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Profiles of liver fibrosis evolution during long-term tenofovir treatment in HIV-positive patients coinfecting with hepatitis B
Running title: **Liver fibrosis evolution in HIV/HBV coinfection**

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1 **Title Page**

2 **Profiles of liver fibrosis evolution during long-term tenofovir treatment in HIV-positive patients**
3 **coinfected with hepatitis B**

4

5 **Running title:** Liver fibrosis evolution in HIV/HBV coinfection

6

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30

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34

35 **List of abbreviations in order of appearance**

36 HIV, human immunodeficiency virus

37 HBV, hepatitis B virus

38 ESLD, end-stage liver disease

39 HCC, hepatocellular carcinoma

40 TDF, tenofovir

41 ART, antiretroviral therapy

42 HBsAg, hepatitis B surface antigen

43 IFN, interferon

44 Peg-IFN, pegylated interferon

45 HCV, hepatitis C virus

46 HDV, hepatitis D virus

47 ALT, alanine aminotransferase

48 AST, aspartate aminotransferase

- 49 HBeAg, hepatitis B “e” antigen
- 50 OR, odds ratio
- 51 CI, confidence interval
- 52 PWID, people who inject drugs
- 53 d4T, stavudine
- 54 ddl, didanosine
- 55 ddC, zalcitabine
- 56 PI, protease inhibitor
- 57 NRTI, nucleoside/nucleotide reverse-transcriptase inhibitor
- 58 NNRTI, nonnucleoside/nucleotide reverse-transcriptase inhibitor
- 59 LAM, lamivudine
- 60 TAF, tenofovir alafenamide
- 61 AIDS, acquired immunodeficiency syndrome
- 62 HSC, hepatic stellate cell

63

64 **Potential conflicts of interest**

65 None to declare.

66

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70 analysis and manuscript writing.

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77

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79 writing the original draft of the manuscript. S.M., A.G. and C.D. were responsible for interpretation of the
80 data and reviewing and editing the manuscript. H.R., P.M., C. L-C., and J.C. acquired data for the cohort,
81 assisted in interpreting data, and gave critical revisions of the manuscript. K.L. helped design,
82 conceptualize, and obtain funding for the French HIV-HBV cohort study, coordinated data collection, and
83 reviewed and edited the manuscript. A.B. coordinated data analysis, gave important comments on data
84 interpretation, wrote parts of the original draft of the manuscript, and provided critical revisions of the
85 manuscript. All authors approved the final version.

86

87 **Abstract**

88 **Background & Aims**

89 Data on liver fibrosis evolution and its involvement in liver-related morbidity are scarce in individuals
90 with human immunodeficiency virus (HIV) and hepatitis B virus (HBV) co-infection during treatment. We
91 identified profiles of liver fibrosis evolution in coinfecting patients undergoing tenofovir (TDF).

92 **Methods**

93 We included 169 HIV-HBV-coinfecting patients on TDF-based antiretroviral therapy. Virological and
94 clinical data were obtained at TDF-initiation and every 6-12 months. From data on non-invasive liver
95 fibrosis assessments collected yearly (FibroTest®), we established clusters of individuals with similar liver
96 fibrosis evolution using group-based trajectory models.

97 **Results**

98 Four profiles of liver fibrosis evolution were established from a median follow-up of 7.6 years (IQR=3.1-
99 13.1): low fibrosis with no progression (29.6%, Profile A), low fibrosis with progression (22.5%, Profile B),
100 moderate fibrosis with high fluctuation (39.6%, Profile C), and cirrhosis with no regression (8.3%, Profile
101 D). When compared to profile A, baseline HBeAg-positive status was associated with profiles B ($p=0.007$)
102 and C ($p=0.004$), older age with profiles C ($p<0.001$) and D ($p=0.001$), exposure to second-generation
103 protease inhibitors with profile C ($p=0.004$), and CD4+ $<500/\text{mm}^3$ at the last visit with profiles C ($p=0.02$)
104 and D ($p=0.002$). Incident liver-related events occurred in profiles other than A (B, $n=1/38$; C, $n=6/67$; D,
105 $n=3/14$) and all 5 cases of hepatocellular carcinoma occurred in profiles C ($n=2$) and D ($n=3$).

106 **Conclusions**

107 TDF-treated HIV-HBV coinfecting individuals do not seem to benefit from comparable levels of liver
108 fibrosis regression as in HBV mono-infection. Liver-related morbidity occurs mainly in those with
109 fluctuating or consistently high fibrosis levels.

110

111 **Key-words:** hepatitis B virus, human immunodeficiency virus, hepatic fibrosis, group-based trajectory
112 models

113

114 **Lay summary**

115 In individuals who have HBV infection, treatment with tenofovir can help decrease liver damage (i.e. liver
116 fibrosis). In this study of individuals living with human immunodeficiency infection and chronic hepatitis
117 B, few patients saw decreases in liver fibrosis after long-term tenofovir treatment, although almost all
118 patients were able to control their HBV infection. Severe liver-related problems were linked to
119 individuals whose fibrosis levels fluctuated or were high and never decreased, suggesting that regular
120 monitoring of liver fibrosis could provide useful information for tenofovir-treated HIV-HBV coinfecting
121 patients.

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134 **Introduction**

135

136 In human immunodeficiency virus (HIV)-positive individuals, roughly 5-12% also have chronic hepatitis B
137 virus (HBV) infection depending on the region (1). HIV-HBV coinfecting individuals have an increased risk
138 of liver fibrosis, end stage liver disease (ESLD), hepatocellular carcinoma (HCC) and liver-related death
139 compared to HIV mono-infected individuals (2). These results can be explained by liver damage from
140 continuous HBV-DNA replication in the absence of effective treatment against HBV (3–6). Tenofovir
141 (TDF) is a nucleotide analogue able to potently suppress HBV and HIV replication, with a virtually null risk
142 of developing TDF-resistant HBV variants (7).

143

144 In HBV mono-infected individuals, it has been clearly established that the vast majority of individuals
145 experience regression of liver fibrosis within 5 years of TDF therapy (8). In HIV-HBV coinfection, much of
146 the research on liver fibrosis evolution during TDF-based antiretroviral therapy (ART) is limited to 1-3
147 years of follow-up (9–12). For coinfecting individuals with advanced fibrosis/cirrhosis, significant
148 decreases in liver fibrosis were generally observed during the first year of TDF, after which fibrosis levels
149 became stabilized. For those with none to moderate fibrosis, levels of liver fibrosis remained consistently
150 low. When examining changes of liver fibrosis in coinfecting individuals with 5 years of TDF, our research
151 group observed that liver fibrosis was still advanced for the majority with baseline fibrosis and roughly
152 25% with no baseline fibrosis had increases in liver fibrosis despite extensive HBV-DNA suppression (13).
153 Similar results have been observed in other cohorts of extensively treated coinfecting individuals (14),
154 while others have found a predominance of stable liver fibrosis without any increase (15).

155

156 We recently conducted an extension of the French HIV-HBV cohort, in which HIV-HBV coinfecting
157 patients were followed for up to 15 years during TDF-based treatment (16). We therefore aimed to

158 describe the evolution of liver fibrosis, using a non-invasive biochemical score, during long-term TDF-
159 based ART in coinfecting individuals. Given the previously observed patterns of liver fibrosis and high
160 degree of variation in non-invasive scores (13), we took on a group-based trajectory approach to identify
161 groups of differing liver fibrosis evolution. We additionally examined characteristics at baseline and
162 during treatment, along with serological endpoints and liver-related morbidity and mortality, associated
163 with these groups.

164

165 **Patients and Methods**

166 ***Study population***

167 Patients were selected from the French HIV-HBV Cohort Study (16). Briefly, this is a prospective,
168 longitudinal cohort study including 308 HIV-positive patients with chronic HBV infection from four
169 centers located in Paris and Lyon, France. Patients were included if they had HIV-positive serological
170 results confirmed by western blot and hepatitis B surface antigen (HBsAg)-positive serological results for
171 >6 months. Participants were recruited in 2002-2003 and followed prospectively every 6-12 months until
172 2017-2018. The cohort design and procedures are described elsewhere (16,17). For this analysis, we
173 included patients undergoing TDF-based ART for at least 6 consecutive months. We did not include
174 patients who had concomitant interferon/pegylated interferon (Peg-IFN) or detectable hepatitis C virus
175 (HCV) or hepatitis D virus (HDV) RNA, less than two consecutive visits or no baseline liver fibrosis
176 assessment.

177

178 ***Ethics***

179 All patients provided written informed consent to participate in the study and the protocol was
180 approved by an appropriate Hospital Ethics Committee (Pitié-Salpêtrière, Saint-Antoine, Hôtel Dieu;
181 Paris, France) in accordance with the Helsinki Declaration.

182

183 Data collection

184 Demographic information was collected at study inclusion. Medical history on antiretroviral and anti-
185 HBV treatments, and the presence of comorbidities, including diabetes, cardiovascular, renal and other
186 liver diseases, were collected at study entry and at each follow-up visit. Alcohol consumption was
187 assessed by daily quantity of alcohol intake and was collected at baseline and at each follow-up visit until
188 2011. HIV-related variables included HIV-RNA VL and CD4+ cell count, and were collected before TDF-
189 initiation and at each follow-up visit. HBV-related variables included HBV-DNA viral load, alanine
190 aminotransferase (ALT) levels, aspartate aminotransferase (AST) levels, qualitative hepatitis B “e”
191 antigen (HBeAg), anti-HBe antibodies, HBsAg, and anti-HBs antibodies, and were collected before TDF-
192 initiation and at each follow-up visit. At TDF-initiation, L-nucleoside-associated HBV mutations at
193 positions rt173, rt180, and rt204 of the *pol* gene and at nucleotide 1896 of the *precore* gene were
194 determined using DNA chip technology (bioMérieux, Marcy l’Etoile, France).

195

196 Assessing liver fibrosis levels

197 Liver fibrosis was assessed at study entry and each yearly interval using the FibroTest® calculated from a
198 standard battery of biochemical markers (18). Scores ranged from 0 to 1. METAVIR equivalents of this
199 measure, as established in the HIV-HBV coinfecting population, were used to grade liver fibrosis (F2: 0.48-
200 0.58, F3: 0.59-0.73, F4 \geq 0.74) (19).

201

202 Statistical analysis

203 Baseline was defined as the study visit at or directly before TDF-initiation. Follow-up began at TDF-
204 initiation and continued until the last study visit, TDF-discontinuation, meeting any of the non-inclusion
205 criteria, or death, whichever occurred first.

206
207 On-treatment FibroTest® scores were used to further classify profiles of liver fibrosis evolution. We used
208 group-based trajectory models (20), allowing us to identify groups of individuals with distinctive
209 individual-level trajectories within a study population. Group-based trajectory models are a form of
210 finite-mixture models that use a multinomial modeling strategy to identify clusters of trajectories within
211 a population and have already been applied in the context of HBV infection (21,22). The goal of this
212 study was to distinguish groups of patients with similar FibroTest® trajectories during TDF treatment.
213 Each trajectory could be estimated with up to a fifth-order polynomial function of the dependent
214 variable, in this case FibroTest® scores, over time. The model was fit using the STATA “traj” plug-in with a
215 beta distribution for FibroTest® scores (23). For model selection, we followed a two-step procedure as
216 suggested by Nagin (20). In the first step, the optimal number of groups was determined. In the second
217 step, the appropriate degree of the polynomial equations giving rise to each trajectory was determined.
218 We then selected a final model with the best fit according to Bayesian Information Criteria. Based on the
219 final model, a total of four distinct profiles of liver fibrosis evolution during TDF-based treatment were
220 identified.

221
222 Patients were then assigned a profile group of liver fibrosis evolution to which they were more likely to
223 belong. Comparisons between profile groups were performed for all clinical parameters at baseline and
224 during follow-up using the Kruskal-Wallis test for continuous variables and Pearson χ^2 test or Fisher exact
225 test for categorical variables. Locally weighted scatterplot smoothing plots were used to illustrate the
226 evolution of FibroTest® levels according to liver fibrosis evolution profiles.

227
228 Considering that group membership is based on a finite-mixture distribution (i.e. group membership
229 contains some degree of misclassification), we modeled the probability of belonging to a group across

230 levels of determinants directly in the group-based trajectory model. Univariable odds ratios (OR) of time-
231 stable determinants associated with group membership and their 95% confidence intervals (CI) were
232 calculated from this model. A multivariable model was constructed by adding covariables with a p -value
233 <0.20 in univariable analysis and removing nonsignificant variables of all group profiles in backward-
234 stepwise fashion.

235
236 All statistical analyses were performed using STATA (v15.1; College Station, Texas, USA) and significance
237 was determined using a p value <0.05 .

238

239 **Results**

240 ***Description of the study population***

241 Of the 308 patients included in the cohort, 139 were not included in analysis for the following reasons:
242 did not initiate TDF ($n=51$), used TDF for less than 6 months ($n=12$), used concomitant PEG-IFN or IFN
243 ($n=20$), had baseline or incident HCV/HDV coinfection ($n=30$), had less than two consecutive visits ($n=1$),
244 had insufficient information at baseline ($n=7$), or did not have baseline FibroTest® levels ($n=18$). Thus,
245 169 individuals were included in the present analysis (Supplementary Figure S1).

246

247 Of these 169 patients, most were male (83.4%) with a median age of 41.5 years (interquartile range
248 [IQR]: 36.0-48.3) at TDF-initiation. The majority of patients included in our study were men who had sex
249 with men (59.2%), followed by heterosexual individuals (37.3%) and persons who inject drugs (PWID)
250 (2.9%). Almost all patients had initiated ART prior to TDF (99.8%), with a median duration of 6.9 years
251 (IQR: 4.1-9.1), and 73.4% and 63.3% of them had previously exposure to D-drugs (stavudine [d4T],
252 didanosine [ddI] or zalcitabine [ddC]) or any first-generation protease inhibitor (PI) at TDF initiation,
253 respectively. Accordingly, baseline median CD4+ count was 402/mm³ (IQR: 280-577) and 97 (57.4%) had

254 undetectable HIV-RNA. At TDF initiation, ART backbone regimens were nucleoside/nucleotide reverse-
255 transcriptase inhibitor (NRTI) only ($N=29$; 17.2%), NRTI + nonnucleoside/nucleotide reverse-transcriptase
256 inhibitor (NNRTI) ($N=50$; 29.6%), NRTI + protease inhibitor (PI) ($N=64$; 37.8%), NRTI + NNRTI + PI ($N=25$;
257 14.8%). At baseline, 99 (58.6%) patients were HBeAg-positive and 44 (26.0%) had advanced liver fibrosis
258 (F3-F4). Of the 149 patients (88.2%) with previous lamivudine (LAM) exposure, median LAM duration
259 was 4.7 years (IQR: 2.6-6.5) at TDF initiation and 20 (19.4%) had baseline LAM-resistant mutations.
260 Among patients with available data on HBV genotypes ($n=108$), most harbored genotype A (65.7%),
261 followed by G (14.8%), E (11.1%) and D (8.3%).

262
263 Patients were followed for a median 7.6 years (IQR: 3.1-13.1), with a maximum follow-up of 15.7 years.
264 Three patients switched from tenofovir disoproxil fumarate to tenofovir alafenamide (TAF)-based ART
265 during follow-up, while follow-up during TAF was still included in analysis (median TAF duration: 0.5
266 years, range: 0.15-5.56). HBV-DNA was detectable in 76.2% of participants at TDF initiation, and in 23.5%
267 (95% CI: 17.4%-31.0%), 16.5% (95% CI: 11.1%-23.9%) and 7.1% (95% CI: 4.1%-12.1%) after 2 and 3 years
268 of TDF and at the last follow-up visit, respectively. Of the 12 (7.1%) patients with detectable HBV-DNA at
269 their last study visit, median HBV-viral load (VL) was 6.75 \log_{10} IU/ml (range=4.32-8.47).

270
271 ***Profiles of liver fibrosis evolution during TDF treatment***
272 Four distinct profiles of liver fibrosis evolution were observed during TDF treatment and are graphically
273 summarized in Figure 1. Approximately 30% of patients had none to mild fibrosis (F0-F1) at TDF initiation
274 and remained low thereafter (henceforth “low fibrosis with no progression” profile) (Figure 2). A second
275 group of patients (22.5%) also presented with initially low level of fibrosis, but showed a slow and linear
276 increase towards advanced fibrosis during TDF treatment (henceforth “low fibrosis with progression”
277 profile). A third group of patients (39.6%) presented a more heterogenous distribution of fibrosis levels

278 at TDF initiation (ranging from F0-F1 to F4), with equally heterogenous fluctuations in FibroTest® scores
279 during treatment (henceforth “moderate fibrosis with high fluctuation” profile). Finally, 8.3% patients
280 with baseline levels almost exclusively greater than F4 maintained advanced liver fibrosis during TDF
281 treatment (henceforth “cirrhosis with no regression” profile). Two patients of this profile group had
282 outlying values at study visits after 15 years of follow-up (i.e. F0-F1 and F3). These values were excluded
283 to improve model fit. In the final model, individual trajectories fit the data rather well, as seen in the
284 individual fibrosis profiles for each trajectory (Supplementary Figure S2). Additionally, high probabilities
285 of membership were observed for each of the four classes (Supplementary Figure S3), with a high class
286 entropy of 0.773.

287

288 ***Determinants of liver fibrosis evolution during TDF treatment***

289 Baseline characteristics of each profile group are summarized in Table 1. Of note, patients of the
290 cirrhosis with no regression profile were older ($p<0.001$), were more commonly male ($p<0.001$), had a
291 longer estimated duration of both HIV ($p=0.002$) and HBV infection ($p=0.002$), higher proportion of an
292 AIDS-defining condition ($p=0.005$), and longer duration of ART at TDF initiation ($p=0.005$), when
293 compared to the other groups. Patients of the moderate fibrosis with high fluctuation profile and those
294 of the cirrhosis with no regression profile were more frequently exposed to D- ($p=0.02$) and first- or
295 second-generation PI-drugs ($p=0.02$ and $p=0.01$, respectively). Individuals from these groups also had
296 lower CD4+ cell counts at the last visit of follow-up, when compared to all other groups (Table 2). Finally,
297 patients of the low fibrosis with no progression profile ended follow-up with the lowest rates of HBeAg
298 and HBsAg seroclearance, whereas patients of the moderate fibrosis with high fluctuation and of the
299 cirrhosis with no regression profiles achieved the highest rates of HBeAg and HBsAg seroclearance (Table
300 2).

301

302 When modeling the finite-mixture distribution of profile membership from the multivariable group-
303 based trajectory model (Table 3), we observed that patients of the low fibrosis with progression profile
304 had a higher odds of having HBeAg-positive status ($p=0.007$) at TDF initiation, when compared to
305 patients of the low fibrosis with no progression profile (i.e. reference group). Patients of the moderate
306 fibrosis with high fluctuation profile had a higher odds of not only having HBeAg-positive status at
307 baseline, but also having a median age greater than the median (42 years-old; $p<0.001$), having more
308 frequent exposure to second generation PIs ($p=0.004$), and achieving a lower CD4+ cell count at the last
309 study visit ($p=0.02$), when compared to the reference group. Finally, patients of the cirrhosis with no
310 regression group had a higher odds of being older ($p=0.001$) and achieving a lower CD4+ cell count at the
311 last study visit ($p=0.002$), when compared to the reference group. Univariable associations of profile
312 determinants can be found in Supplementary Table S1 (determinants at baseline) and Supplementary
313 Table S2 (determinants during follow-up).

314

315 ***Severe liver-related morbidity and mortality and their relation to liver fibrosis evolution during TDF***
316 ***treatment***

317 At TDF initiation, 4 patients had already experienced a severe liver-related event [portal hypertension
318 $n=2$; hepatocellular carcinoma (HCC), $n=1$; haemorrhagic necrosis of liver, $n=1$]. Of them, 3 were
319 classified as having moderate fibrosis with high fluctuation and 1 cirrhosis with no regression. One
320 patient had fatty liver disease at TDF initiation (low fibrosis with progression). During TDF treatment, 1
321 patient developed portal hypertension (moderate fibrosis with high fluctuation), 1 patient developed
322 portal hypertension and thereafter HCC (cirrhosis with no regression), 4 others developed HCC (2
323 moderate fibrosis with high fluctuation and 2 cirrhosis with no regression), 3 developed fatty liver
324 disease (3 moderate fibrosis with high fluctuation), and 2 developed haemorrhagic necrosis of liver (1
325 low fibrosis with progression and 1 low fibrosis with no progression). One death was the result of HCC,

326 and one from decompensated liver disease with HCC and complications due to septic shock (both with
327 moderate fibrosis with high fluctuation).

328

329 **Discussion**

330 Studies in HBV mono-infected patients have demonstrated that during treatment with potent NA
331 therapy, the majority of patients are either able to exhibit liver fibrosis regression from high levels of
332 fibrosis or maintain low levels of liver fibrosis (8,24,25). In our present work, we observed that
333 approximately a quarter of HIV-HBV coinfecting patients treated with a TDF-containing ART regimen
334 maintained low levels of fibrosis, as measured by a noninvasive biochemical marker. Using group-based
335 trajectory models, we determined three other profiles of liver fibrosis evolution, which include low
336 baseline fibrosis with progression, moderate baseline fibrosis with high fluctuation, and baseline
337 cirrhosis with no regression. In stark contrast to studies of HBV mono-infected individuals, none of the
338 currently identified profiles was characterized by regression of liver fibrosis or cirrhosis.

339

340 To the best of our knowledge, this is likely the first study to identify population-level trajectories of liver
341 fibrosis levels during treated HBV infection. Such attempts to study the full spectrum of liver fibrosis
342 evolution have not been addressed partly because of the decreasing use of liver biopsies or liver fibrosis
343 assessment during treatment in clinical practice. In previous studies from our research group, liver
344 fibrosis levels did not appear to decrease for the majority of HIV-HBV co-infected patients undergoing
345 TDF-based ART when using the FibroTest® or appeared to stabilize at higher levels when using the
346 FibroScan® (11,13). Others have also found that a little over a quarter of co-infected patients undergoing
347 anti-HBV-based ART have liver fibrosis regression, as measured using the FibroScan® or even minimal
348 improvement in liver histology with paired liver biopsies (15,26). However, a substantial amount of
349 variation is observed with non-invasive markers, making it difficult to establish determinants of fibrosis

350 progression or regression. The profiles identified here, with an additional 7 years of maximum duration
351 on TDF from our previous research, provide a much clearer conceptualization of why certain TDF-treated
352 HIV-HBV co-infected patients might have liver fibrosis progression or excessive variation in liver fibrosis
353 measurements.

354
355 Indeed, individuals belonging to the moderate fibrosis with high fluctuation and cirrhosis with no
356 regression profiles were more commonly found to have more extensive immunosuppression at baseline,
357 but importantly a CD4+ cell count <500 cells/ μ L at the last study visit. HIV-induced immunosuppression is
358 associated with increased apoptosis of liver cells, including hepatocytes and hepatic stellate cells (HSC)
359 and consequently, could provoke increased HSC activation and fibrogenesis (27). In addition, depletion
360 of CD4+ T-lymphocytes could also lead to persistent expression of proinflammatory cytokines, such as
361 CXCL10, and thus could also accelerate the progression of chronic liver disease (28,29).

362
363 We also observed that the use of second-generation PIs was associated with the moderate fibrosis with
364 high fluctuation group and the previous use of D-drugs was associated with the moderate fibrosis with
365 high fluctuation and cirrhosis with no regression profiles. However, given that D-drug use was highly
366 collinear with age, it could not be included in the multivariable model. The effect of D-drugs on the liver
367 has been extensively described (30,31). Both D-drugs and several PI agents are also known to increase
368 the risk of lipodystrophy, steatosis, steatohepatitis, and insulin resistance, which could accelerate liver
369 fibrosis in patients with co-existing liver disease (32–34). The fact that duration of ART therapy was
370 associated with groups having moderate to high baseline liver fibrosis in univariable analysis was likely a
371 reflection of the previous exposure to a wide variety of hepatotoxic antiretroviral agents and that the
372 effects of these agents remain even after years from discontinuation (30,31,35). Assuming that the
373 antiretroviral agents currently recommended to treat HIV have minimal effects on liver fibrosis, the

374 contribution of ART on liver fibrosis is likely to bear less weight with newly diagnosed HIV-HBV co-
375 infected patients (36,37).

376
377 HBeAg-positive chronic hepatitis is a phase marked by increased HBV-DNA replication, unstable ALT
378 levels and high liver inflammation (38). Given that the FibroTest® is based mostly on markers of liver
379 inflammation, it was fairly expected that HBeAg-positivity was a determinant of the moderate fibrosis
380 with high fluctuation profile. Approximately half of HIV-HBV coinfecting patients are expected to achieve
381 HBeAg-seroclearance within 5 years of TDF treatment (21), thus it is surprising that fluctuations in
382 fibrosis levels remained even after this time point. In addition, HBeAg-positive individuals were more at
383 risk of belonging to the low fibrosis with progression profile; the reason for which is unclear.

384
385 One of the more important findings from our study was that almost all incident liver-related events
386 occurred in profiles other than the low fibrosis with no progression profile, and all incident cases of HCC
387 occurred in individuals with either moderate fibrosis with high fluctuations or cirrhosis with no
388 regression profiles. Baseline levels of the FibroTest® have shown some utility in predicting liver fibrosis
389 evolution and overall survival in individuals with chronic HBV mono-infection (39,40). Our findings
390 indicate that regular monitoring of liver fibrosis in TDF-treated HIV-HBV coinfecting patients could also
391 provide useful insight on their risk of liver-related morbidity, particularly if fibrosis levels fluctuate or
392 never regress in cirrhotics. Current management guidelines of chronic hepatitis B support the use of non-
393 invasive tests, including elastography, FIB-4, FibroTest® or APRI as alternative tests to stage liver disease
394 when biopsy is not a feasible option (41,42). In contrast, current guidelines for HIV-HBV coinfection
395 provide no clear recommendation on how to assess liver fibrosis, other than with liver biopsies (43,44).
396 Coupled with the data on the high performance of non-invasive markers in the co-infected population
397 (19,45,46), recommendations for non-invasive monitoring of liver fibrosis during treatment should be

398 made for HIV-HBV co-infection, while future studies should address the frequency needed in this
399 population.

400
401 Our study has the strengths of a prospective design with extensive follow-up and regularly collected non-
402 invasive measurements of liver fibrosis. Nonetheless, several limitations need to be discussed. First, non-
403 invasive methods of assessing liver fibrosis carry a certain degree of misclassification and could
404 overestimate fibrosis if high levels of necroinflammation, as reflected by elevated ALT, are present (41).

405 Second, we did not consistently measure alcohol consumption during follow-up and thus we cannot
406 determine if patients with more advanced fibrosis and cirrhosis over time excessively drank alcohol.

407 Third, inclusion for the cohort occurred between 2002 and 2003, and thus our data likely represent a
408 population with longer duration of HIV infection, more severe immunosuppression and previous
409 exposure to ART (including hepatotoxic agents) compared to contemporary patient populations.

410 Therefore, our results may not be completely generalizable to co-infected patients who start treatment
411 earlier with more easily tolerated drugs. Nevertheless, our study population does represent a
412 considerable proportion of patients actively seen in outpatient settings. There was also a considerably
413 lower proportion of PWID compared to other cohorts of HIV-HBV individuals (47), which must be
414 considered when generalizing our findings.

415 Finally, very few patients in the profiles with high levels of
416 liver fibrosis were followed for more than 9 years. It is unclear whether fibrosis would eventually regress
417 in these groups of patients (as demonstrated in the two patients belonging to the cirrhosis with no
418 regression profile who had lower fibrosis levels after 15 years of TDF), while the differential loss to
419 follow-up could have biased the analysis on determinants of profile membership.

420 In conclusion, TDF-treated HIV-HBV coinfecting patients do not seem to benefit from comparable levels
421 of liver fibrosis regression as in HBV mono-infection. Based on profiles of liver fibrosis, co-infected

422 individuals who have been exposed to hepatotoxic antiretroviral drugs, are more immunosuppressed,
423 HBeAg-positive or older are less likely to belong to the group with low fibrosis with no progression, that
424 is, the group with the lowest risk of liver-related morbidity. Monitoring liver fibrosis evolution and
425 identifying patients with higher non-invasive measures of fibrosis is of importance in estimating the risk
426 of liver-related morbidity in HIV-HBV coinfecting patients undergoing TDF-based ART.
427

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Table 1. Baseline characteristics according to liver fibrosis profile.

Characteristics	Profile				<i>p</i>
	Low fibrosis with	Low fibrosis with	Moderate fibrosis	Cirrhosis with no	
	no progression (<i>N</i> = 50)	progression (<i>N</i> = 38)	with high fluctuation (<i>N</i> = 67)	regression (<i>N</i> = 14)	
Gender, male/female (% male)	31/19 (62.0)	32/6 (84.2)	64/3 (95.5)	14/0 (100.0)	<0.001
Age, years [†]	37.9 (31.7-41.5)	40.0 (34.7-43.7)	44.6 (40.2-51.9)	48.6 (41.1-55.6)	<0.001
From zone of high HBV-prevalence [‡]	27 (54.0)	9 (23.7)	9 (13.4)	1 (7.1)	<0.001
BMI, Kg/m ² [<i>N</i> = 160] [†]	22.8 (20.7-25.8)	22.2 (21.0-23.7)	22.6 (21.1-24.2)	21.6 (21.0-23.1)	0.3
BMI, ≥25 Kg/m ² [<i>N</i> = 160] [‡]	15 (31.9)	4 (10.8)	10 (15.9)	1 (7.7)	0.04
Alcohol consumption, >50g/day [<i>N</i> = 150] [‡]	3 (6.7)	1 (3.1)	2 (3.3)	0 (0.0)	0.7
Cardiovascular disease [‡]	7 (14.0)	3 (7.9)	15 (22.4)	3 (21.4)	0.24
Diabetes [‡]	1 (2.0)	0 (0.0)	1 (1.5)	1 (7.1)	0.4
Estimated duration of HIV infection, years [<i>N</i> = 168] [†]	7.4 (4.1-11.6)	10.5 (5.5-13.5)	12.1 (7.9-15.7)	13.1 (9.9-16.3)	0.002
AIDS-defining illness [‡]	8 (16.0)	4 (10.5)	24 (35.8)	6 (42.9)	0.005
HIV transmission risk group [‡]					0.007

Heterosexual	29 (58.0)	13 (34.2)	18 (26.9)	3 (21.4)	
MSM	21 (42.0)	21 (55.3)	48 (71.6)	10 (71.4)	
PWID	0 (0.0)	3 (7.9)	1 (1.5)	1 (7.1)	
Unknown	0 (0.0)	1 (2.6)	0 (0.0)	0 (0.0)	
Previous antiretroviral exposure †					
Any D-drug (d4T, ddI or ddC)	30 (60.0)	26 (68.4)	55 (82.1)	13 (92.9)	0.02
Any first-generation PI-drug (IDV, NFV, SQV or APV)	30 (60.0)	18 (47.4)	46 (68.7)	13 (92.9)	0.02
Any second-generation PI-drug (LPV, ATV, TPV or DRV)	8 (16.0)	5 (13.2)	25 (37.3)	3 (21.4)	0.01
CD4+ cell count, cells/μL [N = 168] †	379 (283-531)	483 (343-689)	400 (249-552)	487 (332-665)	0.16
Nadir CD4+ cell count, cells/μL [N = 154] †	238 (121-321)	248 (134-383)	183 (35-307)	158 (90-321)	0.16
Duration of ART, years †	5.4 (3.2-7.9)	6.0 (3.9-8.9)	7.4 (5.5-9.2)	8.8 (7.5-10.6)	0.005
Estimated duration of HBV infection, years [N = 168] †	5.6 (2.6-8.5)	8.1 (4.4-11.8)	9.4 (4.2-13.3)	13.6 (5.2-19.1)	0.002
Previous use of lamivudine †	40 (80.0)	34 (89.5)	61 (91.0)	14 (100.0)	0.13
Cumulative lamivudine use, years †	3.5 (1.6-5.7)	3.8 (1.5-5.5)	4.7 (2.6-6.5)	6.0 (4.3-6.8)	0.06
Baseline lamivudine-resistant mutations [N = 103] †	2 (7.4)	6 (20.7)	12 (29.3)	0 (0.0)	0.09
Baseline <i>precore</i> mutations [N = 104] †	4 (15.4)	7 (23.3)	10 (23.8)	2 (33.3)	0.7

HBeAg-positive †	21 (42.0)	28 (73.7)	45 (67.2)	5 (35.7)	0.003
HBV-genotype [N = 108] ‡					0.4
A	18 (62.1)	20 (66.7)	27 (64.3)	6 (85.7)	
D	1 (3.5)	4 (13.3)	4 (9.5)	0 (0.0)	
E	5 (17.2)	4 (13.3)	2 (4.8)	1 (14.3)	
G	5 (17.2)	2 (6.7)	9 (21.4)	0 (0.0)	

† Median (IQR).

‡ Number (%).

Abbreviations: ART, antiretroviral therapy; APV, amprenavir; ATV, atazanavir; BMI, body mass index; ddC, zalcitabine; ddi, didanosine; d4T, stavudine; DRV, darunavir; HBeAg, hepatitis B 'e' antigen; HBV, hepatitis B virus; IDV, indinavir; LPV, lopinavir; MSM, men who have sex with men; NFV, nelfinavir; PWID, persons who inject drugs; SQV, saquinavir; TPV, tipranavir.

Table 2. Follow-up characteristics according to liver fibrosis profile.

Characteristics	Profile				<i>p</i>
	Low fibrosis with no progression (<i>N</i> = 50)	Low fibrosis with progression (<i>N</i> = 38)	Moderate fibrosis with high fluctuation (<i>N</i> = 67)	Cirrhosis with no regression (<i>N</i> = 14)	
	Total follow-up, years [†]	7.4 (3.1-12.9)	8.5 (3.1-13.0)	6.6 (3.2-13.2)	
HBV-DNA <60 IU/mL at last study visit [‡]	48 (96.0)	33 (86.8)	63 (94.0)	13 (92.9)	0.4
Proportion of visits with HBV-DNA <60 IU/mL [†]	83.3 (66.7-100.0)	78.9 (54.5-90.9)	75.0 (58.3-92.6)	83.7 (75.0-96.8)	0.22
HIV-RNA <50 copies/mL at last study visit [<i>N</i> = 163] [‡]	43 (91.5)	36 (94.7)	56 (86.2)	12 (92.3)	0.5
Proportion of visits with HIV-RNA ≤50 copies/mL [†]	86.2 (66.7-100.0)	89.6 (72.7-100.0)	85.7 (63.6-100.0)	90.9 (77.4-100.0)	0.7
CD4+ cell count at last study visit, cells/μL [<i>N</i> = 160] [†]	545 (457-708)	641 (494-816)	442 (342-606)	417 (240-481)	<0.001
Proportion of visits during HIV treatment era from 2002 to 2007 [†]	48.8 (25.0-100.0)	40.0 (27.3-100.0)	50.0 (36.4-88.9)	47.2 (33.3-75.0)	0.6
HBeAg seroclearance [‡]	5 (10.0)	11 (29.0)	23 (34.3)	3 (21.4)	0.02
HBsAg seroclearance [‡]	2 (4.0)	2 (5.3)	3 (4.5)	4 (28.6)	0.03
Any liver-related morbidity ^{‡§}	1 (2.0)	1 (2.6)	6 (9.0)	4 (28.6)	0.04
Hepatocellular carcinoma	0 (0.0)	0 (0.0)	2 (3.0)	3 (21.4)	<0.001

Portal hypertension	0 (0.0)	0 (0.0)	1 (1.5)	1 (7.1)	0.15
Liver-related death ‡	0 (0.0)	0 (0.0)	2 (3.0)	0 (0.0)	0.4

† Median (IQR).

‡ Number (%).

§ Any liver-related morbidity includes acute, subacute or non-specified hepatic insufficiency, fatty liver disease, haemorrhagic necrosis of liver, portal hypertension or hepatocellular carcinoma.

Abbreviations: HBeAg, hepatitis B 'e' antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus.

Table 3. Determinants of liver fibrosis profile.

Characteristics	Low fibrosis with progression (<i>N</i> = 38) versus low-fibrosis with no progression		Moderate fibrosis with high fluctuation (<i>N</i> = 67) versus low-fibrosis with no progression		Cirrhosis with no regression (<i>N</i> = 14) versus low-fibrosis with no progression	
	OR (95% CI)	<i>p</i> [†]	OR (95% CI)	<i>p</i> [†]	OR (95% CI)	<i>p</i> [†]
	Age at study entry, ≥42 years-old	2.32 (0.72-7.40)	0.16	9.49 (3.17-28.39)	<0.001	9.12 (2.42-34.31)
Previous exposure to 2 nd generation PI (LPV, ATV, TPV or DRV)	1.18 (0.27-5.13)	0.8	3.56 (1.04-12.17)	0.04	2.15 (0.49-9.42)	0.3
HBeAg-positive at study entry	4.83 (1.54-15.17)	0.007	4.75 (1.64-13.79)	0.004	1.24 (0.35-4.33)	0.7
CD4+ cell count at last study visit, <500 cells/μL	0.97 (0.30-3.11)	0.9	3.54 (1.22-10.24)	0.02	8.19 (2.16-30.97)	0.002

[†] Significance between each profile and the low-fibrosis with no progression (N=50) was determined using group-based trajectory modeling.

All odds ratios (OR) and 95% confidence intervals (95% CI) were adjusted for variables listed.

Abbreviations: ATV, atazanavir; DRV, darunavir; HBeAg, hepatitis B “e” antigen; LPV, lopinavir; TPV, tipranavir.

Figure legends

Figure 1. Profiles of liver fibrosis evolution during tenofovir (TDF)-treatment.

Figure 2. Fibrosis levels during follow-up according to profile of liver fibrosis evolution: a) low fibrosis with no progression; b) low fibrosis with progression; c) moderate fibrosis with high fluctuation; and, d) cirrhosis with no regression. Means are expressed as bold lines from a LOWESS curve and individual levels are expressed as gray lines.

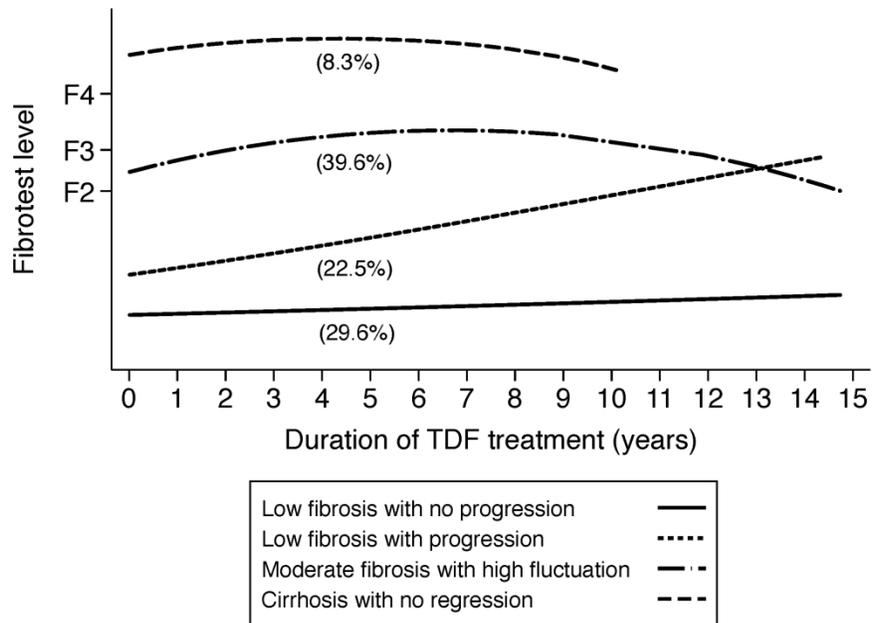


Figure 1. Profiles of liver fibrosis evolution during tenofovir (TDF)-treatment.

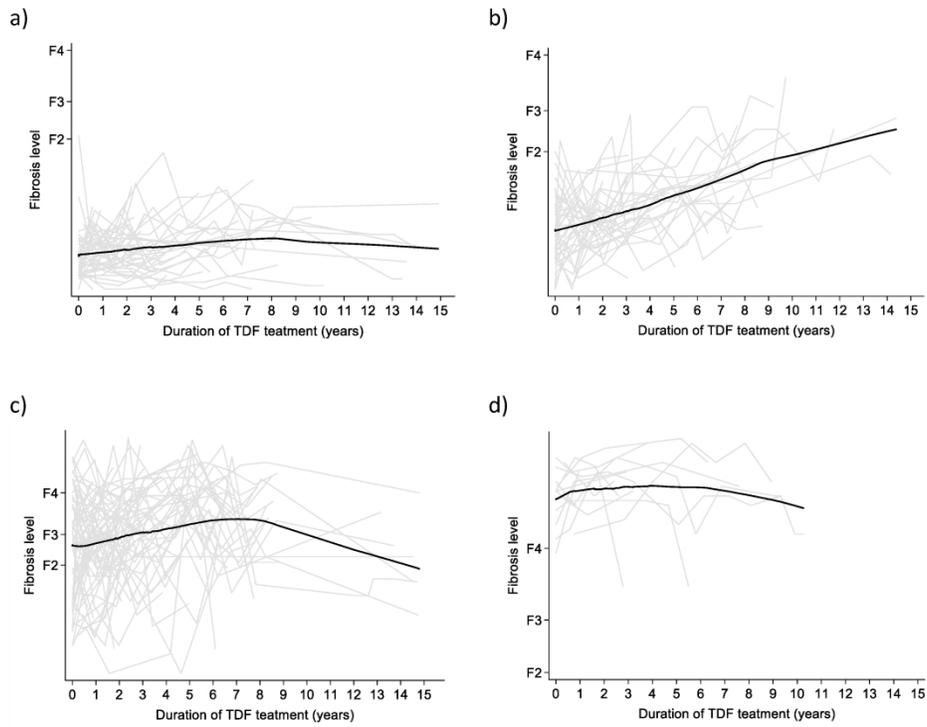


Figure 2. Fibrosis levels during follow-up according to profile of liver fibrosis evolution: a) low fibrosis with no progression; b) low fibrosis with progression; c) moderate fibrosis with high fluctuation; and, d) cirrhosis

SUPPLEMENTARY TABLES

Table S1. Determinants at baseline of liver fibrosis evolution per profile group (univariable analysis).

Characteristics	Low fibrosis with progression (<i>N</i> = 38)	Moderate fibrosis with high fluctuation (<i>N</i> = 67)	Cirrhosis with no regression (<i>N</i> = 14)
	OR (95% CI) [†]	OR (95% CI) [†]	OR (95% CI) [†]
Gender, male/female (% male)	3.08 (0.94-10.02) *	17.84 (3.92-81.15) **	--
Age, ≥ 42 years-old	1.98 (0.66-5.96)	8.03 (3.29-19.62) **	6.03 (1.50-24.29) **
From zone of high HBV-prevalence	0.26 (0.09-0.76) **	0.11 (0.04-0.29) **	0.06 (0.01-0.55) **
BMI, ≥ 25 Kg/m ² [<i>N</i> = 160]	0.28 (0.07-1.13) *	0.44 (0.16-1.19) *	0.15 (0.02-1.26) *
Alcohol consumption, >50g/day [<i>N</i> = 150]	0.44 (0.04-4,72)	--	1.36 (0.19-9.60)
Cardiovascular disease	0.52 (0.09-2.83)	1.63 (0.58-4.60)	1.70 (0.35-8.34)
Diabetes	--	0.71 (0.04-12.18)	3.74 (0.20-69.28)
Estimated duration of HIV infection, ≥ 15.5 years [<i>N</i> = 168]	0.48 (0.06-3.57)	3.90 (1.29-11.82) **	6.95 (1.56-30.97) **
AIDS-defining illness	0.31 (0.04-2.39)	2.73 (1.03-7.24) **	3.69 (1.03-13.24) **
Mode of HIV transmission			
MSM	1.50 (0.56-4.02)	3.71 (1.62-8.51)**	3.96 (0.92-17.13)*
IDU	--	--	--

Unknown	--	--	--
Previous antiretroviral exposure			
Any D-drug (d4T, ddi or ddC)	1.52 (0.54-4.29)	3.28 (1.31-8.22) **	8.18 (0.94-71.03) *
Any first-generation PI-drug (IDV, NFV, SQV or APV)	0.51 (0.19-1.36) *	1.39 (0.61-3.19)	(--)
Any second-generation PI-drug (LPV, ATV, TPV or DRV)	0.88 (0.22-3.50)	3.08 (1.16-8.16) **	1.65 (0.35-7.84)
CD4 ⁺ cell count, > 350 cells/μL [N = 168]	1.38 (0.48-4.01)	0.92 (0.39-2.13)	1.02 (0.31-3.41)
Nadir CD4 ⁺ cell count, > 350 cells/μL [N = 154]	1.52 (0.44-5.26)	0.78 (0.26-2.35)	0.44 (0.08-2.35)
Nadir CD4 ⁺ cell count, > 200 cells/μL [N = 154]	0.90 (0.30-2.68)	0.52 (0.22-1.25) *	0.56 (0.19-1.67)
Duration of ART, ≥ 7 years [N = 167]	1.76 (0.62-5.00)	3.47 (1.48-8.12) **	8.57 (1.93-37.97) **
Estimated duration of HBV infection, ≥ 8 years	3.27 (1.13-9.53) **	5.35 (2.18-13.11) **	6.21 (1.50-25.68) **
Previous use of lamivudine	1.92 (0.46-8.02)	3.30 (0.97-11.27) *	--
Cumulative lamivudine use, ≥ 4 years	1.26 (0.47-3.41)	1.72 (0.75-3.94)	6.79 (1.53-30.16) **
Baseline LAM-resistant mutations [N = 103]	2.10 (0.34-12.84)	6.67 (1.21-36.77) **	2.50 (0.28-22.71)
Baseline <i>precore</i> mutations [N = 104]	2.14 (0.40-11.45)	1.79 (0.37-8.67)	2.80 (0.46-17.21)
HBeAg-positive	4.23 (1.43-12.47) **	3.11 (1.39-6.92) **	0.54 (0.11-2.53)

[†] Significance between each profile and the low-fibrosis with no progression (N=50) was determined using group-based trajectory modeling.

* 0.05 < $p \leq 0.20$; ** $p \leq 0.05$

Abbreviations: APV, amprenavir; ATV, atazanavir; ART, antiretroviral therapy; BMI, body mass index; ddC, zalcitabine; ddi, didanosine; DRV, darunavir; d4T, stavudine; IDV, indinavir; LAM, lamivudine; LPV, lopinavir; NFV, nelfinavir; SQV, saquinavir; TPV, tipranavir.

Table S2. Determinants during follow-up of liver fibrosis evolution per profile group (univariable analysis).

Characteristics	Low fibrosis with progression (<i>N</i> = 38)	Moderate fibrosis with high fluctuation (<i>N</i> = 67)	Cirrhosis with no regression (<i>N</i> = 14)
	OR (95% CI) †	OR (95% CI) †	OR (95% CI) †
HBV-DNA <60 IU/mL at last study visit	0.21 (0.02-1.88)	--	0.15 (0.02-1.26) *
Proportion of visits with HBV-DNA <60 IU/mL, ≥80%	0.75 (0.28-2.03)	0.42 (0.19-0.92) **	1.63 (0.33-7.98)
HIV-RNA <50 copies/mL at last study visit [<i>N</i> = 164]	3.99 (0.04-422,79)	0.50 (0.13-1.85)	2.35 (0.23-24.00)
Proportion of visits with HIV-RNA <50 copies/mL, ≥80%	1.71 (0.57-5.07)	1.09 (0.48-2.45)	1.27 (0.33-4.99)
CD4 ⁺ cell count at last study visit, <500 cells/μL [<i>N</i> = 160]	0.87 (0.28-2.68)	3.46 (1.41-8.50) **	6.13 (1.81-20.68) **
CD4 ⁺ cell count at last study visit, <350 cells/μL [<i>N</i> = 160]	1.29 (0.30-5.53)	2.26 (0.69-7.41) *	2.81 (0.69-11.37) #
% visits during HIV treatment era from 2002 to 2007	0.60 (0.21-1.69)	0.86 (0.38-1.96)	1.05 (0.34-3.23)

† Significance between each profile and the low-fibrosis with no progression (*N*=50) was determined using group-based trajectory modeling.

* $0.05 < p \leq 0.20$; ** $p \leq 0.05$

Abbreviations: HBV, hepatitis B virus; HIV, human immunodeficiency virus.

Figure S1. Patient flow.

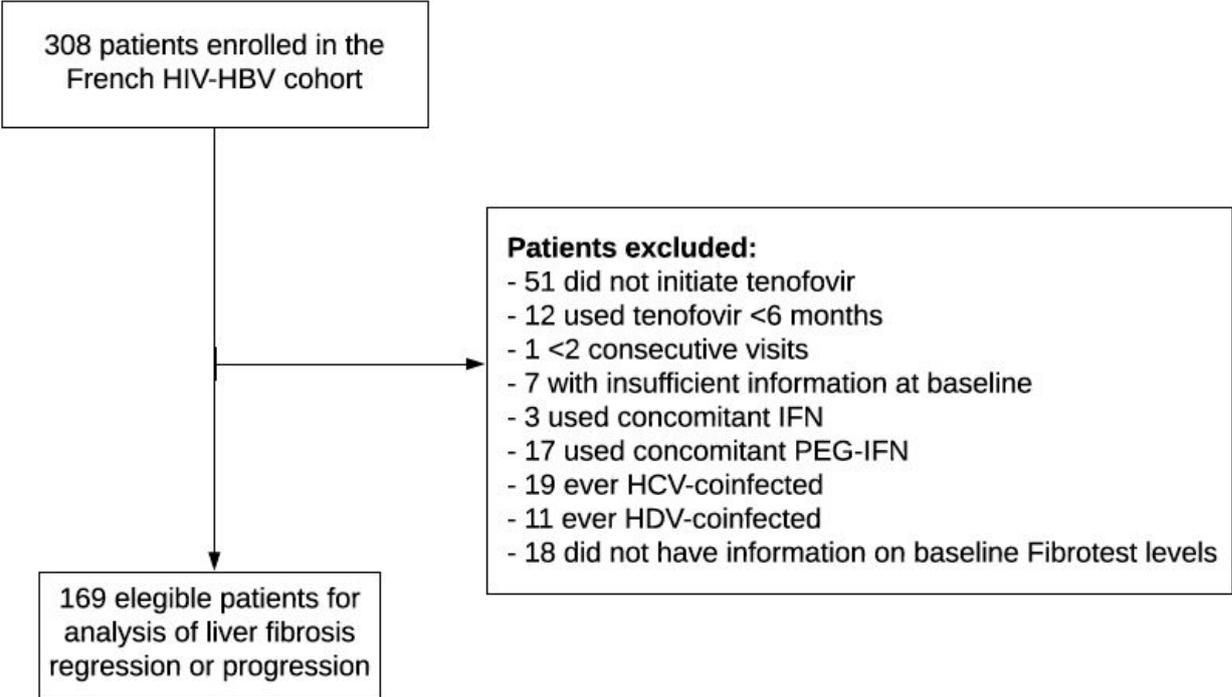
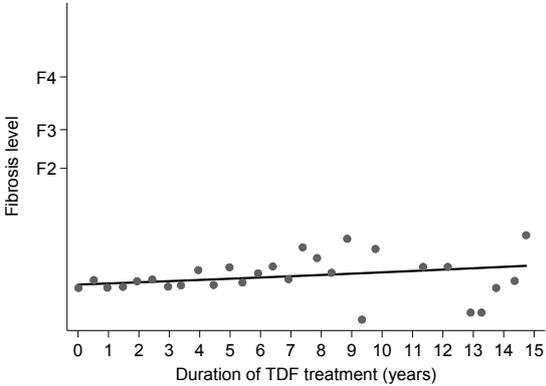
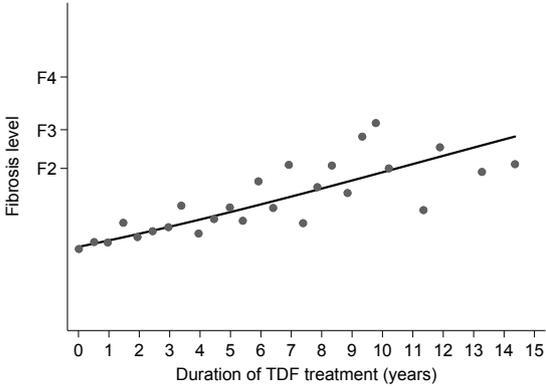


Figure S2. The fit of each profile of liver fibrosis evolution during treatment with tenofovir, as given by the group-based trajectory model: a) low fibrosis with no progression; b) low fibrosis with progression; c) moderate fibrosis with high fluctuation; d) cirrhosis with no regression. Dots represent mean levels of all available FibroTest® measurements at a given time point and lines represent the expected function of the FibroTest® according to the group-based trajectory model.

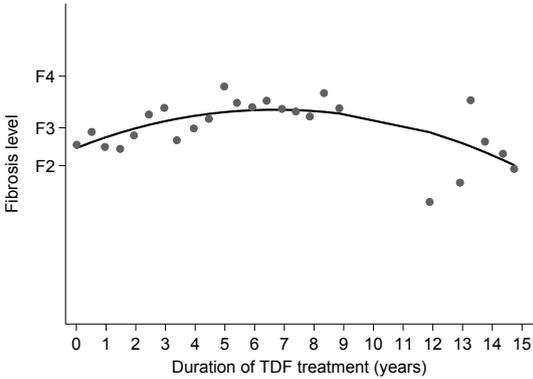
a)



b)



c)



d)

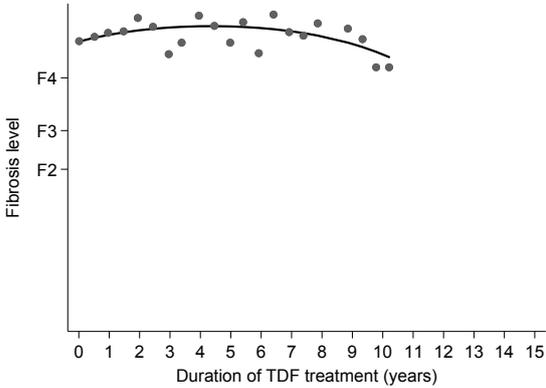


Figure S3. The distribution of posterior group-membership probabilities, as given by the group-based trajectory model: a) low fibrosis with no progression; b) low fibrosis with progression; c) moderate fibrosis with high fluctuation; d) cirrhosis with no regression.

