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Effect of viral replication and liver fibrosis on all-cause mortality in HIV/HBV coinfecting individuals: a retrospective analysis of a 15-year longitudinal cohort

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1 **Effect of viral replication and liver fibrosis on all-cause mortality in HIV/HBV coinfecting**
2 **individuals: a retrospective analysis of a 15-year longitudinal cohort**

3

4 **Running title:** Viral loads and mortality in HIV/HBV

5

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36

37 **Key-points:** Both current and cumulative HBV-DNA levels over time increased the risk of all-cause

38 mortality in HIV-HBV co-infected individuals. Fibrosis was a major determinant of mortality;

39 however, the leading causes of death were mostly extra-hepatic and non-AIDS related.

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45

46 **Abstract**

47 **Background**

48 In individuals co-infected with HIV and hepatitis B virus (HBV), widespread tenofovir (TDF)-
49 containing antiretroviral therapy (ART) has led to substantial decreases in HBV-DNA and HIV-RNA
50 detection. However, the link between viral replication, liver fibrosis, and mortality remains unclear.

51 **Methods**

52 300 HIV-HBV co-infected individuals undergoing ART were prospectively followed. Virological and
53 clinical data were obtained at baseline and every 6-12 months. We quantified the association
54 between HBV-DNA, HIV-RNA, and liver fibrosis with risk of all-cause mortality using a joint
55 longitudinal-survival model. Viral detection, viral loads, and time-averaged cumulative viral loads of
56 HIV and HBV were modeled as three separate exposures.

57 **Results**

58 During a median 10.5 years (IQR=4.0-14.6), the proportion undergoing TDF-containing ART
59 (baseline=18.7%, end of follow-up=79.1%) and with undetectable HBV-DNA (baseline=36.7%, end of
60 follow-up=94.8%) substantially increased. HIV-RNA was mostly undetectable during follow-up
61 (76.6%). 42 participants died (incidence rate=1.30/100person-years, 95%CI=0.96-1.76). The leading
62 causes of death were non-AIDS/non-liver-related malignancies (28.6%), followed by liver-related
63 (16.7%), AIDS-related (16.7%), and other (16.7%). All-cause mortality was associated with HBV-DNA
64 viral load (adjusted-HR per \log_{10} IU/mL=1.41, 95%CI=1.04-1.93, $p=0.03$) or time-averaged cumulative
65 HBV-DNA (adjusted-HR per \log_{10} IU-years=1.37, 95%CI=1.03-1.83, $p=0.03$), but not undetectable HBV-
66 DNA (adjusted-HR=0.30, 95%CI=0.08-1.09, $p=0.08$). Advanced liver fibrosis at baseline was also
67 associated with increased mortality rates (adjusted-HR=2.35, 95%CI=1.16-4.76, $p=0.02$). No
68 significant association between HIV-RNA replication and mortality was observed.

69 **Conclusions**

70 Concurrent and historical HBV replication and liver fibrosis are important drivers of all-cause
71 mortality in largely TDF-treated HIV-HBV co-infected individuals, despite one-fifth of deaths being

72 liver-related. HBV-DNA and liver fibrosis remain important prognostic indicators for this patient
73 population.

74

75 **Key-words:** hepatitis B virus, HIV, mortality, tenofovir, joint models

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77

78

79 **Introduction**

80 Roughly 8% of individuals living with human immunodeficiency virus (HIV) are chronically co-infected
81 with hepatitis B virus (HBV) [1]. Without effective treatment, HIV-HBV co-infected individuals are at
82 increased risk of liver-related and all-cause mortality when compared to HIV-positive individuals
83 without HBV infection [2].

84

85 Treating co-infected individuals primarily involves controlling both HIV and HBV replication. Higher
86 levels of serum HIV-RNA have been associated with increased risk of AIDS-related morbidity and
87 mortality in HIV-positive individuals [3]. With effective antiretroviral therapy (ART), HIV replication is
88 suppressed and the risk of HIV-related morbidity and mortality greatly decreases [4,5]. Likewise,
89 higher levels of circulating HBV-DNA have been linked to fibrosis, hepatocellular carcinoma (HCC),
90 and liver-related and overall death in HBV mono-infected individuals [6–8]. Currently available
91 nucleoside/nucleotide analogues (NA) can suppress HBV-DNA replication, which coincides with liver
92 fibrosis regression [9] and reduced risk of HCC [10]. Conveniently, some antiretroviral agents, such as
93 tenofovir (TDF) and tenofovir alafenamide (TAF), possess potent anti-HIV and anti-HBV activity
94 [11,12], and are thus ideal therapeutic options for HIV-HBV co-infected individuals [13]. It could be
95 hypothesized that reductions in HIV and HBV replication would give way to lower incidence of both
96 HIV- and HBV-related causes of morbidity and mortality.

97

98 Nevertheless, epidemiological studies in co-infected populations are not entirely clear on the
99 relationship between active replication of these viruses and mortality. Recent research has
100 demonstrated clear reductions in AIDS-related, liver-related and overall mortality in the years
101 coinciding with widespread TDF-use [9,14]. However, incidence of HCC in TDF-treated co-infected
102 individuals above the age of 45 remains high enough to warrant increased HCC screening [15].
103 Furthermore, large studies from Tanzania and Côte d'Ivoire have shown that HIV-HBV co-infected
104 individuals, particularly when their HBV-DNA levels are high, are still at increased risk of overall

105 mortality despite TDF-containing ART [16,17]. The principal causes of death in these studies seem
106 related to HIV-related illness or invasive bacterial infections. The common limitation shared across
107 the studies conducted thus far is the lack of consistently collected data on both HIV- and HBV-
108 replication.

109

110 The aim of the present study was then to describe detailed causes of mortality in a cohort of HIV-
111 HBV co-infected individuals followed for up to 15 years with highly effective anti-HIV and anti-HBV
112 treatment. We further develop our analysis by exploring the effect of HBV-DNA and HIV-RNA
113 replication over time and liver fibrosis on all-cause mortality in this study population.

114

115 **Methods**

116 ***Study population***

117 We analyzed HIV-HBV co-infected participants of the French HIV-HBV Cohort Study. Briefly, this was
118 a closed, longitudinal cohort study including 308 HIV-positive patients with chronic HBV infection
119 from four centers located in Paris and Lyon, France. Individuals were included if they had an HIV-
120 positive serological result confirmed by western blot and HBsAg-positive serological results for >6
121 months. Participants were recruited in 2002-2003 and followed every 6-12 months until 2017-2018.
122 The cohort design and procedures are described elsewhere [18]. For this analysis, we included
123 participants who had at least two consecutive visits and available information on vital status.

124

125 All individuals provided written informed consent to participate in the study and the protocol was
126 approved by a Hospital Ethics Committee (Paris, France) in accordance with the Helsinki Declaration.

127

128 ***Data collection***

129 Demographic information was collected at study inclusion. Medical history on antiretroviral and anti-
130 HBV treatments, alcohol consumption and the presence of comorbidities, including diabetes,

131 cardiovascular disease (CVD), renal and other liver diseases, were collected at study entry and at
132 each follow-up visit.

133

134 Laboratory data were collected at study entry and at each follow-up visit. Commercial polymerase
135 chain reaction (PCR)-based assays were used to quantify HBV-DNA viral load (VL; COBAS
136 AmpliPrep/COBAS TaqMan; detection limit=12 or 38 IU/mL; COBAS Amplicor; detection limit=60
137 IU/mL; Roche Diagnostic Systems, Meylan, France). We defined undetectable HBV-DNA at the
138 highest threshold (<60 IU/mL). HIV-RNA VL was measured using a commercial PCR-based assay and
139 CD4⁺ cell count using standard methods. Antibodies to hepatitis C virus (HCV) and hepatitis D virus
140 (HDV) were measured with an ELISA-based assay and if positive, serum HCV-RNA and/or HDV-RNA
141 was quantified by either commercial PCR-based assay (for HCV-RNA) or in-house assay (for HDV-
142 RNA).

143

144 Liver fibrosis was assessed at study entry and each yearly interval using the FibroTest® [19].

145 METAVIR equivalents for HIV-HBV coinfecting individuals were used to grade liver fibrosis (F2=0.48-
146 0.58, F3=0.59-0.73, F4≥0.74) [20].

147

148 ***Mortality outcome assessment***

149 Deaths observed during follow-up, along with the underlying cause of death and date of death, were
150 reported by the treating physician. To obtain vital status for individuals lost to follow-up (LTFU), a
151 trusted third party (Inserm U1018) was requested to link data from the French HIV-HBV cohort to a
152 national identification registry (*Répertoire national d'identification des personnes physiques*). For
153 individuals reported as deceased, the cause of death was then obtained by a separate trusted third
154 party (*Centre d'épidémiologie sur les causes médicales de décès*, CépiDc), linking data from the
155 French HIV-HBV cohort to a national registry of death certificates and death notifications. Both
156 registries are managed by the *Institut National de la Statistique et des Etudes Economiques*. Causes

157 of death were classified by ICD-10 codes. We recategorized causes of death as liver-related, AIDS-
158 related, non-AIDS and non-liver-related malignancies, CVD-related, other, or unknown [5].

159

160 ***Statistical analysis***

161 Baseline was defined as the date of study entry. Follow-up began at baseline and continued until the
162 date of death or the last study visit.

163

164 We assessed treatment efficacy with undetectable HBV-DNA (<60 IU/mL) and HIV-RNA (<50
165 copies/mL) VLs. The extent of replication was assessed with HBV-DNA (\log_{10} IU/mL) and HIV-RNA
166 (\log_{10} copies/mL) levels. Finally, the historical extent of replication was assessed with time-averaged,
167 cumulative copy-years over follow-up time (\log_{10} copy-year_{STAVG}), as detailed elsewhere [21].

168

169 To analyze the contribution of HIV and HBV replication on mortality during follow-up, we
170 simultaneously modeled (i) HBV replication, (ii) HIV replication, and (iii) all-cause mortality. We
171 carried out a generalized, multivariate, joint longitudinal-survival model approach by which the link
172 between these outcomes could be taken into account. First, we ran two submodels (on HBV-DNA
173 and HIV-RNA) for three separate sets of replication outcomes (detectable VL, log-transformed VL,
174 and \log_{10} copy-years_{STAVG}). The probability of having a detectable VL was assumed to be Bernoulli-
175 distributed and modeled using logistic regression, while mean log-transformed VL and mean \log_{10}
176 copy-years_{STAVG} were assumed to be continuous Poisson-distributed and modeled using Poisson
177 regression. We adjusted, in the model regressing HBV-DNA, for HBeAg serostatus at baseline and
178 cumulative tenofovir use (as a cubic-spline function using 4 knots) and, in the model regressing HIV-
179 RNA, for squared CD4⁺ cell count and HIV treatment era (2002-2007, \geq 2008). Second, the hazards of
180 death were assumed to have an exponential survival function and were estimated using a
181 parametric survival model. We included the two submodels of HBV-DNA and HIV-RNA to estimate
182 the hazards ratio (HR) and 95% confidence intervals (CI) of increasing expected probability of

183 detectable VL, expected mean log-transformed VL, or expected mean \log_{10} copy-years_{TAVG} on all-
184 cause mortality. The survival model also included age, previous history of an AIDS-defining illness
185 and level of fibrosis at study entry (F0-F1-F2 and F3-F4) as covariates, selected from the analysis
186 described in the Supplementary Materials. We included a random-intercept across all models to
187 account for between-patient variance at baseline, while the random-intercepts were constrained at
188 1 for the two submodels. Parameters from the three models were jointly estimated via maximum
189 likelihood using the 'merlin' program in STATA [22].

190

191 All statistical analyses were performed using STATA (v15.1; College Station, Texas, USA). Significance
192 was defined as a p value <0.05.

193

194 **Results**

195 ***Description of the study population***

196 Of the 308 cohort participants, 8 were excluded (only one visit, $n=7$; no information on vital status,
197 $n=1$). The 300 included participants were mostly male (84.0%), with a median age of 40 years
198 (IQR=35-45) at study entry. Participants had a median CD4+ count of 400/mm³ (IQR=268-557) and
199 160 (53.5%) had undetectable HIV-RNA. Participants were mostly HBeAg-positive (52.0%) and 63.3%
200 ($n=190/300$) had detectable HBV-DNA. Of the 281 individuals (94.9%) with previous lamivudine
201 (LAM) exposure, median LAM duration was 3.5 years (IQR=1.4-5.5) at study entry and 90 (32.0%)
202 harbored LAM-resistant mutations.

203

204 Participants were followed for a median 10.5 years (IQR=4.0-14.6), totaling 2934.5 person-years. 111
205 (37.0%) were LTFU, including 41 with known vital status and 70 with unknown vital status.

206 Individuals LTFU had a significantly higher median HBV-DNA (for those with detectable levels), ALT
207 and AST levels at inclusion (supplementary table 2). The proportion of participants undergoing ART
208 was high at study inclusion (90.0%) and increased to 100% from the first year until the end of follow-

209 up. Consequently, improvements in CD4⁺ cell counts (p for trend <0.001) were observed over time
210 (Figure 1A). In addition, the proportion of individuals undergoing TDF-based ART increased from
211 18.7% at baseline to 40.1% at the first year and 79.1% at the end of follow-up (Figure 1B, p for trend
212 <0.001).

213

214 ***Description of all-cause mortality***

215 42 deaths (cumulative incidence=14.0%; 95%CI=10.3%-18.4%) occurred after a median 6.2 years
216 (IQR=3.4-7.9) of follow-up (incidence=1.43/100 person-years) 7 of these deaths were obtained
217 through linkage.

218

219 The most common causes of death were non-AIDS/non-liver related malignancies ($n=12$ [28.6%];
220 0.41/100 person-years), liver-related ($n=7$ [16.7%]; 0.24/100 person-years), AIDS-related ($n=7$
221 [16.7%]; 0.24/100 person-years) and CVD-related ($n=6$ [14.3%]; 0.20/100 person-years) (Table 1).

222 HCC and hepatic failure accounted for most liver-related deaths ($n=4$ and 1, respectively). 7
223 individuals (16.7%; 0.24/100 person-years) died from others causes of death, while for three (7.0%),
224 the cause of death was unknown.

225

226 At study entry, individuals who died, compared to those alive, were older ($p<0.001$), more likely to
227 come from zones of low/moderate HBV-prevalence ($p<0.004$), have acquired HIV infection by
228 injecting drug use (IDU) ($p<0.02$), have other liver diseases or hepatic decompensation ($p=0.001$),
229 have an AIDS-defining event ($p<0.001$), have longer duration since first positive HIV test ($p=0.01$),
230 lower nadir CD4⁺ cell counts ($p=0.03$), longer duration of ART ($p=0.05$), higher levels of AST ($p=0.01$),
231 HBeAg-positive status ($p=0.02$), and F3-F4 fibrosis ($p<0.001$) (Table 2).

232

233 During follow-up, individuals who died, when compared to those alive, were more often diagnosed
234 with HDV coinfection ($p=0.01$), had shorter duration of cumulative tenofovir use ($p<0.001$), lower
235 CD4+ cell counts ($p=0.001$), and detectable HBV-DNA at last follow-up visit ($p=0.03$) (Table 2).

236

237 ***HBV and HIV viral replication and all-cause mortality***

238 The average individual proportion of detectable HBV-DNA VL during follow-up was higher in
239 deceased versus alive individuals (52.6%, 95%CI=25.0-77.8 versus 25.0%, 95%CI=6.7-58.8;
240 respectively, $p<0.003$). When HBV-DNA was detectable, median levels were at 5646 IU/mL
241 (IQR=446-3,802,281). Deceased individuals were exposed to a higher level of time-averaged copy-
242 years of HBV-DNA ($\log_{10}\text{Copy-years}_{\text{TAVG}}$) than those remaining alive (Figure 2) (overall p -value
243 <0.001). Conversely, the average individual proportion of detectable HIV-RNA VL during follow-up
244 was no different between alive and deceased individuals (15.4%, 95%CI=4.0-48.1 versus 10.0%,
245 95%CI=0.0-60.0, respectively, $p=0.6$). When HIV-RNA was detectable, median levels were at 232
246 copies/mL (IQR=3,159-22,972). Time-averaged copy-years of HIV-RNA was similarly low in both
247 groups during follow-up ($p=0.6$, supplementary Figure S1).

248

249 When jointly modeling HBV and HIV replication on all-cause mortality, we found no significant
250 associations with undetectable HBV-DNA on mortality rates ($p=0.08$, Table 3). However, we did
251 observe a higher rate of all-cause mortality with higher expected mean log-transformed HBV-DNA VL
252 ($p=0.03$) and cumulative time-averaged copy-years of HBV-DNA ($p=0.03$) after adjustment for age,
253 AIDS-defining illness and F3-F4 fibrosis level at study entry (Table 3). F3-F4 fibrosis level at study
254 entry was the only covariate consistently and significantly associated with overall mortality. No
255 significant association was found between any of the HIV-RNA outcomes and all-cause mortality
256 (Table 3).

257

258 **Discussion**

259 In this long-term, prospective study of treated HIV-HBV co-infected individuals, the most common
260 cause of death observed in our study was non-AIDS/non-liver-related malignancies, representing
261 approximately one-third of deaths. Coupled with the high proportion of deaths due to CVD, the
262 spectrum of mortality causes in HIV-HBV co-infection would appear to mirror that of aging HIV-
263 positive individuals in general [23]. Nevertheless, liver-related and AIDS-related causes together
264 represented one-third of deaths, which occurred even during periods when use of TDF-containing
265 ART and suppression of HIV-RNA and HBV-DNA were common at the population level.

266

267 Interestingly, the concurrent and historical extent of HBV viremia over time, but not HIV, seemed to
268 play a major role in all-cause mortality. This effect was not observed when modeling undetectable
269 HBV-DNA over time. Other studies in HIV-HBV co-infected individuals have shown an association
270 between higher HBV-DNA at treatment initiation and all-cause mortality [17,24]. The curious part of
271 this previous finding was that it was observed in the presence of almost exclusively TDF-treated
272 individuals, with assumedly extensive HBV DNA suppression. Given the findings in our study, perhaps
273 the historical exposure of high HBV DNA explained the increased risk in mortality in these studies
274 despite effective anti-HIV and anti-HBV treatment.

275

276 Conversely, HIV-RNA had no effect on all-cause mortality in our study. This result was somewhat
277 surprising considering the well-described effects of HIV replication on AIDS and non-AIDS-related
278 death [25]. Although a high proportion of individuals had detectable HIV-RNA levels at inclusion,
279 most were able to achieve HIV-RNA suppression early in the cohort study. Under these conditions,
280 AIDS-related mortality is generally associated with the time spent at lower CD4+ cell counts [26,27],
281 while most individuals in our cohort attained levels of CD4+ associated with reduced risk of both
282 AIDS-related and overall mortality. The low overall proportion of AIDS-related deaths is also in
283 accordance with findings from European cohorts of HIV-positive individuals [28]. When comparing
284 HIV and HBV replication, there were comparable degrees of HIV-RNA suppression for individuals

285 who remained alive and died during follow-up, while HBV-DNA was more frequently detectable in
286 deceased than alive individuals. These differences were similarly observed in historical exposure and
287 perhaps drove HBV-DNA, rather than HIV-RNA, to contribute more towards overall mortality in our
288 study.

289

290 The question is then what aspects of HBV-DNA replication are driving overall mortality. Naturally,
291 immunological responses against prolonged HBV infection are responsible for liver-related disease
292 [29], which partly explains the association between advanced liver fibrosis and all-cause mortality.
293 Although liver fibrosis is mostly known to affect liver-related mortality (i.e. death due to HCC or end-
294 stage liver disease), we observed few liver-related deaths. These results are rather surprising. Most
295 individuals were undergoing TDF-containing ART and hence this study population represents those
296 with well-controlled HBV-DNA replication. The risk of HCC would be reduced at these HBV-DNA
297 levels [10]. Nevertheless, individuals with liver cirrhosis do have a high risk of death due to invasive
298 bacterial pathogens [30] and many of the other causes of death in our cohort were related to
299 bacteremia. Although effective anti-HBV treatment is expected to decrease fibrosis in HBV mono-
300 infected individuals [31], fibrosis levels are mostly unchanged or can progress during TDF-based ART
301 in co-infected individuals [32]. Continued risk of death due to this comorbidity should be elucidated
302 in larger cohorts.

303

304 One intriguing finding was that certain extra-hepatic malignant tumors caused several deaths in this
305 study population. In fact, 40% of the 20 individuals who died from cancer developed an extra-
306 hepatic tumor (i.e. anal cancer, cholangiocarcinoma, pancreatic adenocarcinoma, and non-Hodgkin
307 lymphoma [NHL]). Previous research has found that HBsAg-positive individuals with high-risk human
308 papillomavirus had an increased risk of high-grade anal squamous intraepithelial lesions compared
309 to those who were HBsAg-negative [33]. Furthermore, HBsAg-positive individuals exhibiting high
310 levels of HBV activity (i.e. HBeAg-positivity and detectable HBV-DNA) have a significantly higher risk

311 of pancreatic carcinoma compared to HBsAg-negative individuals [34]. Finally, although NHL is
312 normally related to immunocompromised individuals and is classified as an AIDS-defining illness,
313 growing evidence suggests that ART-treated individuals with chronic HBV infection are at increased
314 risk for NHL [35]. How HBV activity participates in the tumorigenesis of extra-hepatic tumors is not
315 clearly understood.

316

317 Similar to others [36], HDV-co-infected individuals had a higher all-cause mortality rate.

318 Unfortunately, due to its close association with fibrosis, we decided not to include HDV-co-infection
319 in multivariable analysis. Of the 22 individuals in our cohort with HIV-HBV-HDV infection, 7 died.

320 Despite the fact that 6 of these deceased individuals had advanced liver fibrosis and at least one
321 liver-related complication, only one died from liver-related diseases. In contrast to others [36], only
322 one HCV-positive individual (without HDV) died in our cohort, which could be a reflection of the low
323 overall proportion of IDU (7.7%) and the increasingly effective direct acting antivirals available by the
324 end of follow-up [37]. Given the very few individuals with tri-/quad-infection who remained in
325 follow-up, generalizability of our data to this population would be limited.

326

327 Our study has certain limitations. First, the study population involves HIV-positive individuals with
328 extensive ART-experience and larger degrees of immunosuppression compared to contemporary
329 patient populations, but still actively seen in outpatient settings. Second, HBsAg-seroclearance has
330 been shown to reduce all-cause mortality in HBV mono-infected individuals [38], yet we had an
331 insufficient number of events to validate this in our cohort. Third, there was a rather high rate of
332 LTFU. Individuals who were LTFU had higher HBV DNA, ALT/AST, but not fibrosis levels at cohort
333 inclusion, suggesting a higher risk of more HBV activity. It is difficult to assess the direction of this
334 differential LTFU bias without knowing whether they were virally suppressed after being LTFU.

335 Fourth, we had limited to no data on HBV-DNA and HIV-RNA replication prior to inclusion, alcohol
336 use, smoking, treatment adherence, and metabolic diseases, all of which could not be considered in

337 analysis. We also used a non-invasive measure to assess liver fibrosis, which involves some error
338 [20]; however, the FibroTest® does accurately predict liver fibrosis evolution [39] and overall survival
339 [40] in individuals with chronic HBV infection. Finally, we did not have enough power to determine
340 which causes of death were more associated with HBV-DNA replication.

341

342 In conclusion, our findings provide strong evidence that HIV-HBV co-infected individuals who have
343 been exposed to higher levels of HBV-DNA over time are at elevated risk for all-cause mortality. The
344 lack of association with HIV-RNA replication could be due to the more extensive viral suppression
345 overall compared to HBV-DNA. Accompanied by the strong association between advanced liver
346 fibrosis and overall mortality, monitoring liver fibrosis and HBV-DNA VL should be an essential
347 component to help assess the prognosis of co-infected individuals. The noticeably common deaths
348 due to extra-hepatic malignancies should be further studied and perhaps increased screening is
349 called for in the HIV-HBV co-infected patient population.

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354

355 **Potential conflicts of interest**

356 None to declare.

357

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363

364 **Role of each author.** L.N.C.D. was responsible for the statistical analysis, interpretation of the data,
365 and drafting the manuscript. R.K. obtained and verified vital status on participants, assisted in the
366 statistical analysis, and gave critical revisions of the manuscript. H.R., P.M., C. L-C., and J.C. acquired
367 data for the cohort, assisted in interpreting data, and gave critical revisions of the manuscript. S.M.,
368 A.G. and C.D. were responsible for interpretation of the data and drafting the manuscript. K.L.
369 helped design, conceptualize, and obtain funding for the French HIV-HBV cohort study, coordinated
370 data collection, and drafted the manuscript. A.B. coordinated data analysis, gave important
371 comments on data interpretation, drafted parts of the manuscript, and provided critical revisions of
372 the manuscript. All authors have approved the final version of the article.

373

374

375 **References**

- 376 1. Leumi S, Bigna JJ, Amougou MA, Ngouo A, Nyaga UF, Noubiap JJ. Global Burden of Hepatitis
377 B Infection in People Living With Human Immunodeficiency Virus: A Systematic Review and Meta-
378 analysis. *Clin Infect Dis* **2020**; 71:2799–2806.
- 379 2. Falade-Nwulia O, Seaberg EC, Rinaldo CR, Badri S, Witt M, Thio CL. Comparative Risk of Liver-
380 Related Mortality From Chronic Hepatitis B Versus Chronic Hepatitis C Virus Infection. *Clin Infect Dis*
381 **2012**; 55:507–513.
- 382 3. Mellors JW, Muñoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic
383 markers of HIV-1 infection. *Ann Intern Med* **1997**; 126:946–954.
- 384 4. The antiretroviral therapy cohort collaboration (ART-CC). Prognosis of patients treated with
385 cART from 36 months after initiation, according to current and previous CD4 cell count and plasma
386 HIV-1 RNA measurements. *AIDS* **2009**; 23:2199–2208.
- 387 5. Smith CJ, Ryom L, Weber R, et al. Trends in underlying causes of death in people with HIV
388 from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet* **2014**; 384:241–248.
- 389 6. Iloeje UH, Yang H, Jen C, et al. Risk and Predictors of Mortality Associated With Chronic
390 Hepatitis B Infection. *Clin Gastroenterol Hepatol* **2007**; 5:921–931.
- 391 7. Hawkins C, Agbaji O, Ugoagwu P, et al. Assessment of Liver Fibrosis by Transient
392 Elastography in Patients With HIV and Hepatitis B Virus Coinfection in Nigeria. *Clin Infect Dis* **2013**;
393 57:e189–e192.
- 394 8. Nina Kim H, Newcomb CW, Carbonari DM, et al. Risk of Hepatocellular Carcinoma with
395 Hepatitis B Viremia among HIV/Hepatitis B Virus-Coinfected Persons in North America. *Hepatology*
396 **2021**; hep.31839.
- 397 9. van Welzen BJ, Smit C, Boyd A, et al. Decreased All-Cause and Liver-Related Mortality Risk in
398 HIV/Hepatitis B Virus Coinfection Coinciding With the Introduction of Tenofovir-Containing
399 Combination Antiretroviral Therapy. *Open Forum Infect Dis* **2020**; 7:ofaa226.
- 400 10. Papatheodoridis GV, Chan HL-Y, Hansen BE, Janssen HLA, Lampertico P. Risk of

- 401 hepatocellular carcinoma in chronic hepatitis B: assessment and modification with current antiviral
402 therapy. *J Hepatol* **2015**; 62:956–967.
- 403 11. Boyd A, Gozlan J, Maylin S, et al. Persistent viremia in human immunodeficiency
404 virus/hepatitis B coinfecting patients undergoing long-term tenofovir: Virological and clinical
405 implications. *Hepatology* **2014**; 60:497–507.
- 406 12. Huang Y-S, Cheng C-Y, Liou B-H, et al. Efficacy and safety of
407 elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide as maintenance treatment in HIV/HBV-
408 coinfecting patients. *J Acquir Immune Defic Syndr* **2021**; 86:473–481.
- 409 13. European AIDS Clinical Society. Guidelines Version 10.1. October 2020.
- 410 14. Tsai W-C, Hsu W-T, Liu W-D, et al. Impact of antiretroviral therapy containing tenofovir
411 disoproxil fumarate on the survival of patients with HBV and HIV coinfection. *Liver Int* **2019**;
412 39:1408–1417.
- 413 15. Wandeler G, Mauron E, Atkinson A, et al. Incidence of hepatocellular carcinoma in HIV/HBV-
414 coinfecting patients on tenofovir therapy: Relevance for screening strategies. *J Hepatol* **2019**;
415 71:274–280.
- 416 16. Christian B, Fabian E, Macha I, et al. Hepatitis B virus coinfection is associated with high early
417 mortality in HIV-infected Tanzanians on antiretroviral therapy. *AIDS* **2019**; 33:465–473.
- 418 17. Kouamé G-M, Boyd A, Moh R, et al. Higher Mortality Despite Early Antiretroviral Therapy in
419 Human Immunodeficiency Virus and Hepatitis B Virus (HBV)-Coinfecting Patients With High HBV
420 Replication. *Clin Infect Dis* **2018**; 66:112–120.
- 421 18. Boyd A, Dezanet LNC, Kassime R, et al. Subclinical and Clinical Outcomes in Patients
422 Coinfecting With HIV and Chronic Hepatitis B Virus From Clinical Outpatient Centers in France:
423 Protocol for an Ambispective, Longitudinal Cohort Study. *JMIR Res Protoc* **2021**; 10:e24731.
- 424 19. Poynard T, Ngo Y, Munteanu M, Thabut D, Ratziu V. Noninvasive Markers of Hepatic Fibrosis
425 in Chronic Hepatitis B. *Curr Hepat Rep* **2011**; 10:87–97.
- 426 20. Bottero J, Lacombe K, Guéchet J, et al. Performance of 11 biomarkers for liver fibrosis

427 assessment in HIV/HBV co-infected patients. *J Hepatol* **2009**; 50:1074–1083.

428 21. Boyd A, Gozlan J, Miaillhes P, et al. Rates and determinants of hepatitis B ‘e’ antigen and
429 hepatitis B surface antigen seroclearance during long-term follow-up of patients coinfecting with HIV
430 and hepatitis B virus: *AIDS* **2015**; 29:1963–1973.

431 22. Crowther MJ. merlin—A unified modeling framework for data analysis and methods
432 development in Stata. *Stata J* **2020**; 20:763–784.

433 23. Joint United Nations Programme on HIV/AIDS. HIV and aging. 2013. Available at:
434 http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2013/20131101_JC2563_hiv-and-aging_en.pdf. Accessed 19 November 2020.

435 24. Velen K, Charalambous S, Innes C, Churchyard GJ, Hoffmann CJ. Chronic hepatitis B increases
436 mortality and complexity among HIV-coinfected patients in South Africa: a cohort study. *HIV Med*
437 **2016**; 17:702–707.

438 25. Phillips AN, Neaton J, Lundgren JD. The role of HIV in serious diseases other than AIDS. *AIDS*
439 **2008**; 22:2409–2418.

440 26. Battegay M, Nüesch R, Hirschel B, Kaufmann GR. Immunological recovery and antiretroviral
441 therapy in HIV-1 infection. *Lancet Infect Dis* **2006**; 6:280–287.

442 27. Opportunistic Infections Project Team of the Collaboration of Observational HIV
443 Epidemiological Research in Europe (COHERE) in EuroCoord, Young J, Psychogiou M, et al. CD4 cell
444 count and the risk of AIDS or death in HIV-Infected adults on combination antiretroviral therapy with
445 a suppressed viral load: a longitudinal cohort study from COHERE. *PLoS Med* **2012**; 9:e1001194.

446 28. Vandenhende M-A, Roussillon C, Henard S, et al. Cancer-Related Causes of Death among
447 HIV-Infected Patients in France in 2010: Evolution since 2000. *PloS One* **2015**; 10:e0129550.

448 29. Singh KP, Crane M, Audsley J, Avihingsanon A, Sasadeusz J, Lewin SR. HIV-hepatitis B virus
449 coinfection: epidemiology, pathogenesis, and treatment. *AIDS* **2017**; 31:2035–2052.

450 30. Nahon P, Lescat M, Layese R, et al. Bacterial infection in compensated viral cirrhosis impairs
451 5-year survival (ANRS CO12 CirVir prospective cohort). *Gut* **2017**; 66:330–341.

452

- 453 31. Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir
454 disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* **2013**;
455 381:468–475.
- 456 32. Boyd A, Bottero J, Mialhes P, et al. Liver fibrosis regression and progression during
457 controlled hepatitis B virus infection among HIV-HBV patients treated with tenofovir disoproxil
458 fumarate in France: a prospective cohort study. *J Int AIDS Soc* **2017**; 20:21426.
- 459 33. McCloskey JC, Kast WM, Flexman JP, McCallum D, French MA, Phillips M. Syndemic synergy
460 of HPV and other sexually transmitted pathogens in the development of high-grade anal squamous
461 intraepithelial lesions. *Papillomavirus Res* **2017**; 4:90–98.
- 462 34. Illoeje UH, Yang H-I, Jen C-L, et al. Risk of pancreatic cancer in chronic hepatitis B virus
463 infection: data from the REVEAL-HBV cohort study. *Liver Int* **2010**; 30:423–429.
- 464 35. Wang Q, De Luca A, Smith C, et al. Chronic Hepatitis B and C Virus Infection and Risk for Non-
465 Hodgkin Lymphoma in HIV-Infected Patients: A Cohort Study. *Ann Intern Med* **2017**; 166:9.
- 466 36. Béguelin C, Moradpour D, Sahli R, et al. Hepatitis delta-associated mortality in HIV/HBV-
467 coinfecting patients. *J Hepatol* **2017**; 66:297–303.
- 468 37. Smit C, Boyd A, Rijnders BJA, et al. HCV micro-elimination in individuals with HIV in the
469 Netherlands 4 years after universal access to direct-acting antivirals: a retrospective cohort study.
470 *Lancet HIV* **2021**; 8:e96–e105.
- 471 38. Arase Y, Ikeda K, Suzuki F, et al. Long-term outcome after hepatitis B surface antigen
472 seroclearance in patients with chronic hepatitis B. *Am J Med* **2006**; 119:71.e9–16.
- 473 39. Poynard T, Munteanu M, Deckmyn O, et al. Validation of liver fibrosis biomarker (FibroTest)
474 for assessing liver fibrosis progression: proof of concept and first application in a large population. *J*
475 *Hepatol* **2012**; 57:541–548.
- 476 40. de Ledinghen V, Vergniol J, Barthe C, et al. Non-invasive tests for fibrosis and liver stiffness
477 predict 5-year survival of patients chronically infected with hepatitis B virus. *Aliment Pharmacol Ther*
478 **2013**; 37:979–988.

Table 1. Causes of death observed in the French HIV-HBV cohort, 2002-2018.

Cause of death	All periods (N=42)	2002-2007 (N=10)	2008-2018 (N=32)
Liver-related disease	7 (16.7%)	2	5
<i>Hepatocellular carcinoma</i>	4	1	3
<i>Cholangiocarcinoma</i>	1	0	1
<i>Liver failure</i>	2	1	1
AIDS-related disease	7 (16.7%)	2	5
<i>Kaposi sarcoma</i>	1	1	0
<i>Burkitt lymphoma</i>	1	0	1
<i>Other non-Hodgkin's lymphoma</i>	1	0	1
<i>Progressive multifocal leukoencephalopathy</i>	1	1	0
<i>HIV-associated neurocognitive disorder</i>	1	0	1
<i>Opportunistic infections</i>	2	0	2
Non-AIDS and non-liver-related malignancy	12 (28.6%)	0	12
<i>Anal cancer</i>	5	0	5
<i>Colorectal cancer</i>	1	0	1

<i>Pancreatic cancer</i>	1	0	1
<i>Prostate cancer</i>	1	0	1
<i>Lung cancer</i>	1	0	1
<i>Chronic lymphocytic leukaemia</i>	1	0	1
<i>Cancer of unknown primary origin</i>	2	0	2
Cardiovascular disease	6 (14.3%)	2	4
<i>Stroke</i>	4	1	3
<i>Sudden cardiac arrest</i>	2	1	1
Others	7 (16.7%)	3	4
<i>Septic Shock</i>	5*	2	3
<i>Pneumonia</i>	1	1	0
<i>Empyema</i>	1	0	1
<i>Crohn's disease</i>	1	1	0
<i>Quadriplegia</i>	1	0	1
<i>Subdural hematoma</i>	1	0	1
<i>Suicide</i>	1	1	0
Unknown	3 (7.0%)	1	2

*4 patients died from multiple causes: hepatocellular carcinoma and sepsis ($N=1$, considered as a liver-related death), non-Hodgkin's lymphoma and sepsis ($N=1$, considered as an AIDS-related death), prostate cancer and sepsis ($N=1$, considered as a non-AIDS and non-liver malignancy death), and empyema and sepsis ($N=1$, considered as a death related to other diseases).

Table 2. Characteristics of study population (at cohort inclusion or during follow-up)

Characteristics	Vital status		<i>p</i> [§]
	Alive (n=258)	Died (n=42)	
Demographics			
Gender, male/female (% male)	213/45 (83)	39/3 (93)	0.09
Age at baseline, years*	39.5 (34.5-43.7)	43.5 (38.0-53.2)	<0.001
From zone of high HBV-prevalence [†]	81 (31.4)	4 (9.5)	0.004
Mode of HIV transmission [†]			
Heterosexual	95 (36.8)	10 (23.8)	0.10
MSM	143 (55.4)	25 (59.5)	0.6
IDU	16 (6.2)	7 (16.7)	0.02
Other/Unknown	4 (1.6)	0 (0)	0.9
Clinical characteristics at study entry			
BMI, Kg/m ² [N = 284]*	22.6 (21.0-24.3)	21.9 (20.6-23.5)	0.11
Alcohol consumption, glasses/day [N = 288]*	1 (0 – 2.7)	1 (0 – 2)	0.4
Comorbidities [†]			
Cardiovascular disease	28 (10.9)	6 (14.3)	0.5

Diabetes	5 (1.9)	0 (0)	0.4
Renal disease	6 (2.3)	0 (0)	0.3
Other liver diseases [‡]	5 (1.9)	5 (11.9)	0.001
HIV infection variables at study entry			
Time since first HIV-positive test, years [<i>N</i> = 299]*	9.4 (3.2-13.2)	13.1 (7.1-15.6)	0.01
AIDS-defining illness [†]	57 (22.1)	21 (50.0)	<0.001
HIV-RNA > 50 copies/mL [†]	125 (48.5)	14 (34.2)	0.09
HIV-RNA, log ₁₀ copies/mL [<i>N</i> = 139]*	3.9 (2.2-4.4)	3.8 (2.6-4.1)	0.7
CD4 ⁺ cell count, cells/μL*	405 (277-577)	370 (249-474)	0.25
Nadir CD4 ⁺ cell count, cells/μL [<i>N</i> = 268]*	223 (108-329)	128 (65-304)	0.03
Initiated ART [†]	230 (89.2)	40 (95.2)	0.22
Duration of ART, years [<i>N</i> = 270]*	5.5 (2.6-7.3)	6.7 (3.7-8.8)	0.05
Viral hepatitis at study entry			
Time since first HBsAg-positive test, years [<i>N</i> = 297]*	6.1 (2.2-10.6)	6.7 (2.3-13.5)	0.19
HBV-genotype [<i>N</i> = 165] [†]			
A	84 (61.3)	17 (60.7)	0.9
D	12 (8.8)	4 (14.3)	0.4

E	18 (13.1)	1 (3.6)	0.15
G	21 (15.3)	6 (21.4)	0.4
HBeAg-positive [†]	127 (49.2)	29 (69.1)	0.02
HBV-DNA > 60 IU/mL [N = 299] [†]	163 (63.2)	27 (65.9)	0.7
HBV-DNA, log ₁₀ IU/mL [N = 190]*	5.1 (3.0-6.9)	5.0 (2.8-7.1)	0.6
ALT level, IU/mL [N = 294]*	40 (24-72)	42 (31-67)	0.4
AST level, IU/mL [N = 294]*	36 (26-52)	51 (31-82)	0.01
Metavir F3-F4 fibrosis [†]			
Estimated using <i>Fibrotest</i> [®] [N = 298]	62 (24.1)	24 (58.5)	<0.001
Determined by liver biopsy [N = 138] [#]	33 (28.7)	10 (41.7)	0.22
Metavir F4 fibrosis [†]			
Estimated using <i>Fibrotest</i> [®] [N = 298]	31 (12.1)	18 (43.9)	<0.001
Determined by liver biopsy [N = 138] [#]	9 (7.9)	8 (33.3)	0.001
Cumulative lamivudine use at study entry, years*	2.95 (0.83-5.45)	4.22 (2.10-5.54)	0.09
Variables assessed during follow-up			
Follow-up time, years*	14.2 (4.8-14.7)	6.2 (3.4-7.9)	<0.001
Cumulative tenofovir use, years*	7.0 (2.1-12.6)	3.1 (1.5-5.4)	<0.001

Cumulative lamivudine use, years*	7.32 (3.31-9.92)	7.46 (0.08-9.71)	0.5
Cumulative HBV-DNA (log ₁₀ copy-years _{TAVG}) at last follow-up visit*	2.01 (1.81-2.58)	2.43 (1.92-3.24)	0.01
HBV-DNA <60 IU/mL at last follow-up visit [†]	220 (85.3)	30 (71.4)	0.03
Cumulative HIV-RNA (log ₁₀ Copy-years _{TAVG}) at last follow-up visit*	1.79 (1.71-2.30)	1.75 (1.70-2.27)	0.6
HIV-RNA <50 copies/mL at last follow-up visit [†]	221 (85.7)	34 (80.1)	0.4
CD4 ⁺ cell count at last follow-up visit, cells/μL*	524 (369-696)	423 (201-531)	0.001
Ever HCV coinfecte [†] ^ϕ	23 (8.9)	3 (7.1)	0.7
Ever HDV coinfecte [†] ^ϕ	15 (5.8)	7 (16.7)	0.01
HBeAg loss [‡]	72 (56.7)	13 (44.8)	0.25
HBsAg loss	27 (10.5)	3 (7.4)	0.8

*Median (IQR).

[†] Number (%).

[§] Significance determined using Kruskal-Wallis test for continuous variables and Pearson χ^2 test or Fisher exact test for categorical variables.

¥Other liver diseases or hepatic decompensation: acute, subacute or unspecified hepatic failure; hemorrhagic necrosis of liver; fatty liver disease; portal hypertension; and hepatocellular carcinoma.

In a subgroup of 138 individuals, liver biopsies were performed within 12 months before or at study entry, based on concomitant guidelines from the European Association for the Study of the Liver. Histological fibrosis and activity were scored with the METAVIR classification.

ϕ Established by a positive ELISA-based assay for HCV or HDV, and confirmed by a positive PCR-based assay for HCV-RNA or HDV-RNA, respectively.

‡In 156 HBeAg-positive individuals.

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; IDU, injection drug use; MSM, men who have sex with men; TAVG; time-averaged.

Table 3. Association of HBV-DNA and HIV-RNA replication with all-cause mortality (joint models) ^a

Parameter	Model: treatment efficacy ^b		Model: extent of replication ^c		Model: historical extent of replication ^d	
	Risk estimate ^e		Risk estimate ^e		Risk estimate ^e	
	(95% CI)	<i>p</i>	(95% CI)	<i>p</i>	(95% CI)	<i>p</i>
HBV replication	OR		RR		RR	
HBeAg at baseline	0.04 (0.03-0.05)	<0.001	1.88 (1.79-1.97)	<0.001	2.02 (1.93-2.11)	<0.001
HIV replication	OR		RR		RR	
CD4+ cell count ($\sqrt{\text{mm}^3}$) [§]	1.10 (1.08-1.12)	<0.001	0.987 (0.984-0.991)	<0.001	0.988 (0.985-0.992)	<0.001
HIV treatment era from 2002 to 2007 ^ϕ	0.14 (0.11-0.17)	<0.001	1.22 (1.17-1.27)	<0.001	1.16 (1.11-1.20)	<0.001
Time to all-cause mortality	HR		HR		HR	
Age [§]	1.06 (1.02-1.10)	0.001	1.06 (1.02-1.10)	0.004	1.05 (1.01-1.09)	0.007
AIDS-defining illness at baseline	1.97 (1.00-3.87)	0.05	1.83 (0.94-3.54)	0.07	1.92 (1.00-3.71)	0.05
F3-F4 fibrosis at baseline [#]	2.33 (1.16-4.70)	0.02	2.35 (1.16-4.76)	0.02	2.37 (1.17-4.81)	0.02
HBV replication [¥]	0.30 (0.08-1.09)	0.08	1.41 (1.04-1.93)	0.03	1.37 (1.03-1.83)	0.03

HIV replication [‡]	0.42 (0.02-10.53)	0.6	2.43 (0.45-13.05)	0.30	2.80 (0.49-15.85)	0.25
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^a Analysis included 298 individuals, 41 of whom were deceased. Two individuals were not considered in this analysis due to missing data for level of fibrosis at baseline; one of them died during study follow-up.

^b HBV replication was measured as longitudinal HBV-DNA VL detectability (<60 IU/mL versus ≥60 IU/mL); whereas HIV replication as longitudinal HIV-RNA detectability (<50 copies/mL versus ≥50 copies/mL).

^c HBV replication was measured as longitudinal HBV-DNA (per log₁₀ IU/mL); whereas HIV replication as longitudinal HIV-RNA (per log₁₀ copies/mL).

^d HBV replication was measured as time-averaged cumulative HBV-DNA (per log₁₀copy-years_{TAVG}); whereas HIV replication as cumulative HIV-RNA (per log₁₀copy-years_{TAVG}).

^e Risk estimates are additionally adjusted for the cumulative duration of tenofovir according to a spline function restricted by 4 knots (0.021, 3, 8 and 11.978 years).

[§] Time-updated covariate.

^ϕ Variable included percentage of visits during two HIV treatment eras, 2002-2007 and ≥2008.

[#] Estimated using the Fibrotest[®].

[‡] Expected value as estimated from the respective submodels (HBV replication and HIV replication) as outcomes.

Abbreviations: AIDS, acquired immunodeficiency syndrome; HBV, hepatitis B virus; HIV, human immunodeficiency virus; TAVG, time-averaged.

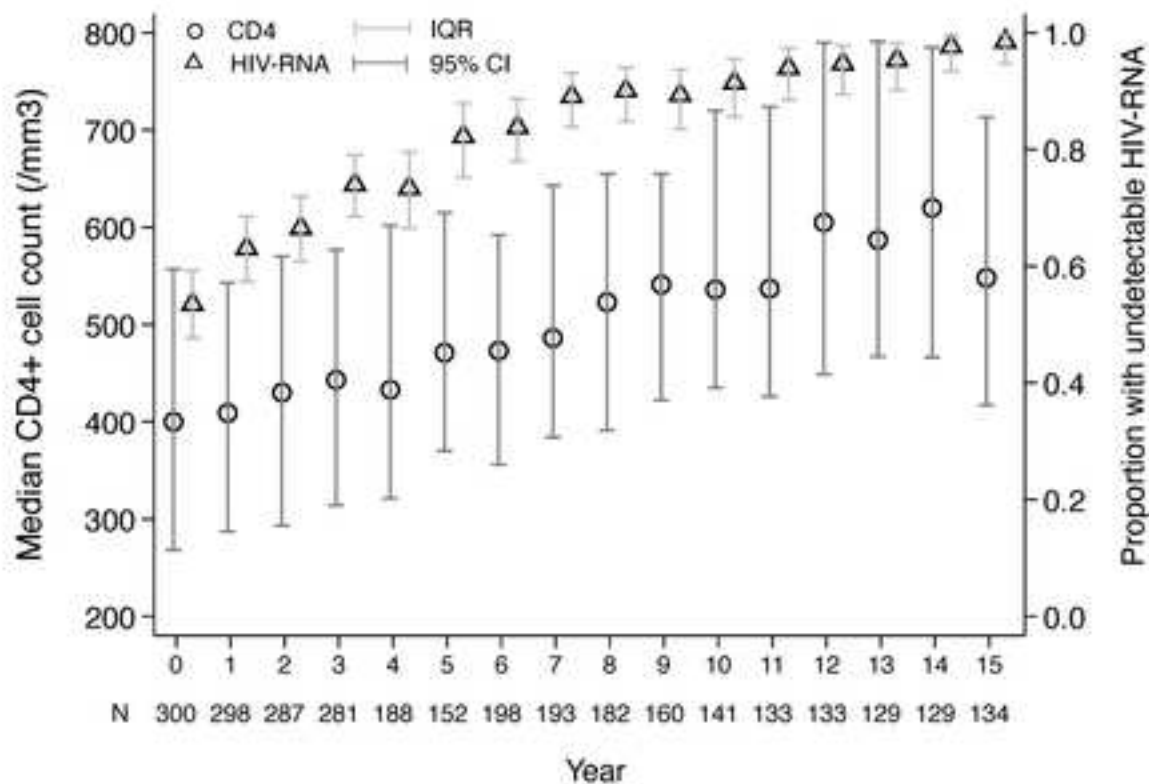
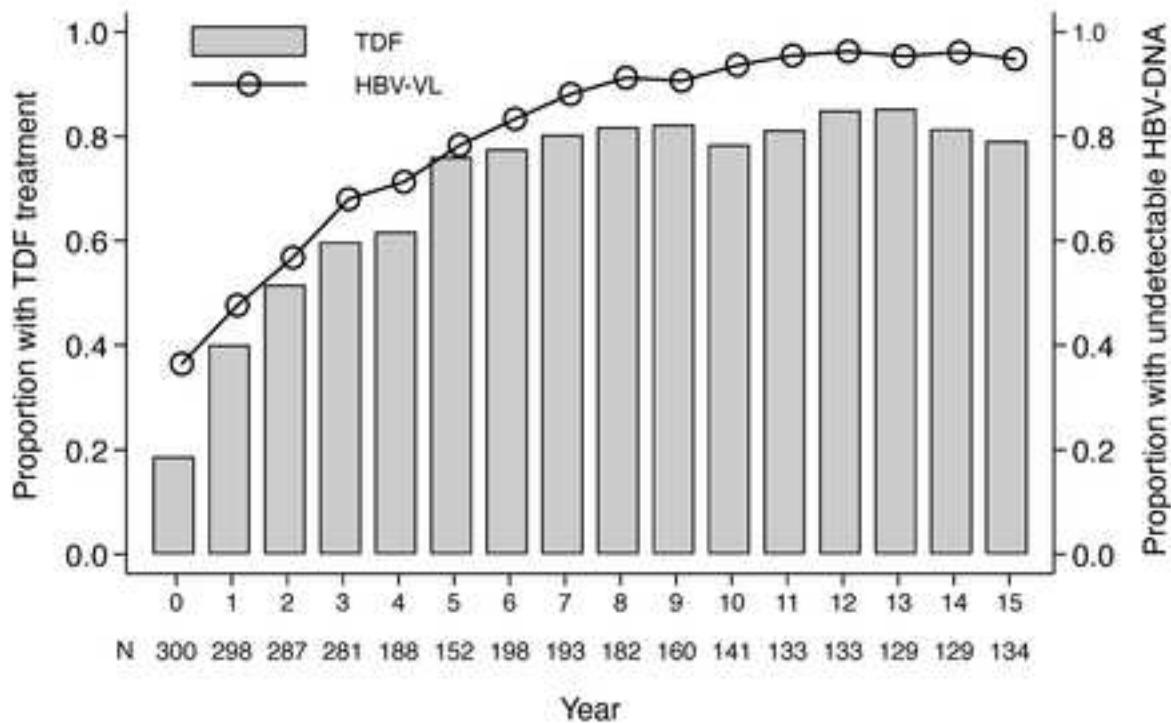
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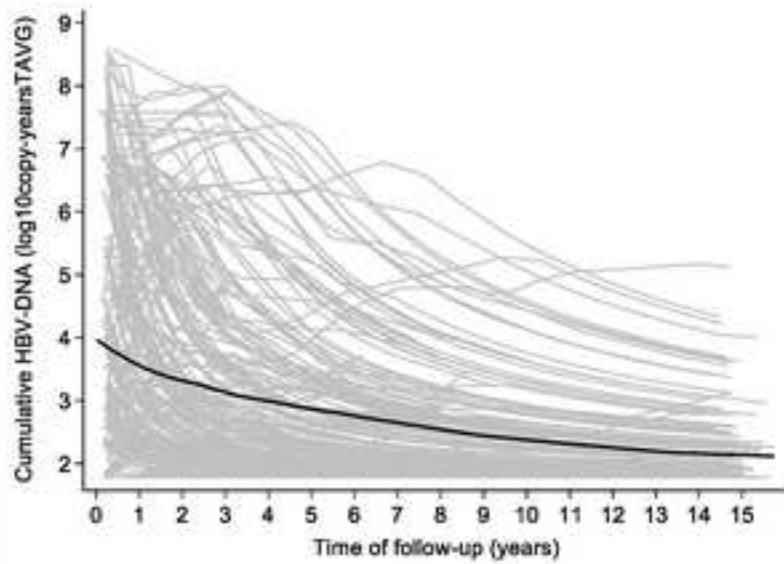
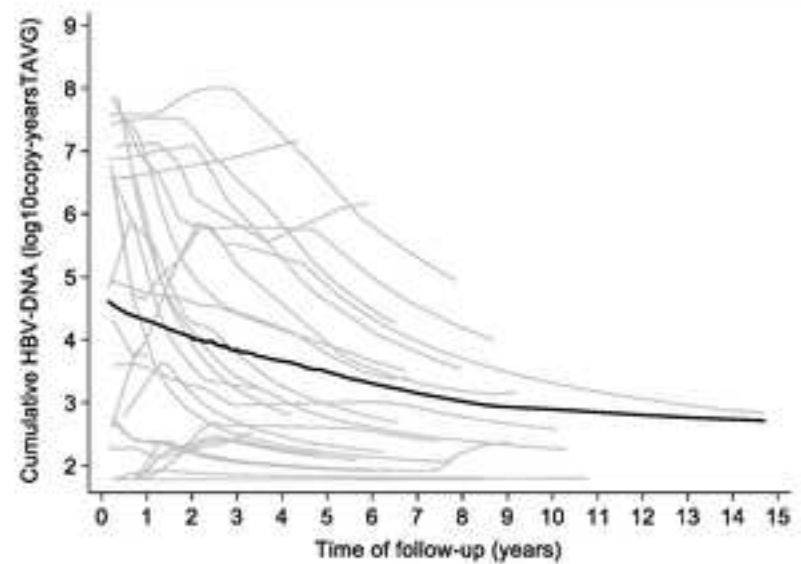
Figure 1. Evolution of HIV, hepatitis B virus (HBV), and antiviral treatment against hepatitis B virus over time.

The number of individuals continuing follow-up, as divided in yearly intervals, are provided at the bottom of each figure. In (A), undetectable HIV-RNA and median (IQR) CD4+ T-cell counts are displayed for each year. In (B), the proportion of individuals with undetectable HBV-DNA viral loads and the proportion undergoing antiviral therapy containing tenofovir are given for each year. Abbreviations: CI, confidence interval; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IQR, interquartile range; TDF, tenofovir.

Figure 2. Evolution of the cumulative extent of viral replication over time

Evolution of cumulative HBV-DNA (\log_{10} copy-years_{TAVG}) over time in (A) alive and (B) deceased individuals. Means are expressed as bold lines from a LOWESS curve and individual levels are expressed as gray lines. Abbreviation: TAVG, time-averaged.

A**B**

A**B**

Supplementary materials to: Lorenza N. C. Dezanet, Raisha Kassime, Patrick Mialhes, et al. Effect of viral replication and liver fibrosis on all-cause mortality in HIV/HBV coinfecting individuals: a retrospective analysis of a 15-year longitudinal cohort.

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Supplementary Table S1. Determinants for all-cause mortality.

Characteristics	Univariable		Multivariable ^a	
	HR (CI 95%)	<i>p</i>	HR (CI 95%)	<i>p</i>
Demographics				
Gender, male/female (% male)	2.62 (0.81-8.47)	0.11		
Age at baseline, years	1.07 (1.04-1.11)	<0.001	1.05 (1.01-1.09)	0.01
From zone of high HBV-prevalence	0.25 (0.09-0.71)	0.009		
Clinical characteristics at study entry				
BMI, Kg/m ²	0.92 (0.82-1.03)	0.15		
Alcohol consumption, glasses/day	1.03 (0.89-1.21)	0.7		
HIV infection variables at study entry				
Time since first HIV-positive test, years	1.08 (1.02-1.14)	0.01		
AIDS-defining illness	2.99 (1.63-5.47)	<0.001	2.35 (1.25-4.41)	0.008
CD4 ⁺ cell count, per 100 cells/μL	0.92 (0.81-1.05)	0.23		
Nadir CD4 ⁺ cell count, per 100 cells/μL	0.80 (0.64-1.00)	0.05		
Initiated ART	1.08 (0.99-1.17)	0.08		
Duration of ART, years	1.03 (0.98-1.09)	0.25		
Viral hepatitis				
Time since first HBsAg-positive test, years	1.03 (0.98-1.09)	0.22		
HBeAg-positive	2.07 (1.08-3.99)	0.03		
ALT level, IU/mL	1.00 (0.99-1.00)	0.8		
AST level, IU/mL	1.008 (1.002-1.01)	0.01		
Metavir F3-F4 fibrosis[†]				
Estimated using <i>Fibrotest</i> ®	3.48 (1.87-6.48)	<0.001	2.12 (1.06-4.21)	0.03
Determined by liver biopsy [#]	1.70 (1.18-2.45)	0.005		
Ever HCV coinfecting	0.28 (0.04-2.01)	0.20		
Ever HDV coinfecting	3.13 (1.39-7.04)	0.006		
Variables assessed during the follow-up study				
Cumulative tenofovir use, years	0.98 (0.91-1.06)	0.6		
Time-updated CD4 ⁺ cell count, cells/μL	0.997 (0.996-0.999)	0.003		
CD4 ⁺ cell count at last follow-up visit, cells/μL	0.996 (0.995-0.998)	<0.001		
Time-updated F3-F4 fibrosis level	4.48 (1.43-14.08)	0.01		

^a In multivariable modeling, BMI had too many missing data and was not considered further; AST levels, HDV coinfection, F3-F4 fibrosis (estimated using FibroTest) and time-updated F3-F4 fibrosis levels were collinear and we preferred F3-F4 fibrosis (estimated using FibroTest) at inclusion; CD4⁺ cell count at the last follow-up visit, initiation of ART and nadir CD4⁺ cell count were collinear and we preferred nadir CD4⁺ cell count; HBeAg status and time-updated CD4⁺ cell count were included in the submodels of the joint models analysis and were not considered further. The following variables were removed as their association was no longer significant in the multivariable model: male gender (*p*=0.63), from zone of high prevalence (*p*=0.17), and nadir CD4⁺ cell count (*p*=0.42). The final multivariable model was adjusted by all covariates listed in the column.

[#] In a subgroup of 138 patients, liver biopsies were performed within 12 months before or at study entry, based on concomitant guidelines from the European Association for the Study of the Liver. Histological fibrosis and activity were scored with the METAVIR classification.

Abbreviations: ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; HBeAg, hepatitis B “e” antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IDU, injection drug use; MSM, men who have sex with men; ULN, upper limit of normal.

Supplementary Table S2. Characteristics of the study population at cohort inclusion, stratified by lost to follow-up.

Characteristics	Patients		p^{\S}
	Completed follow-up (n=147)	Lost to follow-up (n=118) [¶]	
Demographics			
Gender, male/female (% male)	119/28 (81.0)	99/19 (84.7)	0.53
Age at baseline, years*	40.0 (34.6-44.1)	38.1 (34.1-42.0)	0.10
From zone of high HBV-prevalence [†]	47 (32.0)	35 (29.7)	0.69
Mode of HIV transmission [†]			0.42
Heterosexual	57 (38.8)	42 (35.6)	
MSM	80 (54.4)	64 (54.2)	
IDU	7 (4.8)	11 (9.3)	
Other/Unknown	3 (2.0)	1 (0.9)	
Clinical characteristics at study entry			
BMI, Kg/m ² [N = 251]*	22.4 (21.0-24.3)	22.7 (21.0-24.6)	0.58
Alcohol consumption, glasses/day [N = 254]*	1 (0-2)	1 (0-2.9)	0.67
Comorbidities[†]			
Cardiovascular disease	17 (11.6)	11 (9.3)	0.55
Diabetes	3 (3.0)	2 (1.7)	1.00
Renal disease	3 (2.0)	3 (2.5)	1.00
Other liver diseases [*]	4 (2.7)	2 (1.7)	0.58
HIV infection variables at study entry			
Time since first HIV-positive test, years [N = 264]*	9.4 (3.8-13.1)	9.5 (2.8-14.5)	0.93
AIDS-defining illness [†]	30 (20.4)	29 (24.6)	0.42
HIV-RNA > 50 copies/mL [†]	64 (43.5)	63 (53.4)	0.14
HIV-RNA, log ₁₀ copies/mL [N = 127]*	3.9 (4.5-4.4)	3.9 (2.8-4.5)	0.74
CD4 ⁺ cell count, cells/ μ L*	403 (283-557)	406 (249-586)	0.87
Nadir CD4 ⁺ cell count, cells/ μ L [N = 235]*	212 (107-309)	236 (110-372)	0.20
Initiated ART [†]	135 (91.8)	102 (86.4)	0.17
Duration of ART, years [N = 230]*	5.9 (2.4-7.4)	4.8 (2.7-6.8)	0.33
Viral hepatitis at study entry			
HBV-genotype [N = 142][†]			
A	51 (68.0)	36 (53.7)	0.09
D	7 (9.3)	5 (7.5)	0.77
E	8 (10.7)	11 (16.4)	0.32
G	9 (12.0)	13 (19.4)	0.22
HBeAg-positive [†]	73 (49.7)	60 (50.9)	0.85
HBV-DNA > 60 IU/mL [†]	95 (64.6)	74 (62.7)	0.75
HBV-DNA, log ₁₀ IU/mL [N = 169]*	4.3 (2.9-6.6)	5.3 (3.6-6.9)	0.04
ALT level, IU/mL [N = 260]*	38 (22-64)	43 (28-88)	0.02
AST level, IU/mL [N = 260]*	33 (25-52)	38 (29-59)	0.03
Metavir F3-F4 fibrosis[†]			
Estimated using <i>Fibrotest</i> [®] [N = 264]	38 (26.0)	27 (22.9)	0.56
Determined by liver biopsy [N = 119] [#]	22 (31.0)	13 (27.1)	0.65
Metavir F4 fibrosis[†]			
Estimated using <i>Fibrotest</i> [®] [N = 264]	21 (14.4)	13 (11.0)	0.42
Determined by liver biopsy [N = 119] [#]	6 (8.5)	5 (10.4)	0.72
Ever HCV coinfect [†] ϕ	13 (8.8)	8 (6.8)	0.54
Ever HDV coinfect [†] ϕ	7 (4.8)	9 (7.6)	0.33
Cumulative lamivudine use at study entry, years*	2.8 (0.9-5.5)	3.1 (0.4-5.3)	0.74

*Median (IQR).

[†] Number (%).

[¶] Does not include the 35 deceased individuals whose deaths were observed during follow-up.

[§] Significance determined using Kruskal-Wallis' test for continuous variables and Pearson's χ^2 test or Fisher's exact test for categorical variables.

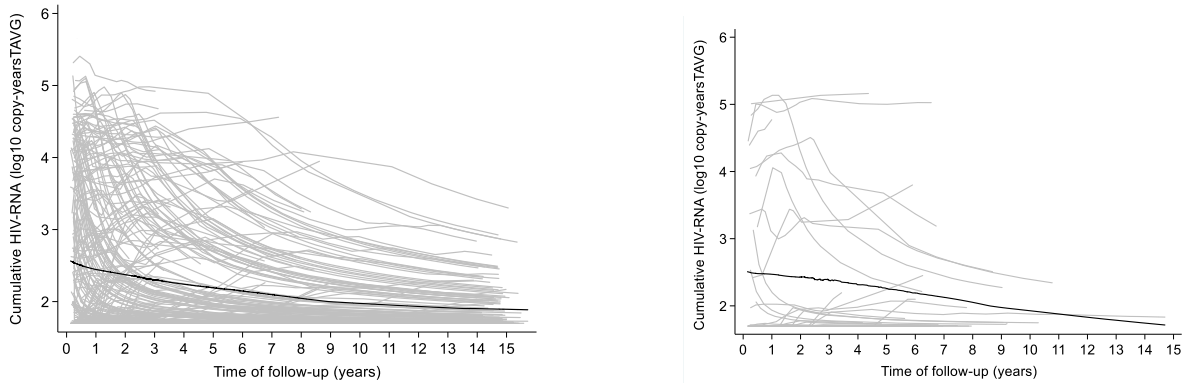
^{*}Other liver diseases or hepatic decompensation: acute, subacute or unspecified hepatic failure; haemorrhagic necrosis of liver; fatty liver disease; portal hypertension; and hepatocellular carcinoma.

In a subgroup of 138 patients, liver biopsies were performed within 12 months before or at study entry, based on concomitant guidelines from the European Association for the Study of the Liver. Histological fibrosis and activity were scored with the METAVIR classification.

‡ Established by a positive ELISA-based assay for HCV or HDV, and confirmed by a positive PCR-based assay for HCV-RNA or HDV-RNA, respectively.

Abbreviations: ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; HBeAg, hepatitis B “e” antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IDU, injection drug use; MSM, men who have sex with men; TAVG; time-averaged.

Supplementary Figure S1. Evolution of cumulative HIV-RNA (\log_{10} copy-years_{TAVG}) over time according to mortality outcome



Evolution of HIV-RNA \log_{10} copy-years_{TAVG} is given for alive patients in the left panel and for deceased patients in the right panel. Means are expressed as bold lines from a LOWESS curve and individual levels are expressed as gray lines. Abbreviation: TAVG, time-averaged.