Mortality in relation to hepatitis B virus (HBV) infection status among HIV-HBV co-infected patients in sub-Saharan Africa after immediate initiation of antiretroviral therapy

Running head: HBV profiles and mortality in HIV

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ABSTRACT

Background

It is unknown how past and active hepatitis B virus (HBV) infection affect immunorecovery and mortality in people with HIV who initiate tenofovir-based anti-retroviral therapy (ART).

Methods

Using data collected between 2008- 2015, we studied people with HIV in sub-Saharan Africa initiating immediate ART in the Temprano randomized control trial. We classified participants into HBV groups at ART-initiation: hepatitis B surface antigen (HBsAg)-positive with HBV DNA ≥ 2000 IU/mL; HBsAg-positive with HBV DNA < 2000 IU/ml; isolated HBcAb-positive; resolved infection (HBsAb-positive/HBcAb-positive); and HBV non-immune/vaccinated (HBcAb-). We compared square-root CD4-cell count increases using a mixed-effect, non-linear regression adjusted for age, sex, baseline CD4 cell count, and HIV RNA. We compared all-cause mortality using Bayesian parametric survival regression.

Results

Among 879 participants, 24 (2.7%) had HBsAg with high HBV DNA, 76 (8.6%) HBsAg with low HBV DNA, 325 (37.0%) isolated anti-HBcAb, 226 (25.7%) resolved HBV infection, and 228 (25.9%) HBV non-immune/vaccinated. We found no significant difference in CD4 cell increases between the four HBV-infection groups after adjustment

($p=0.16$). Participants with HBsAg and high HBV DNA had the highest incidence of all-cause mortality [1.9/100 person-years, 95% Credible Interval (CrI)=1.0-3.4]. By comparison, incidence rates of mortality were reduced by 57% (95%CrI=-79%,-13%), 60% (95%CrI=-82%,-12%), and 66% (95%CrI=-84%,-23%) in those who had isolated anti-HBcAb-positive, resolved HBV infection, and HBV non-immune/vaccinated, respectively.

Conclusion

Individuals with HIV and past HBV infection or isolated anti-HBcAb-positive serology, much like HBV non-immune/vaccinated, experience lower mortality than those with HBsAg and high HBV DNA. Additional HBV-related management would not be necessary for these individuals.

INTRODUCTION

The burden of hepatitis B virus (HBV) co-infection among people with HIV remains high in sub-Saharan Africa, despite effective strategies to prevent and treat both infections. The prevalence of HBV, as determined by hepatitis B surface antigen (HBsAg), among people with HIV is approximately 10% worldwide¹ and is even higher in many parts of sub-Saharan Africa.^{1,2} Antiretroviral therapy (ART), particularly with regimens containing HBV-active drugs such as tenofovir disoproxil fumarate (TDF), are able to suppress both HIV and HBV replication.³ The differential mortality benefit of TDF-containing ART in HIV-HBV co-infected versus HIV mono-infected patients has been uncertain in epidemiological studies to date.⁵

Many observational studies have shown no difference in the HIV virologic response to ART among people with HIV-HBV co-infection compared to HIV mono-infection.^{6–8} In Nigeria, people with HIV-HBV co-infection, including those with positive Hepatitis B e-antigen (HBeAg), had comparable rates of HIV virologic suppression after 48 weeks of ART compared to those with HIV mono-infection.⁹ These data support no difference in HIV suppression between people with HIV-HBV co-infection and HIV mono-infection, even at varying degrees of HBV activity. However, conflicting data are available regarding the differences in immunologic recovery following ART initiation between people with HIV-HBV co-infection and HIV mono-infection. Some studies have shown impaired CD4 count recovery among people with HIV-HBV co-infection initiating ART,⁸

others have shown no difference,^{7,10} and some have even suggested accelerated immunorecovery when compared to people with HIV mono-infection.^{6,11}

Furthermore, individuals with HIV-HBV co-infection are at strongly increased risk of all-cause mortality compared to those who are HIV mono-infected in the absence of effective ART.¹² Since higher levels of both HIV and HBV replication are individually associated with higher mortality rates^{13–15}, the antiviral activity of TDF-containing ART would be assumed to reduce mortality rates in people with HIV-HBV co-infection. However, some studies in treated co-infected individuals have demonstrated higher mortality compared to those with HIV mono-infection, even after adjustment for HIV-related factors^{10,16–20}, and thus reduced viral replication might not fully translate to lower rates of mortality.

A number of factors may explain these heterogeneous findings. One of the more important reasons could be that some of these studies defined the presence or absence of HBV infection solely on the basis of HBsAg. Data are indeed emerging on the prevalence of other HBV profiles in HIV co-infection: namely, isolated Hepatitis B core antibody (HBcAb) positivity; occult HBV infection (presence of HBV DNA without HBsAg); and resolved infection (presence of HBcAb and HBV surface antibody [HBsAb]).^{6,17,21–23} To build further on these data, our objective was to determine the trajectory of immunologic recovery as measured by CD4+ cell count and the rate of all-cause mortality among people with HIV initiating ART in Côte d'Ivoire according to the following different HBV serologic profiles: active HBV infection (including with high and

146 low HBV DNA); resolved HBV infection; isolated anti-HBcAb antibody; non-infected/
147 non-immune to HBV; and vaccinated against HBV.

METHODS

Participants and study design

We conducted a secondary analysis of participants in the Temprano ANRS 12136 study, a randomized controlled, 2x2 factorial, superiority trial, conducted in nine clinics in Côte d'Ivoire. The trial design and results have been previously reported²⁴. In brief, from March 18, 2008 and July 16, 2012, patients were included based on the following criteria: newly diagnosed HIV infection, 18 years of age or older, CD4+ count below 800 cells/mm³, and not yet eligible for ART-initiation according to concomitant guidelines from World Health Organization (WHO) guidelines^{25–27}. Patients were excluded if they had active tuberculosis (TB) or severe liver disease, defined by plasma aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels more than 4 times the upper limit of normal (ULN) or any other severe liver diseases determined by the treating physician.

Trial arms and treatment

Participants were randomly assigned to one of four arms: two “deferred ART” arms (arms 1 and 2), in which ART initiation was deferred until patients met concurrent WHO starting criteria; and two “immediate ART” arms (arms 3 and 4), in which ART was initiated immediately on inclusion. In arms 2 and 4, participants received 6-month isoniazid prophylaxis for tuberculosis. First-line ART regimen in all arms contained TDF/emtricitabine. The third agent was preferably efavirenz. In the case of

contraindication to efavirenz, the third drug was either zidovudine or lopinavir/ritonavir. In this analysis, we only included individuals randomized to the “immediate ART” arms.

Follow-up

We analysed data collected between March 18, 2008 and Jan 5, 2015. The baseline time point was the time of randomization. All participants were followed for 30 months. The first Temprano study participant completed 30 months of follow-up in September 2010. From this date, all patients who reached the 30-month visit were asked to continue follow-up in a post-trial phase (PTP) until the last patient completed their 30-month trial visit (closing date: 5 January 2015). Both the Temprano study and PTP had similar procedures: patients had quarterly visits at their healthcare center and were requested to present for additional visits any time they encountered a medical event; CD4+ count and plasma HIV-1 RNA were measured every 6 months; consultations, CD4+ count, viral load, and antiretroviral drugs were free of charge; and patients who did not present at a trial visit were traced by experienced social workers. In the Temprano trial, transportation for unscheduled visits, consultations, hospitalization, and nonantiretroviral drugs were free of charge when morbidity events occurred during the trial. In PTP, patients were required to pay out-of-pocket, similar to other patients followed in routine care at the same center.

Study parameters

Blood tests including CD4+ count, plasma HIV-1 RNA, and serum transaminases were performed at baseline and every six months. HBsAg serology was performed at

baseline using an enzyme-linked immune-assay (ELISA) test (Monolisa® AgHBS Ultra, Bio-Rad, Marnes la Coquette, France). From frozen samples stored at -80°C, anti-HBcAb and anti-HBsAb serology were also performed using an ELISA test (Monolisa® anti-HBs plus, anti-hepatitis B core antibody-anti-HBc-plus, Bio-Rad). HBV infection status was based on AASLD HBV guidance and was defined as follows: HBsAg-positive; isolated anti-HBcAb+; resolved HBV infection (HBcAb and HBsAb positive); non-immunized (HBcAb negative and HBsAb negative); vaccinated (HBcAb negative and HBsAb positive) (Box 1).

Baseline HBV DNA viral loads were quantified for HBsAg-positive (to assess HBV activity) and non-immunized individuals (to assess seronegative occult HBV infection),²⁸ using an in-house polymerase chain reaction (PCR)-based assay (QuantiFast SYBR® Green PCR kit, detection limit: 12 copies/mL; Qiagen, Courtaboeuf, France; Light Cycler 480; Roche, Boulogne-Billancourt, France) or a commercially-available PCR assay (COBAS®Amplicor HBV Monitor, detection limit: 60 IU/mL; Roche Diagnostics, Meylan, France). To ensure comparability between assays, viral loads were reported in IU/mL [conversion factor: 1 IU/mL=2.8 copies/mL].²⁹

Statistical analysis

Patient characteristics at baseline were compared across HBV-infection groups using Pearson's χ^2 or Fisher's Exact tests for categorical variables and Kruskal-Wallis for continuous variables. Follow-up time began at baseline and ended at death, loss to follow-up (LTFU), or 5 January 2015, whichever occurred first.

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217 To examine the effect of HBV status on immunologic recovery, we used mixed-effect,
218 non-linear regression and modeled increase in CD4+ cell count as a square-root
219 function over time. We obtained stratum-specific estimates by including an interaction
220 term between HBV-infection group and follow-up time. We used random-intercept and
221 random-coefficient for time to account for variation between individuals with respect to
222 CD4+ T-cell count at ART-initiation and during follow-up, with unstructured covariance
223 between random-intercept and random-coefficient. Models were both unadjusted and
224 adjusted for CD4+ cell count at ART-initiation, HIV RNA level at inclusion (>5.0 versus
225 $\leq 5.0 \log_{10}$ copies/mL), age (>35 versus ≤ 35 years), and sex.

226

227 To examine the effect of HBV status on all-cause mortality, we used parametric survival
228 regression with exponentially distributed survival functions and modeled the proportional
229 hazards of mortality during follow-up. As a reference group, we used HBsAg-positive
230 individuals in a first model and HBsAg-positive individuals with HBV DNA ≥ 2000 IU/mL
231 in a second model. The threshold of 2000 IU/mL was pre-specified in our analysis
232 based on levels corresponding to active infection and treatment indication among HBV
233 mono-infected individuals.³⁰

234

235 Because few deaths were expected, we supposed that parameter estimates from
236 standard regression techniques could be exaggerated and uncertain.³¹ To minimize this
237 bias, we used a penalized regression approach whereby uncertain estimates from the
238 data are pulled towards more realistic ones assumed from prior knowledge.³² Briefly,

survival models were fit using a Bayesian approach. For each HBV infection group, a prior distribution of hazards ratios (HRs) was first specified based on the anticipated strength of association (Supplementary Table 1).³³ These distributions were assigned from a previous analysis of the Temprano study.¹⁷ Since the intercept of this model estimates the incidence rates (IR) of the reference group, the prior distribution of this parameter was based on IR from previous studies of HIV-HBV co-infected individuals, overall and with high HBV-DNA viral loads,^{17,34} estimated at 1.0 per 100 person-years [95% credible interval (CrI) 0.3-4.0] and 2.0 per 100 person-years (95%CrI=0.5-8.0), respectively. Using these priors together with the data, a posterior distribution of HRs was estimated with Markov Chain Monte Carlo methods from the “bayes” prefix commands in STATA. The median of this distribution defined the parameter estimate (termed “posterior-HR”) and their 2.5% and 97.5% quantiles defined the 95%CrI. Since posterior-HR need to be interpreted with the priori distributions, both prior-HR and posterior-HR are provided.

All reported *p*-values were two-sided and no adjustments for multiple comparisons were applied. Statistical analyses were performed using STATA (v15.0, College Station, Texas).

Sensitivity analyses

Since specification of the prior distribution can influence posterior HRs,³⁵ we repeated the analysis using (1) non-informative priors and (2) a different prior HR distribution whose effect size was weaker.

262

263 **Funder, registration, and ethics**

264 The Temprano trial protocol was approved by the Côte d'Ivoire National Ethics

265 Committee for Health Research. It was registered at Clinical Trials.gov (NCT00495651).

266 Signed informed consent was provided prior to participating in the trial. The sponsor had

267 no role in the conduct of the study and interpretation of the data.

RESULTS

Description of the study population

In the Temprano trial, 1032 individuals were randomized to receive immediate ART. Of them, 153 (14.8%) did not have available serum samples to determine HBV-infection group and were excluded. In total, 879 participants were included in this secondary analysis. Clinical characteristics were similar between those who were included versus those who were not, except that included individuals had lower CD4+ cell counts and more advanced WHO clinical stage (Supplementary Table 2).

One hundred (11.4%) participants were HBsAg-positive (24 and 76 with HBV DNA ≥ 2000 IU/mL and HBV DNA < 2000 IU/mL, respectively). Of the other HBV-infection groups, 325 (37.0%) had isolated anti-HBcAb positive serology, 226 (25.7%) had resolved infection, 203 (23.1%) were non-immunized/ non-infected, and 25 (2.8%) were vaccinated. All non-immunized individuals were tested for HBV DNA viral loads, and none had detectable HBV DNA. Due to the few numbers of vaccinated individuals, these individuals were grouped with non-immunized individuals in further analysis.

Baseline characteristics are compared across HBV infection groups in Table 1. HBsAg-positive individuals were more frequently male, had higher ALT levels, including a higher proportion with ALT greater than the upper limit of normal (ULN), yet this was mostly observed in those with HBV-DNA ≥ 2000 IU/mL [versus < 2000 IU/mL, respectively: median ALT: 32 versus 19 IU/mL ($p=0.002$); proportion with ALT $> \text{ULN}$:

37.5% versus 18.4% ($p=0.05$)]. HBsAg-positive and isolated anti-HBc antibody-positive individuals were more likely to be treated with LPV/r as a third agent compared to the other HBV subgroups. Non-immunized/ vaccinated individuals were younger than participants from all other groups.

Immunologic Recovery during antiretroviral therapy

Participants were followed for a median of 61 months (IQR=46-71). Median follow-up was no different between HBV-infection groups ($p=0.26$). At baseline, mean CD4+ count was lowest in the isolated anti-HBc antibody-positive group (460/mm³, 95%CI=445-476) and highest in the non-immunized/ vaccinated group (483/mm³, 95%CI=464-502). At the end of follow-up, mean CD4+ count was lowest in the HBsAg-positive group (691/mm³, 95%CI=639-744) and remained highest in the non-immunized/ vaccinated group (757 mm³, 95%CI=717-797). Based on the mixed-effect non-linear regression model, there was no significant difference in CD4+ cell increase during treatment between HBV-infection groups, both unadjusted ($p=0.18$) and adjusted for baseline CD4+ count, HIV RNA, age, and sex ($p=0.16$) (Figure 1A). No significant difference was observed when further stratifying on baseline HBV-DNA level ≥ 2000 and < 2000 IU/mL: unadjusted, $p=0.15$; adjusted, $p=0.13$ (Figure 1B).

Incidence of all-cause mortality with respect to HBV-infection status

During follow-up, there were 37 deaths. IRs and 95%CrI across HBV infection groups (obtained from the Bayesian exponential model) are provided in Figure 2. When compared to HBsAg-positive individuals (modeled IR=1.3/100PY, 95%CrI=0.7-2.1/100

PY) (Table 2), mortality IRs were reduced by 32% (95%CrI=-67%, +28%) in those with isolated anti-HBcAb-positive serology, 35% (95%CrI=-72%,+22%) in those with resolved infection, and 43% (95%CrI=-75%, +11%) in those who were non-immunized or vaccinated (modeled IR=0.7/100PY, 95%CrI=0.3-1.5/100PY). When compared to HBsAg-positive individuals with HBV-DNA ≥ 2000 IU/mL (modeled IR=2.0/100PY, 95%CrI=1.0-3.4/100PY) (Table 2), mortality IRs were reduced by 56% (95%CrI=-83%, +3%) in those with HBsAg-positive serology and HBV-DNA <2000 IU/mL, 57% (95%CrI=-79%, -13%) in those with isolated anti-HBcAb-positive serology, 60% (95%CrI=-82%, -12%) in those with resolved infection, and 66% (95%CrI=-84%, -23%) in those who were non-immunized/ vaccinated (modeled IR=0.7/100PY, 95%CrI=0.3-1.5/100PY).

Sensitivity analysis

In the model with HBsAg-positive individuals without stratification on HBV DNA levels, the posterior-HRs were slightly closer to one (i.e., reductions in mortality were slightly attenuated) when using non-informative priors (Supplementary Table 3). In the model with HBsAg-positive individuals stratified on HBV DNA levels, the posterior-HRs were also closer to one when using a prior with a weaker effect (Supplementary Table 4), with only non-immunized/ vaccinated individuals having a 95%CrI falling below 1. The posterior-HRs were similar when using non-informative priors on this model, yet 95%CrI were much wider (Supplementary Table 3).

DISCUSSION

In this analysis of people with HIV initiating ART in Côte d'Ivoire, participants in different HBV subgroups exhibited no difference in immunologic recovery over the first five years of ART when compared to participants with HIV mono-infection. However, adults with high baseline HBV DNA levels had higher rates of all-cause mortality than other HBV subgroups, including HBV non-immunized/vaccinated, by the end of the follow-up period. These findings provide important information on the clinical evolution of people with HIV with respect to their HBV infection status.

The first notable finding in this analysis is the distribution of HBV serologic profiles among people with HIV. Among individuals with sufficient stored blood for testing, more than 75% had evidence of past or present infection. The largest HBV subgroup in this cohort was comprised of people with isolated anti-HBcAb (37.0%), followed by resolved HBV infection (25.7%) and HBV non-immune/vaccinated (23.1%). This distribution is comparable to a prior cross-sectional study sampling patients from HIV clinics in Côte d'Ivoire[21]. In cohort studies of people with HIV in the U.S., where HBV is not endemic, the most common HBV subgroup noted was non-immune/vaccinated (66-74%) followed by resolved HBV infection (20-23%) and isolated anti-HBcAb (3.5-6%)[6,17]. Possible explanations for these observed subgroup differences among people with past or present HBV infection include: (1) timing of HBV infection, with co-infected individuals in sub-Saharan Africa being mostly infected with HBV long before HIV infection and co-infected individuals in the U.S. more likely to be in the "window phase" of acute HBV

infection; (2) immunologic differences, which may produce a different proportion of individuals due to the timing of waning anti-HBsAb levels following resolved HBV infection; and to a lesser extent (3) genetic differences in HBV, which may result in HBsAg that escapes detection due to mutations on the surface gene.³⁶

We found that no participant in this cohort had seronegative occult HBV infection on study enrolment, defined as a detectable HBV DNA without HBsAg, anti-HBcAb, and anti-HBs antibodies. The estimated prevalence of seronegative occult HBV among people living with HIV ranges from 0% to 18%, depending on the study.^{37–40} Compared to other studies, our cohort had a relatively high median CD4+ count and 90% were in WHO Stage I or II disease on enrolment, indicating a less immunosuppressed population able to control intrahepatic HBV replication more effectively.²⁷ Testing constraints limited our ability to determine the prevalence of seropositive occult HBV (detectable HBV DNA without HBsAg but with anti-HBcAb). However, even people with seropositive occult HBV infrequently have elevated HBV DNA so we would not expect them to incur a higher mortality rate than those who are HBsAg positive with low HBV DNA levels.

Participants with HBsAg positivity, with and without high HBV DNA, exhibited comparable rates of CD4+ count recovery in the first five years of ART as other HBV serologic profiles. These findings are in contrast to an analysis of 4,773 participants initiating ART in the Swiss HIV cohort, which showed impaired CD4 count recovery at 36 weeks in participants who had isolated HBcAb and HBsAg positivity.⁷ That study,

mostly comprised of men, noted several baseline differences between those with and without HBsAg, including lower baseline CD4+ counts (median, 218 cells/mm³) and a higher prevalence of HCV antibody (20.9%), which were not apparent in our study. In a prior analysis in Côte d'Ivoire, participants with HBsAg positivity and a high HBV DNA demonstrated an accelerated rate of CD4+ count recovery compared to other HBV serologic profiles.¹⁰ That study also excluded participants with advanced liver disease, but baseline CD4+ counts were overall lower than the current analysis. Interestingly, a study of people with HIV-HBV co-infection in Nigeria found no difference in rates of immunologic recovery to those with HIV mono-infection despite having lower baseline CD4+ count (median, 107 cells/mm³) and higher HIV viral load (median, 4.96 log copies/mL).⁸ Taken together, the reasons for differences in observed immunorecovery remain unclear.

Despite showing comparable rates of CD4+ count recovery, people with HIV-HBV co- still incurred a higher mortality rate compared to those with HIV mono-infection. This finding was evident for those with positive HBsAg and high HBV DNA levels at baseline and has been noted in other HIV-HBV cohorts in sub-Saharan Africa,^{3,34,41} all of which included participants who initiated ART at lower CD4 thresholds. It is noteworthy that we were able to establish this finding in an analysis restricted to participants who were randomized to receive ART immediately after HIV diagnosis, with extensive use of TDF-based regimens, and a low overall mortality rate. It could be speculated that our estimates were inflated due to sparse data issues (Supplementary table 5); however, we applied a regression technique aimed at mitigating this bias.

404

405 Prior studies have noted that people with HIV-HBV co-infection are more likely to
406 experience hepatitis flares compared to those with HIV mono-infection, but it is not clear
407 if liver disease is itself the driver of excess mortality among people with HIV-HBV co-
408 infection.¹⁵ Participants with HBsAg in our cohort, particularly those with high HBV DNA
409 viral loads, were more likely to have elevated baseline ALT levels, but this frequency
410 was still relatively low (<25%), and no participant had an ALT level >4 x ULN at study
411 entry because of the Temprano inclusion criteria. However, it is unclear whether
412 baseline levels of fibrosis may have contributed to the differences in mortality observed
413 between those with and without HBV co-infection.

414

415 Our study has limitations. First, there was a considerable proportion of participants with
416 missing data at baseline, for whom immunosuppression was less severe and with
417 missing data on causes of death, which made us unable to further analyze the
418 underlying reasons for increased mortality. In addition, individuals with ALT levels >4x
419 ULN were excluded from the Temprano trial, thus, participants were less likely to have
420 severe liver disease. Further, individuals in this cohort were not treated with integrase
421 strand transfer inhibitors, which have now become the standard of care of first-line ART.
422 The included population might not represent the entire HIV-HBV disease spectrum
423 observed currently in sub-Saharan Africa. Second, virologic and biochemical markers of
424 HBV replication fluctuate in the absence of ART,⁴² and individuals could have had their
425 HBV infection status misclassified prior to ART initiation. Finally, despite the large

426 number of individuals with complete HBV serology and regular follow-up, few deaths
427 occurred during follow-up.

CONCLUSION

In a cohort of people with HIV in Côte d'Ivoire who initiated ART immediately after diagnosis, 74.0% of participants had prior HBV exposure with 11.4% being HBsAg positive and 2.7% having HBsAg with a high HBV DNA. All HBV subgroups exhibited similar trajectories of CD4+ count recovery. However, participants with HBsAg and high HBV DNA levels incurred a higher rate of all-cause mortality. We observed no difference in all-cause mortality between HBsAg-negative HBV subgroups, suggesting that HBsAg and HBV DNA (among those HBsAg-positive) are the most important HBV markers to assess in people with HIV initiating ART. Non-immunologic factors, such as the presence of baseline liver disease, should be investigated as possible causes of differences in mortality.

Competing Interests

The authors declare no competing interests.

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Author Contributions

AMM, GMK, and ABo were responsible for the statistical analysis, interpretation of the data and drafting the manuscript. **DG, ABa, and AE** coordinated data collection and management and provided critical revision of the manuscript. **AG, SM, HM, and CDe** gave technical support, were responsible for HBV virologic measurements and provided critical revision of the manuscript. **EPH** provided methodological assistance and provided critical revision of the manuscript. **RM, CDa, XA, KL, SPE** and provided important advice regarding the TEMPRANO, assisted in data collection and management and provided critical revision of the manuscript.

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DATA AVAILABILITY STATEMENT

The data from the Temprano Study are owned by the ANRS. Original data can be requested by submitting a study proposal to the principal investigators of the study. The proposal format can be obtained from the corresponding author (a.c.boyd@amsterdamumc.nl or anders.boyd@iplesp.upmc.fr). The principal investigators of the study will check each proposal for compatibility with the general objectives, ethical approvals, and informed consent forms of the Temprano study, and potential overlap with ongoing work. This study contains original data. **AB, GMK, DG, CD, RM, XA** and **SE** had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

STATEMENT OF SIGNIFICANCE

486 Individuals who have been infected with hepatitis B virus (HBV) can either resolve their
487 infection or become chronically infected. For HIV-positive individuals, this could have an
488 impact on their risk of dying during treatment. In this study, we observed that HIV-
489 positive individuals in Côte d'Ivoire who started their treatment early and had highly
490 active, chronic HBV infection had a higher risk of death. This was in comparison to HIV-
491 positive individuals who were never infected or had resolved HBV infection. These
492 findings suggest that presence of chronic HBV infection, and not resolved HBV
493 infection, warrants closer monitoring in HIV-positive individuals.

REFERENCES

1. Leumi S, Bigna JJ, Amougou MA, Ngouo A, Nyaga UF, Noubiap JJ. Global burden of hepatitis B infection in people living with human immunodeficiency virus: a systematic review and meta-analysis. *Clin Infect Dis*. 2019, , ciz1170, <https://doi.org/10.1093/cid/ciz1170>
2. Barth RE, Huijgen Q, Taljaard J, Hoepelman AIM. Hepatitis B/C and HIV in sub-Saharan Africa: an association between highly prevalent infectious diseases. A systematic review and meta-analysis. *Int J Infect Dis* 2010;14(12):e1024–31.
3. Hawkins C, Christian B, Ye J, et al. Prevalence of hepatitis B co-infection and response to antiretroviral therapy among HIV-infected patients in Tanzania: *AIDS* 2013;27(6):919–27.
4. Singh KP, Crane M, Audsley J, Avihingsanon A, Sasadeusz J, Lewin SR. HIV-hepatitis B virus coinfection: epidemiology, pathogenesis, and treatment. *AIDS* 2017;31(15):2035–52.
5. Chun HM, Mesner O, Thio CL, et al. HIV outcomes in hepatitis B virus coinfecting individuals on HAART: *JAIDS J Acquir Immune Defic Syndr* 2014;66(2):197–205.
6. Wang H, Li Y, Zhang C, et al. Immunological and virological responses to combined antiretroviral therapy in HIV/hepatitis B virus-coinfecting patients from a multicenter cohort: *AIDS* 2012;26(14):1755–63.
7. Wandeler G, Gsponer T, Bihl F, et al. Hepatitis B Virus infection is associated with impaired immunological recovery during antiretroviral therapy in the Swiss HIV Cohort Study. *J Infect Dis* 2013;208(9):1454–8.

8. Idoko J, Meloni S, Muazu M, et al. Impact of Hepatitis B Virus infection on Human Immunodeficiency Virus response to antiretroviral therapy in Nigeria. *Clin Infect Dis* 2009;49(8):1268–73.
9. Omland L, Weis N, Skinhøj P, et al. Impact of hepatitis B virus co-infection on response to highly active antiretroviral treatment and outcome in HIV-infected individuals: a nationwide cohort study. *HIV Med* 2008;9(5):300–6.
10. Houghtaling L, Moh R, Abdou Chekaraou M, et al. CD4+ T Cell recovery and Hepatitis B Virus co-infection in HIV-infected patients from Côte d'Ivoire initiating antiretroviral therapy. *AIDS Res Hum Retroviruses* 2018;34(5):439–45.
11. Falade-Nwulia O, Seaberg EC, Rinaldo CR, Badri S, Witt M, Thio CL. Comparative risk of liver-related mortality from chronic hepatitis B versus chronic hepatitis C virus infection. *Clin Infect Dis* 2012;55(4):507–13.
12. Vlahov D, Graham N, Hoover D, et al. Prognostic indicators for AIDS and infectious disease death in HIV-infected injection drug users: plasma viral load and CD4 cell count. *JAMA* 1998;279(1):35.
13. Iloeje UH, Yang H, Jen C, et al. Risk and Predictors of Mortality Associated With Chronic Hepatitis B Infection. *Clin Gastroenterol Hepatol* 2007;5(8):921–31.
14. Mugavero MJ, Napravnik S, Cole SR, et al. Viremia Copy-Years Predicts Mortality Among Treatment-Naive HIV-Infected Patients Initiating Antiretroviral Therapy. *Clin Infect Dis* 2011;53(9):927–35.
15. Sheng W-H, Chen M-Y, Hsieh S-M, et al. Impact of chronic hepatitis B virus (HBV) infection on outcomes of patients infected with HIV in an area where HBV infection is hyperendemic. *Clin Infect Dis* 2004;38(10):1471–7.

- 540 16. Chun HM, Roediger MP, Hullsiek KH, et al. Hepatitis B virus coinfection negatively
541 impacts HIV outcomes in HIV seroconverters. *J Infect Dis* 2012;205(2):185–93.
- 542 17. Kouamé G-M, Boyd A, Moh R, et al. Higher mortality despite early antiretroviral
543 therapy in Human Immunodeficiency Virus and Hepatitis B Virus (HBV)–coinfected
544 patients with high HBV replication. *Clin Infect Dis* 2018;66(1):112–20.
- 545 18. Christian B, Fabian E, Macha I, et al. Hepatitis B virus coinfection is associated
546 with high early mortality in HIV-infected Tanzanians on antiretroviral therapy: *AIDS*
547 2019;33(3):465–73.
- 548 19. Mbae M, Owen L, Elisha KK, et al. Excess early mortality in HIV/hepatitis B virus
549 co-infected patients initiating antiretroviral therapy in Kenya: *AIDS*
550 2019;33(8):1404–6.
- 551 20. N'Dri-Yoman T, Anglaret X, Messou E, et al. Occult HBV infection in untreated HIV-
552 infected adults in Côte d'Ivoire. *Antivir Ther* 2010;15(7):1029–34.
- 553 21. Landrum ML, Roediger MP, Fieberg AM, et al. Development of chronic hepatitis B
554 virus infection in hepatitis B surface antigen negative HIV/HBV co-infected adults:
555 A rare opportunistic illness. *J Med Virol* 2011;83(9):1537–43.
- 556 22. Gandhi RT, Wurcel A, McGovern B, et al. Low Prevalence of Ongoing Hepatitis B
557 Viremia in HIV-Positive Individuals with Isolated Antibody to Hepatitis B Core
558 Antigen: *JAIDS J Acquir Immune Defic Syndr* 2003;34(4):439–41.
- 559 23. The TEMPRANO ANRS 12136 Study Group. A Trial of Early Antiretrovirals and
560 Isoniazid Preventive Therapy in Africa. *N Engl J Med* 2015;373(9):808–22.

24. World Health Organization, Gilks C, Vítória M. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. 2006. Available from: <http://www.who.int/hiv/pub/guidelines/artadultguidelines.pdf>.
25. World Health Organization, Department of HIV/AIDS. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. 2010; Available from: <http://www.ncbi.nlm.nih.gov/books/NBK138540/>.
26. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. 2013. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK195400/>.
27. Raimondo G, Locarnini S, Pollicino T, et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. *J Hepatol* 2019;71(2):397–408.
28. The ANRS 12240 VarBVA study, Boyd A, Moh R, et al. Low risk of lamivudine-resistant HBV and hepatic flares in treated HIV-HBV co-infected patients from Côte d'Ivoire. *Antivir Ther* 2015;20(6):643–54.
29. Paraskevis D, Beloukas A, Haida C, et al. Development of a new ultra sensitive real-time PCR assay (ultra sensitive RTQ-PCR) for the quantification of HBV-DNA. *Virol J*. 2010;7(1).
30. WHO. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. 2015. Available from: https://apps.who.int/iris/bitstream/handle/10665/154590/9789241549059_eng.pdf

- 583 31. Greenland S, Mansournia MA, Altman DG. Sparse data bias: a problem hiding in
584 plain sight. *BMJ* 2016;i1981.
- 585 32. Greenland S. Bayesian perspectives for epidemiological research. II. Regression
586 analysis. *Int J Epidemiol* 2007;36(1):195–202.
- 587 33. Greenland S, Mansournia MA. Penalization, bias reduction, and default priors in
588 logistic and related categorical and survival regressions. *Stat Med*
589 2015;34(23):3133–43.
- 590 34. Velen K, Charalambous S, Innes C, Churchyard G, Hoffmann C. Chronic hepatitis
591 B increases mortality and complexity among HIV-coinfected patients in South
592 Africa: a cohort study. *HIV Med* 2016;17(9):702–7.
- 593 35. McNeish D. On Using Bayesian Methods to Address Small Sample Problems.
594 *Struct Equ Model Multidiscip J* 2016;23(5):750–73.
- 595 36. Boyd A, Moh R, Maylin S, et al. Effect of hepatitis B virus (HBV) surface-gene
596 variability on markers of replication during treated human immunodeficiency virus-
597 HBV infection in Western Africa. *Liver Int* 2019;39(2):280–9.
- 598 37. Gachara G, Magoro T, Mavhandu L, et al. Characterization of occult hepatitis B
599 virus infection among HIV positive patients in Cameroon. *AIDS Res Ther.*
600 2017;14(1).
- 601 38. Ryan K, Anderson M, Gyurova I, et al. High rates of occult Hepatitis B Virus
602 infection in HIV-positive individuals initiating antiretroviral therapy in Botswana.
603 *Open Forum Infect Dis.* 2017;4(4).
- 604 39. Nebbia G, Garcia-Diaz A, Ayliffe U, et al. Predictors and kinetics of occult hepatitis
605 B virus infection in HIV-infected persons. *J Med Virol* 2007;79(10):1464–71.

- 606 40. Shire NJ, Rouster SD, Rajcic N, Sherman KE. Occult Hepatitis B in HIV-Infected
607 Patients: JAIDS J Acquir Immune Defic Syndr 2004;36(3):869–75.
- 608 41. Matthews GV, Manzini P, Hu Z, et al. Impact of lamivudine on HIV and hepatitis B
609 virus-related outcomes in HIV/hepatitis B virus individuals in a randomized clinical
610 trial of antiretroviral therapy in southern Africa: AIDS 2011;25(14):1727–35.
- 611 42. Boyd A, Kouamé MG, Houghtaling L, et al. Hepatitis B virus activity in untreated
612 hepatitis B e antigen–negative human immunodeficiency virus–hepatitis B virus co-
613 infected patients from sub-Saharan Africa. Trans R Soc Trop Med Hyg
614 2019;113(8):437–45.
- 615

616 **TABLES**

617 **Box 1. Definition of HBV infection status**

HBV infection group	HBV serology [†]		
	HBsAg	anti-HBcAb	anti-HBsAb
HBsAg-positive	+	N/A	N/A
Isolated anti-HBcAb+	-	+	-
Resolved infection	-	+	+
Non-immunized	-	-	-
Vaccinated	-	-	+

618 Note: Anti-HBcAb and anti-HBsAb were not measured for HBsAg-positive individuals.

619 [†]Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBcAb,
620 anti-hepatitis B core antibodies; anti-HBsAb, anti-hepatitis B surface antibodies.
621

A **B**

Supplement to: Mohareb AM, Kouamé GM, Gabassi A, et al. Mortality in relation to HBV-infection status among HIV-HBV co-infected patients in sub-Saharan Africa after immediate initiation of antiretroviral therapy.

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Supplementary Table 1. Prior distributions used in analysis

Assumed direction of association	HR (95% CrI)
Probably protective	0.50 (0.13-2.00)
Possibly protective	0.69 (0.17-2.70)
Unknown direction	1.00 (0.25-4.00)

Abbreviations: CrI, credible interval; HR, hazards ratio.

All priors based on log-normal distribution with mean $\ln(\text{HR})$ and variance 1/2 [1].

Reference:

1. Greenland S. Prior data for non-normal priors. Stat Med. 2007 Aug 30;26(19):3578–90.

Supplementary Table 2. Comparison of individuals included and not included in analysis

	Included <i>n</i> =879	Not included <i>n</i> =153	<i>p</i> -value*
Median age, years (IQR)	35 (30-42)	35 (30-40)	0.88
Female	701 (80)	118 (79)	0.76
Median BMI, kg/m ² (IQR)	22 (20-25)	22 (20-26)	0.76
WHO clinical stage			0.04
1	560 (64)	92 (61)	
2	225 (26)	51 (34)	
3	89 (10)	7 (5)	
4	5 (<1)	0 (0)	
Median CD4 cell count/mm ³ (IQR)	459 (365-561)	543 (445-668)	<0.001
Median HIV-1 RNA, log ₁₀ copies/ml (IQR)	4.7 (4.0-5.3)	4.6 (4.0-5.2)	0.16
Plasma ALT, median (IQR)	18 (14-26)	18 (13-23)	0.18
Plasma ALT >1×ULN, <i>n</i> (%)	109 (12)	12 (8)	0.15
First-line ART regimen			0.14
TDF–FTC plus EFV	602 (68)	112 (75)	
TDF–FTC plus LPV/r	72 (8)	6 (4)	
TDF–FTC plus ZDV	205 (23)	32 (21)	
Randomized to IPT, <i>n</i> (%)	441 (50)	75 (50)	0.97

All statistics are *n* (%) unless otherwise indicated. The table only includes participants randomized to the two ‘immediate ART’ arms (arms 3 and 4) of the ANRS Temprano trial.

n, number in groups; IQR, interquartile range; BMI, body mass index; IPT, Isoniazid Preventive Therapy; WHO, World Health Organization; ART, Antiretroviral Therapy; TDF, tenofovir; FTC, emtricitabine; ZDV, zidovudine; LPV/r, lopinavir/ritonavir; ALT, alanine transaminase; ULN, upper limit of normal. BMI, body mass index; Ag, antigen; Ab, antibodies; HBsAg, hepatitis B surface antigen; anti-HBc, anti-hepatitis B core.

**p*-value for comparison between groups using Pearson’s χ^2 or Fisher’s Exact test for categorical variables and Kruskal-Wallis test for continuous variables.

Supplementary Table 3. Incidence of all-cause death according to hepatitis B virus infection group (using non-informative priors[†])

Infection group [‡]		Posterior HR (95% CrI)
Model 1		
	HBsAg-positive	Ref
	Isolated anti-HBc Ab-positive	0.66 (0.25-1.93)
	Resolved infection	0.60 (0.20-1.82)
	Non-immunized/Vaccinated	0.51 (0.16-1.58)
Model 2 [§]		
	HBsAg-positive, HBV DNA ≥ 2000 IU/mL	Ref
	HBsAg-positive, HBV DNA < 2000 IU/mL	0.31 (0.06-1.72)
	Isolated anti-HBc Ab-positive	0.33 (0.10-1.46)
	Resolved infection	0.29 (0.08-1.33)
	Non-immunized/Vaccinated	0.26 (0.07-1.20)

Abbreviations: anti-HBc Ab, anti-hepatitis B core antibodies; CrI, credible interval; HBsAg, Hepatitis B surface antigen; HBV DNA, Hepatitis B virus deoxyribonucleic acid; HR, hazard ratio.

HRs and 95% CrI were obtained from univariable Bayesian exponential survival models.

[†]Non-informative priors (i.e. those that bear minimal influence when estimated the posterior distribution) were log normal distributions with mean $\ln(1)$ and variance 100.

[‡]Determined from serological results at inclusion, defined in Box 1.

[§]Parameter estimates should be interpreted with caution as a high level of autocorrelation between iterations was observed for all parameter estimates in this model.

Supplementary Table 4. Incidence of all-cause death according to hepatitis B virus infection group (using priors with weaker effects)

Infection group [†]		Prior HR (95% CrI)	Posterior HR (95% CrI)
Model 2			
	HBsAg-positive, HBV DNA ≥2000 IU/mL	Ref	Ref
	HBsAg-positive, HBV DNA <2000 IU/mL	0.69 (0.17-2.77)	0.58 (0.21-1.38)
	Isolated anti-HBc Ab-positive	0.69 (0.17-2.77)	0.54 (0.25-1.10)
	Resolved infection	0.69 (0.17-2.77)	0.50 (0.23-1.06)
	Non-immunized/Vaccinated	0.69 (0.17-2.77)	0.44 (0.20-0.93)

Abbreviations: anti-HBc Ab, anti-hepatitis B core antibodies; CrI, credible interval; HBsAg, Hepatitis B surface antigen; HBV DNA, Hepatitis B virus deoxyribonucleic acid; HR, hazard ratio.

HRs and 95% CrI were obtained from univariable Bayesian exponential survival models.

[†]Determined from serological results at inclusion, defined in Box 1.

Supplementary Table 5. Incidence of all-cause death according to hepatitis B virus infection group (without using Bayesian approach)

Infection group [†]		HR (95% CI)
Model 1		
	HBsAg-positive	Ref
	Isolated anti-HBc Ab-positive	0.63 (0.24-1.66)
	Resolved infection	0.58 (0.20-1.67)
	Non-immunized/Vaccinated	0.50 (0.17-1.49)
Model 2		
	HBsAg-positive, HBV DNA ≥2000 IU/mL	Ref
	HBsAg-positive, HBV DNA <2000 IU/mL	0.31 (0.06-1.56)
	Isolated anti-HBc Ab-positive	0.30 (0.09-1.06)
	Resolved infection	0.28 (0.07-1.04)
	Non-immunized/Vaccinated	0.24 (0.06-0.93)

Abbreviations: anti-HBc Ab, anti-hepatitis B core antibodies; CI, confidence interval; HBsAg, Hepatitis B surface antigen; HBV DNA, Hepatitis B virus deoxyribonucleic acid; HR, hazard ratio.

HRs and 95% CI were obtained from univariable exponential survival models.

[†]Determined from serological results at inclusion, defined in Box 1.