, and travel expenses were reimbursed. Overall 133/230 (57.8%) subjects attended, and 121/133 (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=0.078), with a lower CD4 cell count (median 402 vs 570 cells/mm3; p<0.001), but with no difference in age (median 38 vs 40 years; p=0.13).

**Cross-sectional analysis of tenofovir-naïve subjects receiving lamivudine**

The characteristics of the study population are summarized in Table 1. At the time of TE, 15/121 (12.4%) subjects were ART-naïve, whereas 106/121 (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 median 45 months of ART, plasma HIV-1 RNA was <40 copies/ml in 71/106 (67%) subjects and >1000 copies/ml in 21/106 (19.8%) subjects. Resistance testing was successful in 23/25 subjects with HIV-1 RNA >200 copies/ml. Overall 17/106 (16%) subjects harbored ≥1 HIV-1 RAM, predominantly affecting the NRTIs (n=15) and the NNRTIs (n=16); 14/106 (13.2%) subjects had dual NRTI and NNRTI resistance. NRTI RAMs comprised M184V in all 15 cases; 3 subjects also showed the thymidine analogue mutations (TAMs) T215F, T215NSTY, and D67N+K70R+K219Q, respectively. No major PI RAMs were identified.

After median 45 months of lamivudine, HBV DNA was <14 IU/ml in 52/106 (49.1%) subjects. Among subjects with detectable HBV DNA, 23/54 (42.6%) also had detectable HIV-1 RNA (Spearman’s rho 0.21; p=0.033). Resistance testing was successful in 44/47 subjects with HBV DNA >100 IU/ml. Overall 31/106 (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 >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).

TE values in the total population on lamivudine were median 5.7 kPa (IQR 4.7, 7.2), and median 7.0 (IQR 5.5, 9.5) in HBeAg-positive subjects vs. 5.4 (IQR 4.5, 6.9) in HBeAg-negative subjects (p=0.001) (Table 2). By univariate analysis, longer duration of lamivudine exposure was associated with higher TE measurements. By multivariable analysis, three 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 by: TE (kPa) = 6.37 + 0.54(HBV DNA (log10 IU/ml)) + 0.05(AST (U/l)) – 0.01(Platelet count (109/l)). The model adjusted R2 was 0.280. Including APRI in place of AST and platelets improved the model fit (adjusted R2 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 that introduced tenofovir**

A median of 4.4 months (IQR 2.8, 7.1) 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 a further median 7.8 months (IQR 6.1, 9.3) 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 mean 1.5 log10 IU/ml (95% CI 2.1, 0.9; p<0.001), which reduced HBV DNA detection rates and proportions with HBV DNA >2000 IU/ml (Table 4). HBV DNA levels declined by mean 4.3 log10 IU/ml in subjects that at T0 were HBeAg-positive (95% CI 5.3, 3.3; p<0.001), and by mean 4.9 log10 IU/ml in those that had HBV RAMs (95% CI 5.7, 4.1; p<0.001) (Figure 3). Five of 23 (21.7%) HBeAg-positive subjects lost HBeAg and one acquired anti-HBe antibody at T1. There was no significant change in ALT and AST levels both overall and by HBeAg status (Supplementary Table 1). The mean change in TE values was -0.23 kPa (95% CI -0.72, 0.25; p=0.097), 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 pre-tenofovir 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 transient elastography, and associated markers of liver disease and virologic status, among HIV/HBV co-infected 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 four years of lamivudine-containing ART, over half of patients had persistent HBV replication, one third had HBV DNA levels >2000 IU/ml, nearly a third had HBV drug-resistance, and one in eight 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 reducing TE measurements in those with higher baseline measurements.

The virologic expression of HIV/HBV co-infection 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, co-infected 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 median 45 months of 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 >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 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; two 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 median 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 that pre-tenofovir 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 co-infection receiving tenofovir [16-18, 29].

Transient elastography 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 non-homogenous 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-naïve HIV/HBV co-infected 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 cut-off score of 0.56 excluded TE measurements ≥9.4 kPa with a negative predictive value of 97%, 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 median 45 months of therapy, although immunological 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 sub-Sahara Africa 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, extra-hepatic cholestasis, or marginally after a recent meal.[32]Only one patient had ALT levels more than twice above the upper limit and excluding this patient did not affect the regression model (not shown). ALT and AST were not markedly elevated suggesting HBV suppression is indeed the driver of improved TE measurements. We obtained bilirubin levels in 43 patients and none had raised values (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 (e.g., 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-positive 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 co-infection and lack of defined strategies for the diagnosis and management of viral hepatitis.Our findings that a substantial proportion of HIV/HBV co-infected 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 co-infected 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 co-infection is an achievable goal across Africa.

**Acknowledgements**

We thank all staff involved in the HEPIK study in Ghana and the United Kingdom, and the patients for their participation.

**Declaration of interests**

AMG, SB, GD and DC report personal fees and research grants from Gilead Sciences and Bristol-Myers Squibb, outside the submitted work.

**Source of funding**

The study was supported by a Leverhulme - Royal Society Africa award. An award made by the MAC AIDS Foundation to the Royal Free Charity supported insured transport to Ghana of the portable FibroScan. The funders had no role in the collection, analysis, and interpretation of the data, in the writing of the report, and in the decision to submit the paper for publication. The corresponding author (AMG) had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

**Table 1.** Characteristics of tenofovir-naive HIV/HBV co-infected subjects at the time of their first transient elastography

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Characteristics | | | ART-naive | On lamivudine |
| Number (%) | | | 15 (8.6) | 106 (60.6) |
| Female, n (%) | | | 12 (80.0) | 62 (58.5) |
| Age, median years (IQR) | | | 34 (29, 38) | 40 (36, 47) |
| HIV diagnosis duration, median months (IQR) | | | 13 (6, 46) | 51 (33, 75) |
| BMI, median kg/m2 (IQR) | | | 23 (21, 26) | 24 (21, 27) |
| Hemoglobin, median g/dl (IQR) | | | 12.0 (10.0, 12.8) | 12.6 (11.7, 13.7) |
| Platelet, median count x109/l (IQR) | | | 265 (213, 365) | 240 (188, 283) |
| CD4 count, median cells/mm3 (IQR) | | | 614 (447, 865) | 571 (366, 766) |
| ART duration, median months (IQR) | | | - | 45 (27, 64) |
| NNRTI-based ART, n (%) | | | - | 103 (97.2) |
| PI-based ART, n (%) | | | - | 3 (2.8) |
| Lamivudine duration, median months (IQR) | | | - | 45 (27, 64) |
| Zidovudine, duration, median months (IQR) | | | - | 22 (10, 45) |
| Stavudine duration, median months (IQR) | | | - | 4 (0, 28) |
| Nevirapine duration, median months (IQR) | | | - | 0 (0, 38) |
| Efavirenz duration, median months (IQR) | | | - | 13 (0, 51) |
| HIV-1 RNA, median log10 copies/ml (IQR) | | | 4.8 (3.5, 5.7) | UD (UD, 2.2) |
| HBV DNA, median log10 IU/ml (IQR) | | | 2.9 (1.5, 4.8) | 1.2 (UD, 6.3) |
| HBeAg-positive, n (%) | | | 3 (20.0) | 32 (29.1) |
| Hepatitis C RNA positive, n (%) | | | 0 (0) | 1 (0.9) |
| Hepatitis Delta antibody positive, n (%) | | | 0 (0) | 2 (1.9)**a** |
| ALT, median U/l (IQR) | | | 28 (17, 39) | 25 (18, 37) |
| AST, median U/l (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, median kPa (IQR) | | | 6.9 (4.7, 8.8) | 5.7 (4.7, 7.2) |
| kPa scores, n (%)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) |

aBoth patients tested HDV RNA negative. bMETAVIR interpretive cut-offs based on Mailes et al.[25] ART = Antiretroviral therapy; BMI = Body Mass Index; NNRTI = Non-nucleoside reverse transcriptase inhibitor; PI = Protease inhibitor; UD = Undetectable (HIV-1 RNA <40 copies/ml; HBV DNA <14 IU/ml); ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; APRI = AST to Platelet Ratio Index; FIB-4 = Fibrosis-4.

**Table 2.** HBV-related parameters by HBeAg status at the time of transient elastography among tenofovir-naive subjects receiving lamivudine-based antiretroviral therapy

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristics | | | | HBeAg-positive | HBeAg-negative | P value |
| Number | | | | 30 | 76 | - |
| Female, n (%) | | | | 17 (56.7) | 45 (59.2) | 0.83 |
| Age, median years (IQR) | | | | 44 (35, 50) | 40 (36, 45) | 0.23 |
| BMI, median kg/m2 (IQR) | | | | 21.4 (19.0, 26.0) | 24.5 (22.0, 27.5) | 0.03 |
| Hemoglobin, median g/dl (IQR) | | | | 12.8 (11.7, 13.9) | 12.6 (11.7, 13.7) | 0.80 |
| Platelet, median count x109/l (IQR) | | | | 211 (173, 265) | 253 (210, 289) | 0.06 |
| CD4 count, median cells/mm3 (IQR) | | | | 516 (379, 665) | 589 (355, 783) | 0.36 |
| Lamivudine duration, median months (IQR) | | | | 49 (31, 71) | 42 (26, 62) | 0.46 |
| HIV-1 RNA, median log10 copies/ml (IQR) | | | | UD (UD, 2.5) | UD (UD, 2.0) | 0.65 |
| HBV DNA, median log10 IU/ml (IQR) | | | | 7.1 (4.5, 8.3) | UD (UD, 2.2) | <0.001 |
| ALT, median U/l (IQR) | | | | 31 (20, 52) | 24 (16, 33) | 0.03 |
| AST, median U/l (IQR) | | | | 34 (26, 54) | 28 (22, 36) | 0.03 |
| APRI score, median (IQR) | | | | 0.4 (0.3, 0.7) | 0.3 (0.2, 0.4) | 0.004 |
| FIB-4 score, median (IQR) | | | | 1.3 (0.9, 2.2) | 0.9 (0.7, 1.5) | 0.026 |
| Liver stiffness, median kPa (IQR) | | | | 7.0 (5.5, 9.5) | 5.4 (4.5, 6.9) | 0.001 |
| kPa scores, n (%)a | | <5.9 (F0/F1) | | 10 (33.3) | 46 (60.5) | 0.015 |
|  | | 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, n (%) | <14 IU/ml | |  | 3 (10.0) | 49 (65.3) | <0.001 |
| 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, n (%)b | | | | 26 (86.7) | 18 (23.7) | - |
| HBV RAMs, n (%) | | | | 22 (73.3) | 9 (11.8) | <0.001 |
| 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 | - |

**a**METAVIR interpretive cut-offs based on Mailes et al.[25]**b**The HBV drug-resistance analysis (HBV DNA >100 IU/ml) comprised 47 subjects and 44 yielded a sequence. HBV DNA load was median 7.4 (IQR 6.2, 8.4) vs. 3.4 (IQR 2.6, 6.2) log10 IU/ml in subjects with vs. those without HBV RAMs (p<0.001). HBV genotypes were E (n=43) and A1 (n=1). BMI=Body Mass Index; UD = Undetectable (HIV-1 RNA <40 copies/ml; HBV DNA <14 IU/ml); ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; APRI = AST to Platelet Ratio Index; FIB-4 = Fibrosis-4; RAMs = Resistance-associated mutations

**Table 3.** Factors associated with liver stiffness among tenofovir-naïve subjects receiving lamivudine-based antiretroviral therapya

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristics | Univariate | | | Multivariable | | |
| Coefficient | 95% CI | P | Coefficient | 95% CI | P |
| Gender, male | 0.44 | -1.49, 2.36 | 0.653 |  |  |  |
| Age, years | 0.10 | -0.01, 0.21 | 0.083 |  |  |  |
| HIV diagnosis duration, months | 0.03 | 0.00, 0.07 | 0.058 |  |  |  |
| BMI, kg/m2 | -0.04 | -0.25, 0.16 | 0.685 |  |  |  |
| Hemoglobin, g/dl | -0.84 | -0.68, 0.52 | 0.782 |  |  |  |
| Platelet count, x109/l | -0.02 | -0.03, -0.01 | 0.002 | -0.01 | -0.02, 0.00 | 0.022 |
| CD4 count, cells/mm3 | 0.00 | 0.00, 0.00 | 0.623 |  |  |  |
| Lamivudine duration, months | 0.04 | 0.00, 0.08 | 0.033 |  |  |  |
| Stavudine duration, months | 0.00 | -0.04, 0.05 | 0.858 |  |  |  |
| Nevirapine duration, months | 0.01 | -0.03, 0.05 | 0.579 |  |  |  |
| HIV-1 RNA, log10 copies/ml | -0.27 | -1.07, 0.53 | 0.510 |  |  |  |
| HBV DNA, log10 IU/ml | 0.74 | 0.43, 1.04 | <0.001 | 0.54 | 0.23, 0.85 | 0.001 |
| HBeAg status, positive | 3.14 | 1.13, 5.15 | 0.003 |  |  |  |
| ALT, U/l | 0.08 | 0.03, 0.14 | 0.004 |  |  |  |
| AST, U/l | 0.09 | 0.05, 0.14 | <0.001 | 0.05 | 0.01, 0.10 | 0.02 |

aCoefficients describe unit increase or decrease in each variable per 1 kPa increase in liver stiffness. The linear regression equation is given by TE (kPa) = 6.37 + 0.54(HBV DNA (log10 IU/ml)) + 0.05(AST (U/l)) – 0.01(Platelet count (109/l)).

**Table 4.** Comparison of subjects’ characteristics before (T0) and after (T1) introduction of tenofovira

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Characteristics | | | T0 | T1 | P |
| Total number | | | 76 | 76 | - |
| Lamivudine duration, median months (IQR) | | | 48 (26, 63) | 60 (38, 75) | - |
| Tenofovir duration, median months (IQR) | | | 0 (0, 0) | 7.8 (6.1, 9.3) | - |
| BMI, median kg/m2 (IQR) | | | 24.0 (21.1, 27.1) | 25.0 (21.8, 26.3) | 0.19 |
| Hemoglobin, median g/dl (IQR) | | | 12.7 (11.7, 13.8) | 12.5 (11.9, 13.8) | 0.12 |
| Platelet, median count x109 (IQR) | | | 249 (211, 303) | 254 (217, 308) | 0.28 |
| CD4 count, median cells/mm3 (IQR) | | | 586 (355, 767) | 616 (387, 775) | 0.97 |
| NNRTI-based ART, n (%) | | | 73 (96.1) | 70 (92.1) | 0.49 |
| PI-based ART, n (%) | | | 3 (3.9) | 6 (7.9) |  |
| HIV-1 RNA, median log10 cps/ml (IQR) | | | UD (UD, 1.6) | UD (UD, 1.6) | 0.85 |
| HBeAg-positive, n (%) | | | 23 (30) | 18 (24) | 0.06 |
| HBV DNA, median log10 IU/ml (IQR) | | | UD (UD, 6.0) | UD (UD, UD) | <0.001 |
| HBV DNA, n (%) | <14 IU/ml |  | 44 (57.9) | 55 (78.6) | 0.001 |
| 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, n (%)b | | | 27 | 12 | - |
| HBV RAMs, n (%) | | | 19 (25) | 11 (15)c | 0.04 |
| M204V V173L L180M | |  | 10 | 6 | - |
| M204V L180M | |  | 5 | 3 | - |
| M204I/V L80I L180M | |  | 2 | 0 | - |
| M204I L80I | |  | 1 | 2 | - |
| M204I A181S | |  | 1 | 0 | - |
| ALT, median U/l (IQR) | | | 24 (17, 34) | 24 (18, 33) | 0.96 |
| AST, median U/l (IQR) | | | 28 (24, 37) | 26 (22, 36) | 0.37 |
| Liver stiffness, median kPa (IQR) | | | 5.5 (4.7, 7.1) | 5.5 (4.4, 6.4) | 0.06 |
| Liver stiffness ≥ 9.4kPa, n (%) | | | 7 (9) | 4 (5) | 0.38 |

a76 subjects (59% females) underwent assessment in July 2011 (T0), introduced tenofovir median 4 months later, and underwent a second assessment in July 2012 (T1). At T0 subjects had median 40 years of age (IQR 36, 46) and 48 months of ART (IQR 26, 63). bThe 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. cOnly 2/11 subjects had new HBV RAMs (M204V+L180M) at T1, comprising one patient with HBV DNA <14 IU/ml at T0 and 1475 IU/ml at T1, and one with HBV DNA 7.6 log10 IU/ml and no HBV RAMs at T0 and 2.9 log10 IU/ml at T1. BMI = Body Mass Index; RAMs = Resistance-associated mutations; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase.

**Table 5.** Results of transient elastography (TE) at T0 and T1 according to HBeAg status, HBV DNA levels, and TE values at T0

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| T0 status | N | Median kPa (IQR) | | | | Mean change  (95% CI) | Pa |
| T0 | | T1 | |
| All patients | 76 | 5.5 | (4.7, 7.1) | 5.4 | (4.5, 6.4) | -0.2 (-0.7, 0.3) | 0.097 |
| HBeAg-positive | 23 | 6.2 | (5.0, 7.9) | 5.6 | (4.3, 6.9) | -0.5 (-1.8, 0.7) | 0.026 |
| HBeAg-negative | 53 | 5.4 | (4.5, 6.8) | 5.3 | (4.6, 6.4) | -0.1 (-0.6, 0.3) | 0.56 |
| HBV DNA >2000 IU/ml | 24 | 7.0 | (5.4, 9.8) | 5.9 | (4.5, 8.9) | -0.8 (-2.2, 0.5) | 0.048 |
| HBV DNA <2000 IU/ml | 52 | 5.3 | (4.5, 6.3) | 5.3 | (4.4, 6.1) | -0.1 (-0.5, 0.4) | 0.50 |
| HBV DNA >20,000 IU/ml | 22 | 7.0 | (5.2, 9.9) | 5.9 | (4.4, 9.0) | -0.7 (-2.1, 0.7) | 0.028 |
| HBV DNA <20,000 IU/ml | 54 | 5.4 | (4.5, 6.4) | 5.3 | (4.5, 6.1) | -0.1 (-0.5, 0.4) | 0.56 |
| TE at T0 > 7.6kPa | 63 | 9.7 | (8.1, 15.4) | 8.3 | (5.7,14.0) | -1.2 (-3.5, 1.2) | 0.021 |
| TE at T0 ≤ 7.6kPa | 13 | 5.3 | (4.5, 6.2) | 5.3 | (4.3, 6.1) | -0.0 (-0.4, 0.3) | 0.46 |

aLog10-transformed paired samples T test

**Supplementary Table 1.** HBV virologic parameters at T0 and T1 by HBeAg status

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Characteristics | T0 | | | T1 | |
| HBeAg  Positive | HBeAg  Negative | HBeAg  Positive | | HBeAg  Negative |
| Total number (%) | 23 (30) | 53 (70) | 18 (24) | | 58 (77) |
| Lamivudine duration, median months (IQR) | 48 (28, 64) | 46 (26, 63) | 60 (39, 76) | | 60 (38, 75) |
| Tenofovir duration, median months (IQR) | 0 | 0 | 7 (6, 8) | | 8 (6, 9) |
| HBV DNA, median log10 IU/ml (IQR) | 7.4 (5.7, 8.4) | UD (UD, UD) | 2.1 (UD, 3.6) | | UD (UD, UD) |
| HBV sequences, n (%) | 19 (82) | 8 (15) | 10 (55) | | 2 (3) |
| HBV RAMs, n (%) | 16 (70) | 3 (6) | 9 (50) | | 2 (3) |
| ALT, median U/l (IQR) | 29 (18, 43) | 22 (15, 32) | 27 (18, 37) | | 23 (17, 33) |
| AST, median U/l (IQR) | 29 (26, 49) | 27 (20, 34) | 33 (23, 40) | | 26 (21, 32) |

RAMs = Resistance-associated mutations; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase.

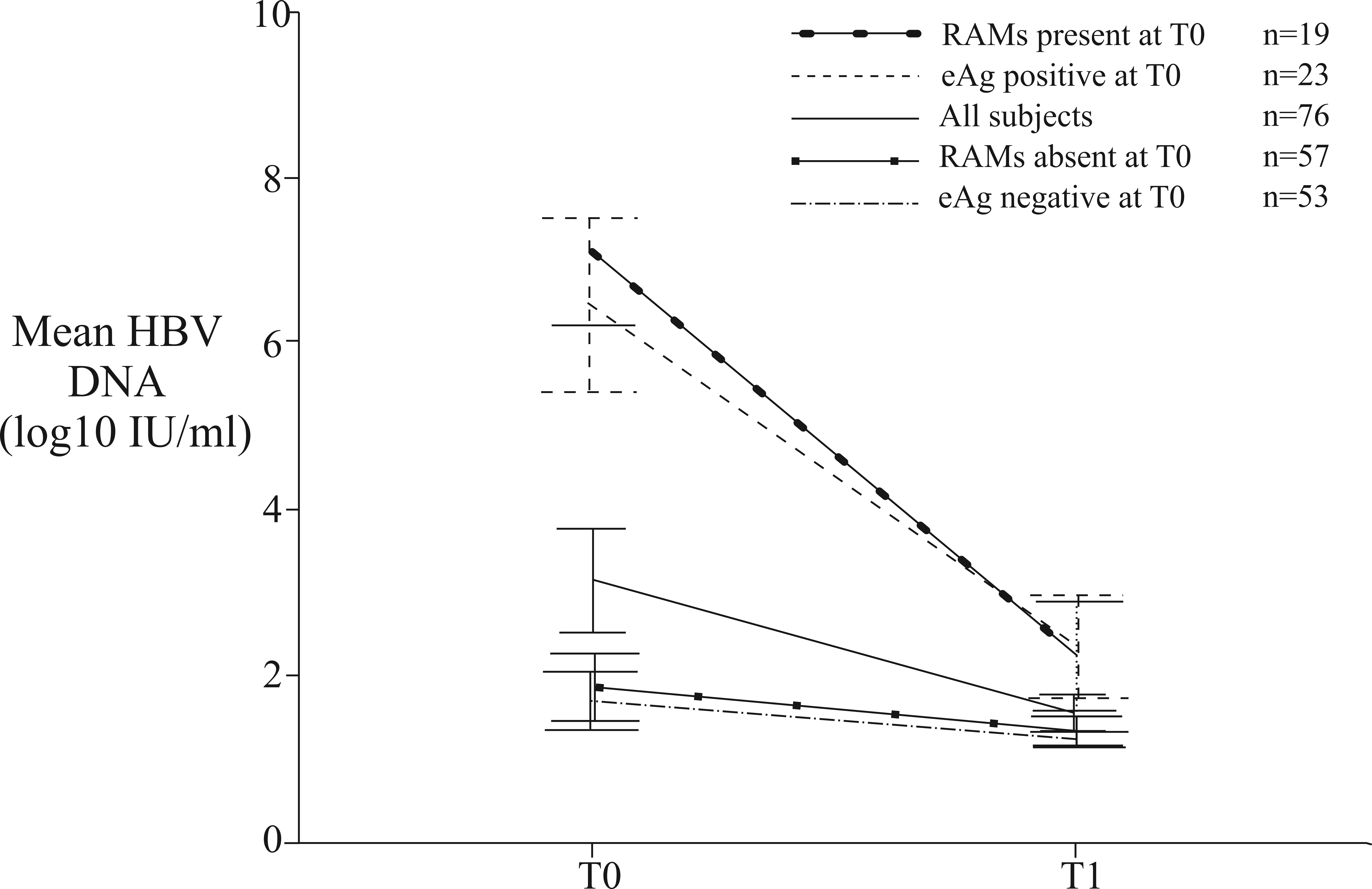
**Figure 1.** Flow diagram of study and analysis plan. TE= Transient elastography

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | HBsAg-positive  n= 230/1643 (14%) | |  |  | |
|  |  |  |  |  |  | |
|  |  | Attended at T0  n= 133 (58%) | |  |  | |
| Invalid T0 TE n=11 (8%) |  |  | Excluded due to pregnancy n=1 | |
|  |  |
|  |  |  |  |  |  | |
|  |  | Valid T0 TE  n= 121/133 (91%) | |  |  | |
|  |  |  | ART-naïve at T0 n=15/121 (12%) | |
|  |
|  |  |  |  |  |  | |
|  |  | On lamivudine at T0  n= 106/121 (88%) | |  | Introduced tenofovir after T0 n= 84/121 (69%) | |
|  |
|  |  |  |  |  |  |  |
|  |  | Cross-sectional analysis population | |  | Attended at T0 and T1 n= 76/84 (70%) | |
|  |  |  |  |  |  |  |
|  |  |  | |  | Prospective analysis population | |

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

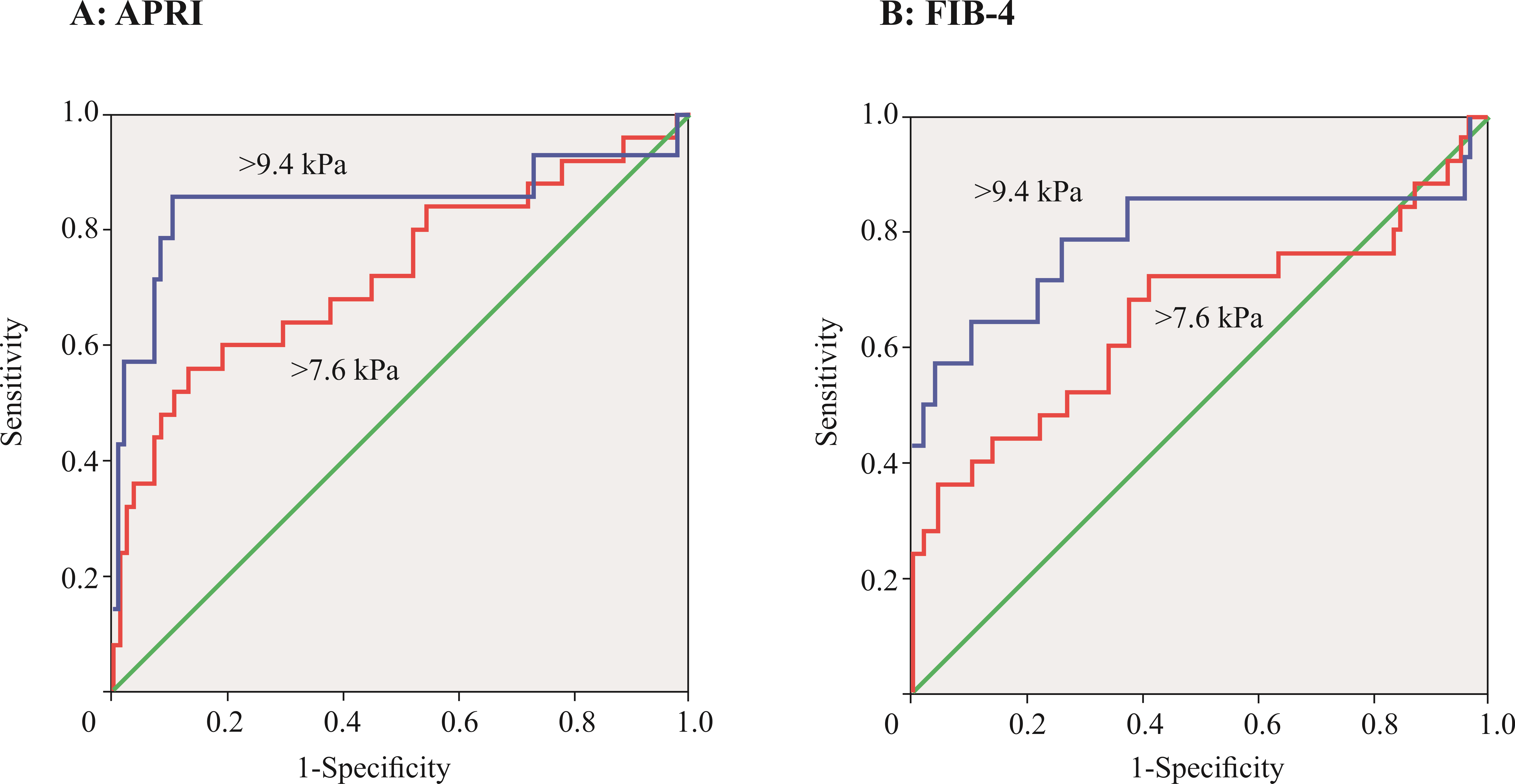
**N = 17 21 28 23 17**

**Figure 3.** Change in HBV DNA levels between T0 and T1, stratified by HBeAg status and presence of HBV resistance-associated mutations (RAMs) at T0



**Figure 4**. Change in transient elastography interpretative value categories between T0 and T1

**Supplementary Figure 1.** ROC curves exploring the relationship between transient elastography (TE) measurements and APRI (left) and FIB-4 (right) scores. For APRI, the area under the ROC curve was 0.73 (95% CI 0.60, 0.85; p=0.001) and 0.85 (95% CI 0.69, 1.0; p<0.001) for TE measurements >7.6 kPa and >9.4 kPa, respectively. APRI cut-off values of 0.45 and 0.56 had sensitivity, specificity, positive predictive value and negative predictive value of 60%, 81%, 48% and 87% for TE measurements >7.6 kPa, and 79%, 90%, 52% and 97% for TE measurements >9.4 kPa. For FIB-4, the area under the ROC curve was 0.65 (95% CI 0.51, 0.79; p=0.023) and 0.79 (95% CI 0.62, 0.96; p<0.001) for TE measurements >7.6 kPa and >9.4 kPa, respectively. FIB-4 cut-off values of 1.0 and 1.3 had sensitivity, specificity, positive predictive value and negative predictive value of 72%, 58%, 33% and 88% for TE measurements >7.6kPa, and 79%, 71%, 28% and 96% for TE measurements >9.4 kPa.



**References**

1. Lacombe K, Rockstroh J. HIV and viral hepatitis coinfections: advances and challenges. Gut **2012**; 61 Suppl 1: i47-58.

2. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach. Geneva, Switzerland, **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(5): 1302-6.

4. Gish R, Jia JD, Locarnini S, Zoulim F. Selection of chronic hepatitis B therapy with high barrier to resistance. The Lancet infectious diseases **2012**; 12(4): 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(6): 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(6): 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. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology **2014**; 61(1): 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. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **2014**; 59(11): 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(3): 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. Journal of acquired immune deficiency syndromes **2013**; 64(2): 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(6): 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(12): 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. Journal of viral hepatitis **2011**; 18(10): 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. Antiviral therapy **2012**; 17(2): 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. Antiviral therapy **2010**; 15(7): 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(9865): 468-75.

17. Marcellin P, Heathcote EJ, Buti M, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. The New England journal of medicine **2008**; 359(23): 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(1): 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(9): 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. The American journal of tropical medicine and hygiene **2010**; 82(3): 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. The International Liver Congress 2014 – 49th Annual meeting of the European Association for the Study of the Liver Vol. 60. London, UK: Elsevier, **2014**:S414-S5.

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. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **2013**; 57(12): 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. Antiviral therapy **2011**; 16(3): 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(3): 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. Journal of viral hepatitis **2011**; 18(1): 61-9.

26. 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. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **2012**; 54(5): 724-32.

27. King S, Adjei-Asante K, Appiah L, et al. Antibody screening tests variably overestimate the prevalence of hepatitis C virus infection among HIV-infected adults in Ghana. Journal of viral hepatitis **2015**; 22(5): 461-8.

28. 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. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association **2013**; 11(12): 1573-84 e1-2; quiz e88-9.

29. 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. Journal of hepatology **2011**; 54(4): 650-9.

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(3): 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. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **2014**; 58(1): 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(1): 65-72.

33. Lemoine M, Eholie S, Lacombe K. Reducing the neglected burden of viral hepatitis in Africa: strategies for a global approach. Journal of hepatology **2015**; 62(2): 469-76.