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Title Page

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List of abbreviations in order of appearance

HIV, human immunodeficiency virus

HBV, hepatitis B virus

ESLD, end-stage liver disease

HCC, hepatocellular carcinoma

TDF, tenofovir

ART, antiretroviral therapy

HBsAg, hepatitis B surface antigen

IFN, interferon

Peg-IFN, pegylated interferon

HCV, hepatitis C virus

HDV, hepatitis D virus

ALT, alanine aminotransferase

AST, aspartate aminotransferase

HBeAg, hepatitis B “e” antigen

OR, odds ratio

CI, confidence interval

PWID, people who inject drugs

d4T, stavudine

ddI, didanosine

ddC, zalcitabine

PI, protease inhibitor

NRTI, nucleoside/nucleotide reverse-transcriptase inhibitor

NNRTI, nonnucleoside/nucleotide reverse-transcriptase inhibitor

LAM, lamivudine

TAF, tenofovir alafenamide

AIDS, acquired immunodeficiency syndrome

HSC, hepatic stellate cell

Potential conflicts of interest

None to declare.

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

Abstract

Background & Aims

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

Methods

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

Results

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

Conclusions

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

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

Lay summary

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

Introduction

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

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

We recently conducted an extension of the French HIV-HBV cohort, in which HIV-HBV coinfected 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 coinfected 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 coinfected 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.

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

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

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

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

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

Results

Description of the study population

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

Of these 169 patients, most were male (83.4%) with a median age of 41.5 years (interquartile range [IQR]: 36.0-48.3) at TDF-initiation. The majority of patients included in our study were men who had sex with men (59.2%), followed by heterosexual individuals (37.3%) and persons who inject drugs (PWID) (2.9%). Almost all patients had initiated ART prior to TDF (99.8%), with a median duration of 6.9 years (IQR: 4.1-9.1), and 73.4% and 63.3% of them had previously exposure to D-drugs (staduvine [d4T], didanosine [ddI] or zalcitabine [ddC]) or any first-generation protease inhibitor (PI) at TDF initiation, respectively. Accordingly, baseline median CD4+ count was 402/mm^3 (IQR: 280-577) and 97 (57.4%) had

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

Patients were followed for a median 7.6 years (IQR: 3.1-13.1), with a maximum follow-up of 15.7 years. Three patients switched from tenofovir disoproxil fumarate to tenofovir alafenamide (TAF)-based ART during follow-up, while follow-up during TAF was still included in analysis (median TAF duration: 0.5 years, range: 0.15-5.56). HBV-DNA was detectable in 76.2% of participants at TDF initiation, and in 23.5% (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 of TDF and at the last follow-up visit, respectively. Of the 12 (7.1%) patients with detectable HBV-DNA at their last study visit, median HBV-viral load (VL) was 6.75 $\log_{10}$ IU/ml (range=4.32-8.47).

Profiles of liver fibrosis evolution during TDF treatment

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

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

Determinants of liver fibrosis evolution during TDF treatment

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

Our study has the strengths of a prospective design with extensive follow-up and regularly collected non-invasive measurements of liver fibrosis. Nonetheless, several limitations need to be discussed. First, non-invasive methods of assessing liver fibrosis carry a certain degree of misclassification and could overestimate fibrosis if high levels of necroinflammation, as reflected by elevated ALT, are present (41). Second, we did not consistently measure alcohol consumption during follow-up and thus we cannot determine if patients with more advanced fibrosis and cirrhosis over time excessively drank alcohol. Third, inclusion for the cohort occurred between 2002 and 2003, and thus our data likely represent a population with longer duration of HIV infection, more severe immunosuppression and previous exposure to ART (including hepatotoxic agents) compared to contemporary patient populations. Therefore, our results may not be completely generalizable to co-infected patients who start treatment earlier with more easily tolerated drugs. Nevertheless, our study population does represent a considerable proportion of patients actively seen in outpatient settings. There was also a considerably lower proportion of PWID compared to other cohorts of HIV-HBV individuals (47), which must be considered when generalizing our findings. Finally, very few patients in the profiles with high levels of liver fibrosis were followed for more than 9 years. It is unclear whether fibrosis would eventually regress in these groups of patients (as demonstrated in the two patients belonging to the cirrhosis with no regression profile who had lower fibrosis levels after 15 years of TDF), while the differential loss to follow-up could have biased the analysis on determinants of profile membership.

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

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

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Table 1. Baseline characteristics according to liver fibrosis profile.

Characteristics	Profile				*p*
	Low fibrosis with no progression (*N* = 50)	Low fibrosis with progression (*N* = 38)	Moderate fibrosis with high fluctuation (*N* = 67)	Cirrhosis with no regression (*N* = 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 [*N* = 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 [*N* = 160] ‡	15 (31.9)	4 (10.8)	10 (15.9)	1 (7.7)	0.04
Alcohol consumption, >50g/day [*N* = 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 [*N* = 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 *precore* 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, stvudine; 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				*p*
	Low fibrosis with no progression (*N* = 50)	Low fibrosis with progression (*N* = 38)	Moderate fibrosis with high fluctuation (*N* = 67)	Cirrhosis with no regression (*N* = 14)	
Total follow-up, years †	7.4 (3.1-12.9)	8.5 (3.1-13.0)	6.6 (3.2-13.2)	7.8 (2.8-14.4)	0.9
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 [*N* = 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 [*N* = 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 (*N* = 38) versus low-fibrosis with no progression		Moderate fibrosis with high fluctuation (*N* = 67) versus low-fibrosis with no progression		Cirrhosis with no regression (*N* = 14) versus low-fibrosis with no progression	
	OR (95% CI)	*p* †	OR (95% CI)	*p* †	OR (95% CI)	*p* †
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)	0.001
Previous exposure to 2nd 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.

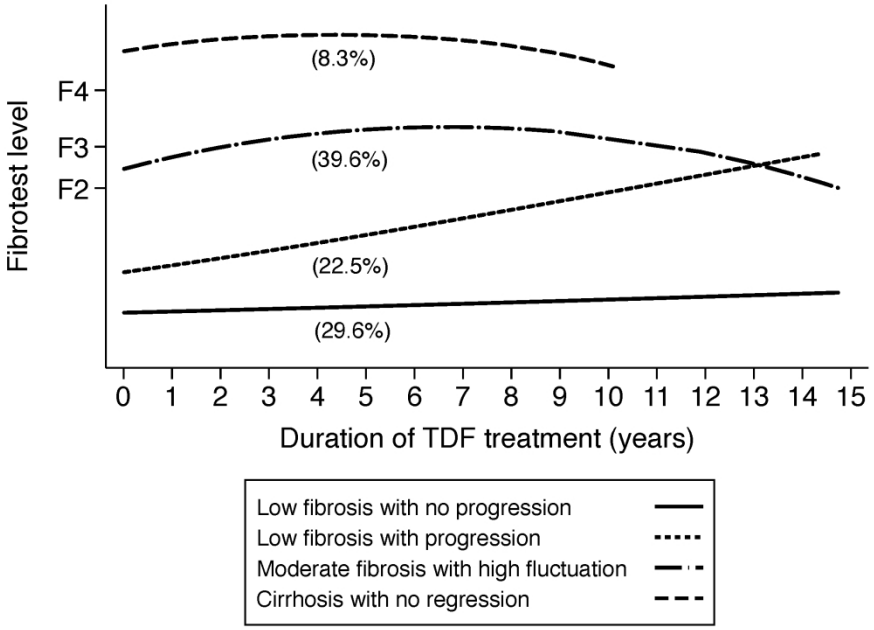


Figure 1. Profiles of liver fibrosis evolution during tenofovir (TDF)-treatment.

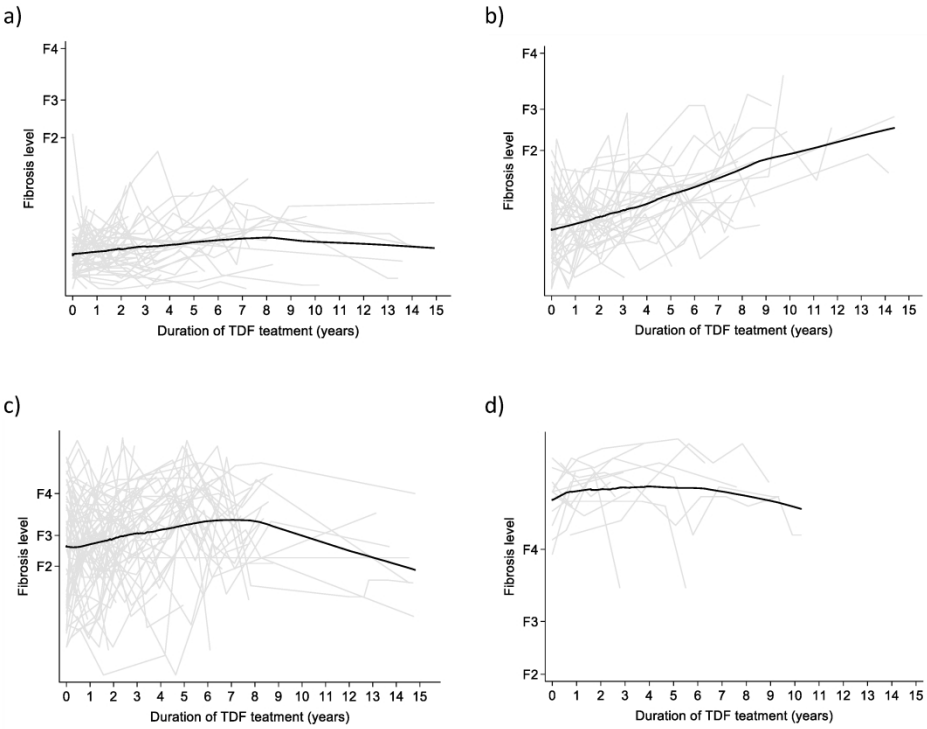


Figure 2. Fibrosis levels during follow-up according to profile of liver fibrosis evolution: a) low fibrosis with no progression; b) low fibrosis with progression; c) moderate fibrosis with high fluctuation; and, d) cirrhosis

SUPPLEMENTARY TABLES

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

Characteristics	Low fibrosis with progression (*N* = 38)	Moderate fibrosis with high fluctuation (*N* = 67)	Cirrhosis with no regression (*N* = 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 [*N* = 160]	0.28 (0.07-1.13) *	0.44 (0.16-1.19) *	0.15 (0.02-1.26) *
Alcohol consumption, >50g/day [*N* = 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 [*N* = 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, $\geq$ 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, $\geq$ 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, $\geq$ 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 *precore* 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 (*N* = 38)	Moderate fibrosis with high fluctuation (*N* = 67)	Cirrhosis with no regression (*N* = 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 [*N* = 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 [*N* = 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 [*N* = 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.

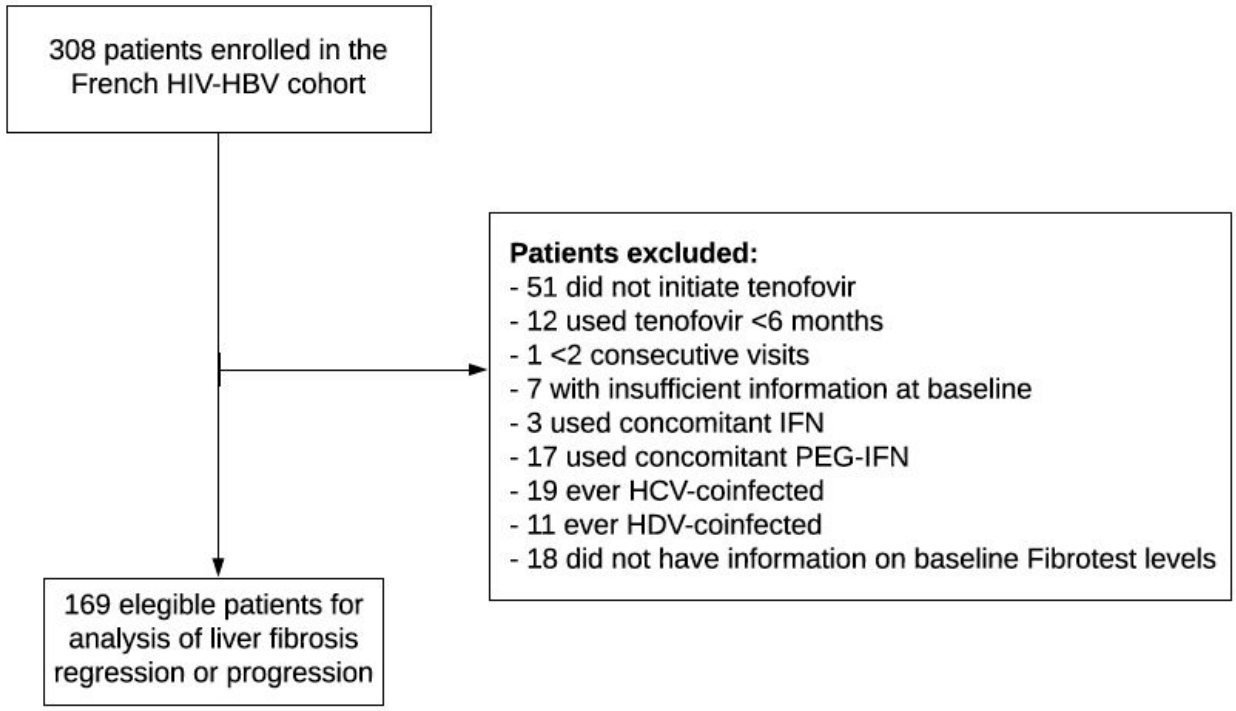


Figure S2. The fit of each profile of liver fibrosis evolution during treatment with tenofovir, as given by the group-based trajectory model: a) low fibrosis with no progression; b) low fibrosis with progression; c) moderate fibrosis with high fluctuation; d) cirrhosis with no regression. Dots represent 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.

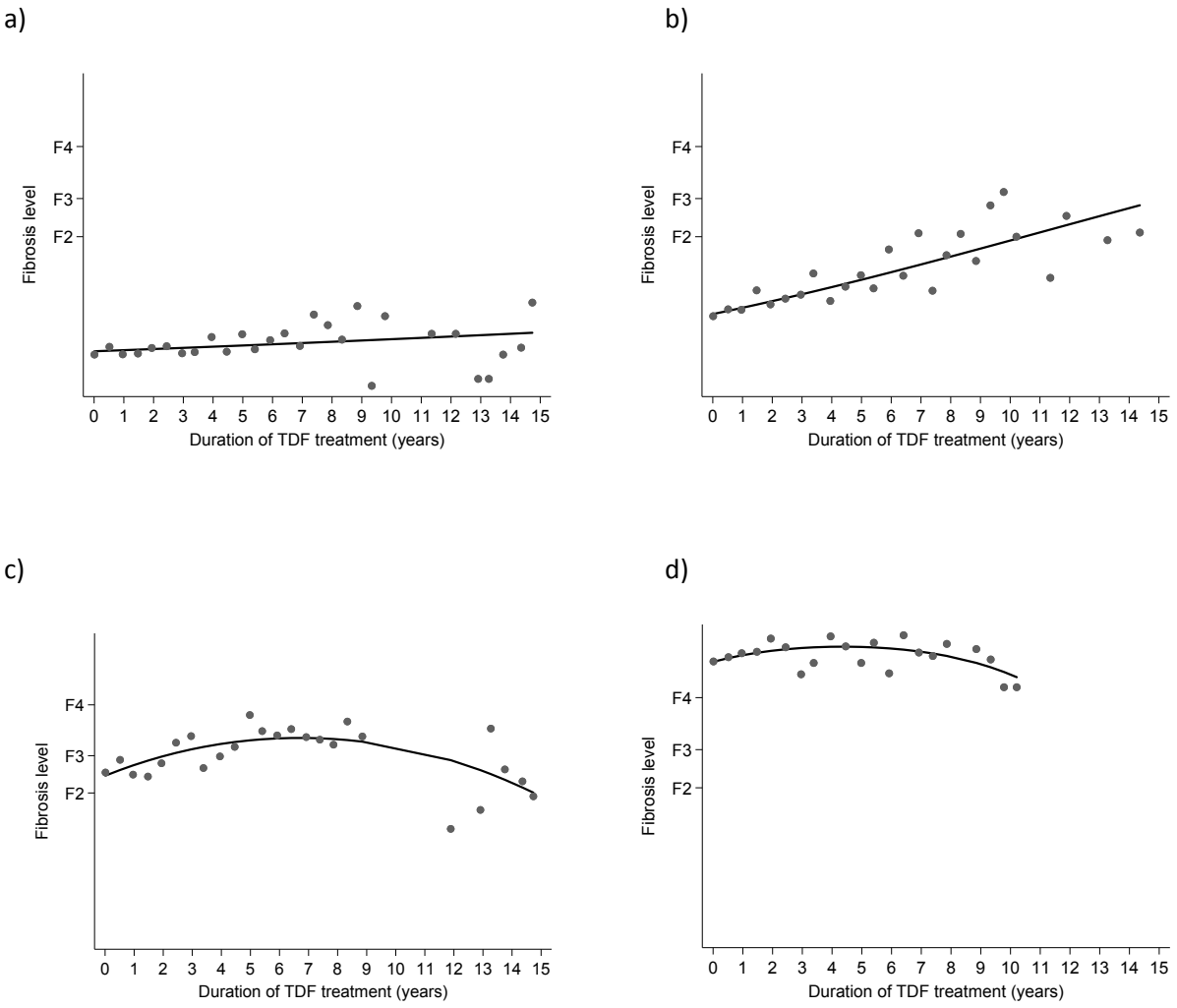
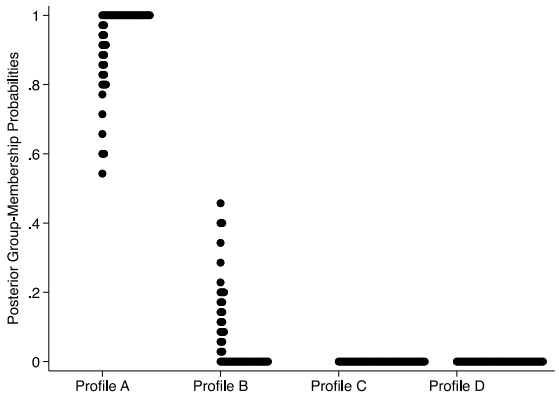
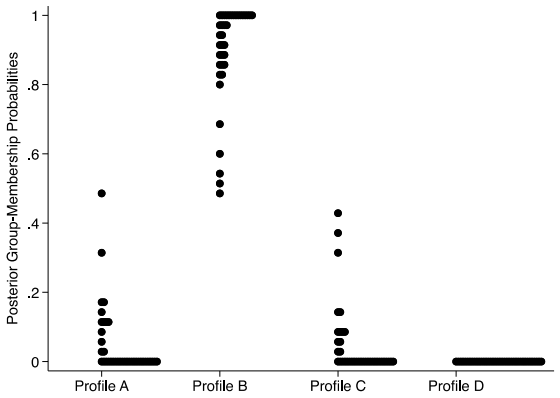


Figure S3. The distribution of posterior group-membership probabilities, as given by the group-based trajectory model: a) low fibrosis with no progression; b) low fibrosis with progression; c) moderate fibrosis with high fluctuation; d) cirrhosis with no regression.

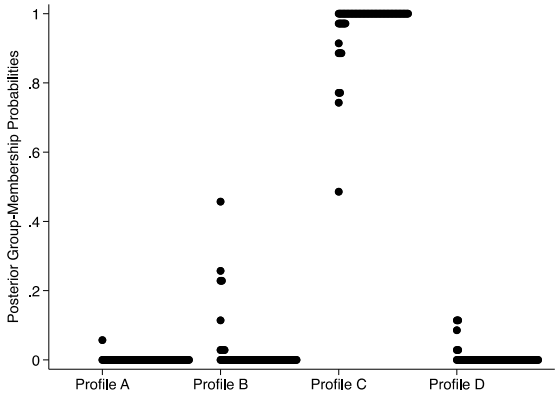
a)



b)



c)



d)