Original article

Changes in biomarkers of liver disease during successful combination antiretroviral therapy in HIV–HCV-coinfected individuals

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Background: We investigated changes in biomarkers of liver disease in HIV–HCV-coinfected individuals during successful combination antiretroviral therapy (cART) compared to changes in biomarker levels during untreated HIV infection and to HIV-monoinfected individuals.

Methods: Non-invasive biomarkers of liver disease (hyaluronic acid [HYA], aspartate aminotransferase-to-platelet ratio index [APRI], Fibrosis-4 [FIB-4] index and cytokeratin-18 [CK-18]) were correlated with liver histology in 49 HIV–HCV-coinfected patients. Changes in biomarkers over time were then assessed longitudinally in HIV–HCV-coinfected patients during successful cART (n=58), during untreated HIV-infection (n=59), and in HIV-monoinfected individuals (n=17). The median follow-up time was 3.4 years on cART. All analyses were conducted before starting HCV treatment.

Results: Non-invasive biomarkers of liver disease correlated significantly with the histological METAVIR stage (P<0.002 for all comparisons). The mean ±SD area under the receiver operating characteristic (AUROC) curve values for advanced fibrosis (≥F3 METAVIR) for HYA, APRI, FIB-4 and CK-18 were 0.86 ±0.05, 0.84 ±0.08, 0.80 ±0.09 and 0.81 ±0.07, respectively. HYA, APRI and CK-18 levels were higher in HIV–HCV-coinfected compared to HIV-monoinfected patients (P<0.01). In the first year on cART, APRI and FIB-4 scores decreased (-35% and -33%, respectively; P=0.1), mainly due to the reversion of HIV-induced thrombocytopaenia, whereas HYA and CK-18 levels remained unchanged. During long-term cART, there were only small changes (<5%) in median biomarker levels. Median biomarker levels changed <3% during untreated HIV-infection. Overall, 3 patients died from end-stage liver disease, and 10 from other causes.

Conclusions: Biomarkers of liver disease highly correlated with fibrosis in HIV–HCV-coinfected individuals and did not change significantly during successful cART. These findings suggest a slower than expected liver disease progression in many HIV–HCV-coinfected individuals, at least during successful cART.

Introduction

HCV infection is a major cause of morbidity and mortality in HIV-infected individuals [1]. One-third of HIV-infected patients in the Swiss HIV Cohort Study (SHCS) are coinfected with HCV [2]. It is well established that coinfection with HIV is associated with higher HCV viraemia [3], a faster progression of liver fibrosis, and higher incidences of end-stage liver disease and hepatocellular carcinoma [4,5]. Previous reports suggest that combination antiretroviral therapy (cART) reduces liver-related mortality [6] and,

accordingly, current guidelines recommend earlier cART initiation in HIV–HCV-coinfected patients [7,8].

We previously demonstrated that successful cART partially restores HCV-specific CD4+ T-cell responses and slightly decreases HCV RNA levels [9]. However, it is unclear whether the improved immunological control of HCV infection during cART is associated with slower progression of liver disease, as most previous studies were either cross-sectional comparisons of HCV-monoinfected with HIV–HCV-coinfected individuals or did not assess liver fibrosis progression during long-term cART [6,10].

Most experts consider liver biopsy as the gold standard for fibrosis staging in HCV-infected individuals. However, considering the expense and limitations of liver histology, and the invasive nature of the procedure, previous studies proposed several non-invasive biomarkers of liver fibrosis for follow-up. Some non-invasive markers correlate well with the histological fibrosis stages in both HCV-monoinfected and HIV–HCV-coinfected patients [11,12]. These non-invasive biomarkers can be measured in stored plasma samples and are the only possibility to retrospectively estimate the longitudinal evolution of liver disease. The biomarkers assessed in this study were hyaluronic acid (HYA), the aspartate aminotransferase (AST)-to-platelet ratio index (APRI), the Fibrosis-4 (FIB-4) index and cytokeratin-18 (CK-18) fragment levels. Several studies demonstrated that these biomarkers accurately predict liver fibrosis (area under the receiver operatic characteristic [AUROC] curve 0.69 to 0.88) and cirrhosis (AUROC 0.80 to 0.98) [13]. The biomarkers correlate with liver histology and, importantly, also predict liver-related mortality in HIV–HCV-coinfected patients [14]. CK-18 fragments are intermediate filament proteins in the liver activated by cysteine caspases during hepatocyte apoptosis and are sensitive markers of liver injury and inflammation [15–17].

The aims of this study were first to evaluate the correlation between non-invasive biomarkers of liver disease with liver histology, and then to assess longitudinally the evolution of liver disease during successful cART, during untreated HIV infection and in HIV-monoinfected individuals.

Methods

Study population and follow-up

We included participants of the SHCS, a prospective multicentre study that is carried out at seven major Swiss hospitals and their local affiliated centres [18,19]. Written informed consent was mandatory for inclusion, and all local ethical committees approved the study. We included HIV–HCV-coinfected patients fulfilling the following criteria, as described before [9]: detectable HCV RNA≥12 months after the first positive HCV serology, assessed by quantitative or qualitative assays; availability of plasma samples after >2 years of successful uninterrupted cART; and infection with HCV genotypes 1 or 3. We restricted our analysis to the most common two HCV genotypes in order to be able to assess virological and immunological covariates as described before [9]. Patients with HCV therapy before or during follow-up were excluded. After the last follow-up for this study until closure of the study database in December 2010, 26/120 (22%) patients started HCV therapy.

Changes in biomarker levels within individuals were assessed longitudinally in 58 HIV-HCV-coinfected patients during successful cART and compared to the changes in 59 HIV-HCV-coinfected patients before commencement of cART. Patient characteristics are outlined in Table 1. After commencing cART, biomarkers were assessed at three time-points: during the first year of cART, and after a median of 3 and 6 years on successful cART (Table 2). As a further comparison group, we included 17 HIV-monoinfected individuals matched for sex, age, median CD4+ T-cell counts at baseline, and with available longitudinal plasma samples during successfully treated HIV-infection (median follow-up time 2.4 years). The median follow-up time during untreated HIV-infection was 2.3 years. Successful cART was defined as HIV RNA levels <400 copies/ml after 6 months from cART initiation and thereafter, as described before [9].

From the HIV–HCV-coinfected study participants, 13 patients died until closure of the study database. Three patients died from end-stage liver disease caused by chronic HCV infection, two from bronchial neoplasm, two from an unknown cause, and one each from rectal neoplasm, cocaine overdose, breast cancer, renal insufficiency, intra-cerebral haemorrhage, and from aspiration pneumonia. Liver-related events have been recorded systematically in the SHCS since November 2005. From 92 study participants with a follow-up time of ≥1 year after this date, 3 (3%) experienced a major liver-related event (esophageal bleeding, hepatorenal syndrome or hepatic encephalopathy).

Liver histology

Liver biopsy results were available in 49 HIV–HCV-coinfected individuals. The correlation between non-invasive biomarkers and liver histology was assessed from a frozen plasma sample that was taken within ±3 months from the liver biopsy date. Liver histology results were classified according to the METAVIR score (fibrosis: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis; activity: A0, no activity; A1, mild activity; A2, moderate activity; and A3, severe activity) [20]. All liver histologies obtained from different centres were reviewed by an independent pathologist.

Table 1. Patient characteristics

Characteristic	Follow-up during cART[a]	Comparison groups	
		Follow-up pre-cART[a]	HIV-monoinfected
HIV and HCV status	HIV-positive and HCV-positive	HIV-positive and HCV-positive	HIV-positive and HCV-negative
Participants, *n*	58	59	17
Sex			
Male, *n* (%)	36 (62)	44 (75)	10 (59)
Female, *n* (%)	22 (38)	15 (25)	7 (41)
HIV transmission			
Intravenous drugs, *n* (%)	41 (71)	42 (71)	0 (0)
Heterosexual, *n* (%)	16 (27)	12 (20)	17 (100)
MSM, *n* (%)	0 (0)	4 (7)	0 (0)
Haemophilia, *n* (%)	1 (2)	1 (2)	0 (0)
Median age, years (IQR)	37 (33–40)	38 (33–42)	36 (30–46)
HCV genotype			
1a/1b, *n* (%)	36 (62)	31 (53)	NA
3a, *n* (%)	22 (38)	28 (47)	NA

[a]A total of 14 patients were included in both pre- and post-combination antiretroviral therapy (cART) analyses. MSM, men who have sex with men; NA, not applicable.

Table 2. Laboratory characteristics and biomarker levels at baseline and during follow-up

Variable	Follow-up during cART[a]				Comparison groups			
					Follow-up pre-cART[a]		HIV-monoinfected	
	Pre-cART	First year on cART[b]	After 3 years on cART	Long-term cART[c]	First presentation	Last presentation before cART	Pre-cART	After 3 years on cART
Duration from baseline, months	0	8 (6–11)	35 (30–38)	75 (56–103)	0	27 (25–33)	0	29 (29–31)
HCV RNA levels, $\log_{10}$ IU/ml	6.5 (5.9–7.0)	6.5 (6.0–6.9)	6.3 (5.7–6.8)	6.1 (5.6–6.4)	6.2 (5.4–6.5)	6.1 (5.6–6.9)	NA	NA
HIV RNA levels, $\log_{10}$ copies/ml	4.7 (4.3–5.1)	1.0 (0–2.5)	1.6 (1.4–2.1)	1.8 (1.4–2.6)	3.9 (3.3–4.5)	4.4 (3.7–4.9)	4.9 (4.2–5.4)	2.2 (1.4–3.9)
CD4$^+$ T-cell count, cells/mm^3	217 (138–355)	360 (171–512)	395 (285–601)	467 (373–609)	473 (359–631)	332 (178–463)	198 (173–346)	435 (397–635)
Aminotransferase levels								
ALT, U/l	52 (34–86)	50 (34–79)	51 (37–97)	61 (42–91)	73 (35–126)	59 (35–107)	24 (12–43)	17 (11–28)
AST, U/l	43 (33–66)	39 (29–51)	46 (32–67)	47 (33–74)	51 (29–77)	41 (30–79)	25 (23–31)	22 (20–24)
Biomarkers of liver disease								
HYA, ng/ml	39 (27–54)	31 (22–48)	32 (25–61)	43 (26–68)	31 (24–44)	32 (26–47)	20 (4–32)	20 (17–26)
CK-18, U/l	83 (72–106)	89 (82–105)	98 (83–131)	108 (86–257)	95 (83–123)	92 (77–121)	76 (64–86)	77 (68–83)
APRI	0.6 (0.4–1.4)	0.4 (0.3–0.8)	0.5 (0.3–0.9)	0.6 (0.3–1.2)	0.7 (0.4–1.2)	0.5 (0.3–1.4)	0.3 (0.2–0.4)	0.2 (0.1–0.3)
FIB-4	1.5 (1.0–3.6)	1.0 (0.6–1.8)	1.0 (0.7–2.0)	1.1 (0.7–2.3)	1.1 (0.8 – 2.2)	1.1 (0.8–3.0)	1.4 (0.8–1.7)	0.9 (0.5–1.3)

Data are median (IQR). [a]A total of 14 patients were included in both pre- and post-combination antiretroviral therapy (cART) analyses. [b]Plasma sample available in 43 patients. [c]Plasma sample available in 28 patients. ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; CK-18, cytokeratin-18; FIB-4, Fibrosis-4; HYA, hyaluronic acid; NA, not applicable.

Quantification of hyaluronic acid

HYA in plasma was measured using a commercially available enzyme-linked binding protein assay (HA Test Kit, Corgenix, Bloomfield, CO, USA) according to the manufacturer's protocol. The colour intensity was detected at 450 nm using a Sunrise microplate absorbance reader (Tekan, Männedorf, Switzerland). HYA levels in patients and control samples were determined against a calibration curve prepared from the reagent blank (0 ng/ml) and calibrator solutions provided with the kit (50, 100, 200, 500 and 800 ng/ml HYA).

Measurement of cytokeratin-18 fragments

Hepatic apoptosis expressed as caspase activity was determined with an ELISA, which monitors the caspase-cleaved CK-18 fragments in plasma (M30-Apoptosense® ELISA, Peviva, Bromma, Sweden). The test was used according to the instructions given in the manual. Absorbance was measured at 450 nm and samples were quantitated with a calibration graph prepared with seven standards (0 to 1,000 U/l).

APRI score and FIB-4 index

APRI scores were calculated based on the proposed formula by Wai *et al*. [21]: ([AST of the sample/upper limit of normal of AST]×100)/platelets (10^9/l). FIB-4 was determined using the formula proposed by Vallet-Pichard *et al*. [22]: (age [years]×AST [U/l]/[platelets (10^9/l)×alanine aminotransferase (ALT [U/l])]$^{1/2}$).

Data analyses

The correlation between non-invasive biomarkers and liver fibrosis was assessed using non-parametric tests for trend across ordered groups [23]. AUROC curve analyses were performed to calculate the optimal cut-off values, the sensitivity and specificity of non-invasive biomarkers for predicting advanced fibrosis (≥F3 METAVIR stage). Comparisons of non-invasive biomarkers at the different time points were performed using a robust variance estimation linear regression model for cluster-correlated data to consider data points representing repeated measurements within individuals [24]. Statistical analyses were performed using STATA/SE 11.1 software (StataCorp LP, College Station, TX USA) and the figures were drawn using GraphPad Prism 5.01 software (La Jolla, CA, USA).

Results

Non-invasive biomarkers correlate with liver fibrosis

All non-invasive biomarkers of liver fibrosis (HYA, APRI score and FIB-4 index) correlated significantly with the METAVIR stage (Figure 1A–1C). HYA levels increased with the METAVIR stages (P<0.001). Similarly, APRI and FIB-4 scores positively correlated with the fibrosis stage (P<0.0001 and P<0.002, respectively). CK-18 fragment level as a marker of hepatic apoptosis also significantly correlated with the hepatic fibrosis stage (Figure 1D). The AUROC curve of HYA for advanced fibrosis (≥F3 METAVIR stage) was 0.86 (SD 0.05) with an optimal cutoff value of 59.1 ng/ml (sensitivity 69%, specificity 89%; Additional file 2). The AUROC values of APRI score, FIB-4 index and CK-18 levels were 0.84 (SD 0.08), 0.80 (SD 0.09) and 0.81 (SD 0.07), respectively. The respective sensitivities were 84%, 83% and 77%, and the specificities were 80%, 74% and 75% for the optimal cutoff values (0.7 U/l, 1.3 U/l and 122 U/l, respectively). The correlation of non-invasive biomarkers with the grade of liver inflammation could not be assessed, because only one patient had a METAVIR inflammatory activation score ≥A2. AST levels positively correlated (P<0.001) with the fibrosis stage.

CK-18 fragment levels positively correlated with AST levels (R^2=0.35, P<0.0001; Additional file 2), whereas there was no significant correlation of CK-18 levels with ALT levels (R^2=0.08, P=0.09).

HYA, APRI score and CK-18 levels were higher in HIV–HCV-coinfected compared to HIV-monoinfected patients (P<0.01), whereas FIB-4 scores did not differ substantially between HIV–HCV-coinfected and HIV-monoinfected individuals (Table 1).

Median baseline values of non-invasive biomarkers of liver disease were significantly higher in the three patients who died of end-stage liver disease compared to individuals with a non-liver-related cause of death, or to those still alive at the end of follow-up (Additional file 3).

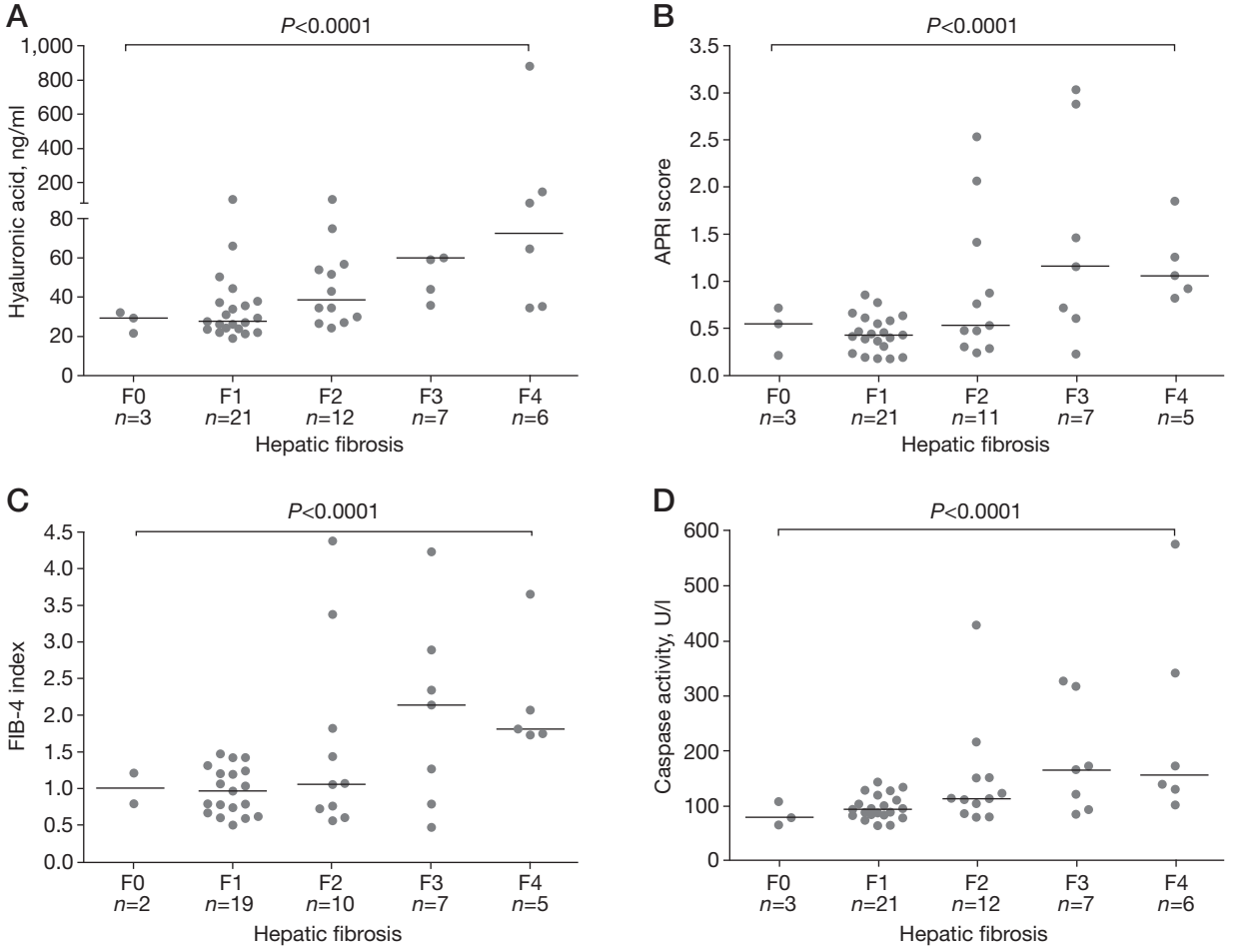
Minimal changes in non-invasive biomarkers of liver disease during successful cART within individuals

During the first year on successful cART, there was a decrease in APRI scores and FIB-4 scores (Figure 2). HYA and CK-18 levels did not change significantly during the first year on successful HIV treatment (3% and -2%, P>0.1 for both comparisons). After the first year of cART, there were no significant changes in biomarkers of liver fibrosis.

In the first year on cART, the change for APRI and FIB-4 scores were -35% and -33% (P=0.1), respectively, whereas there was only a slight and non-significant change per year during long-term cART (<3%). Median changes in HYA and CK-18 levels per year were <5% in the first year on cART and thereafter. Sex, HIV transmission mode, HCV genotype, HCV RNA levels, ethnicity and *IL28B* genotypes did not correlate with the change in biomarkers of liver fibrosis (Table 3).

The individuals in the quartile with the strongest increases in HYA, APRI, FIB-4 and CK-18 did not differ

Figure 1. Non-invasive biomarkers of liver disease significantly correlate with liver fibrosis (METAVIR score)



Correlations between hepatic fibrosis and **(A)** hyaluronic acid, **(B)** aspartate aminotransferase-to-platelet ratio index (APRI), **(C)** Fibrosis 4 (FIB-4) index and **(D)** caspase activity. Horizontal lines represent medians. *P*-values are for trends.

significantly from the remaining patients with regard to sex, baseline and nadir CD4+ T-cell counts, HCV RNA and HCV genotypes, and PI- versus NNRTI-based cART ($P>0.1$ for all comparisons).

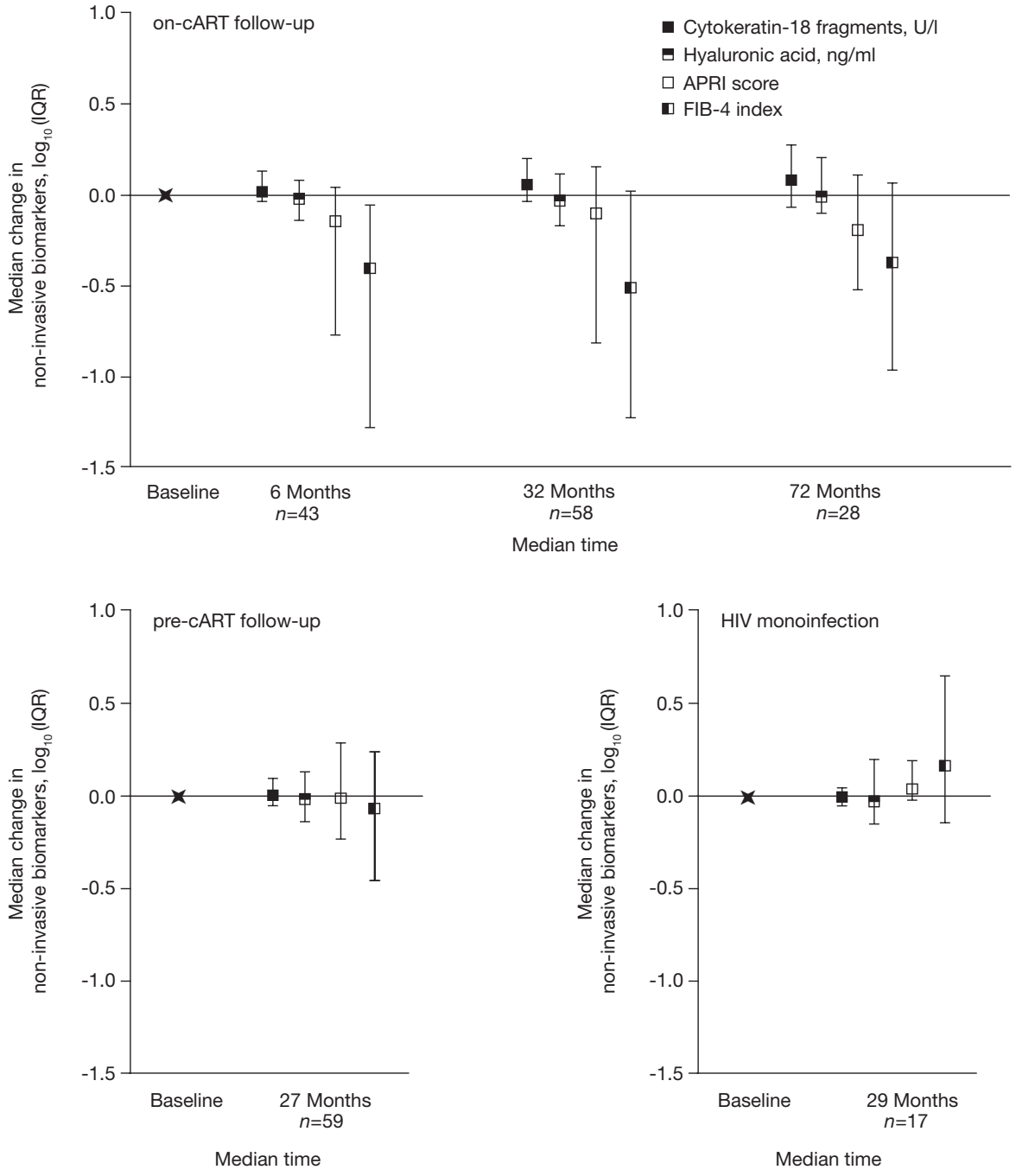
Liver fibrosis progression during untreated HIV infection

During untreated HIV infection, there was no significant change in biomarkers of liver disease ($P>0.3$ for all comparisons; Figure 2). The median change per year in biomarkers was <3% (change/year [IQR] for HYA 0.6 [-4.6–4.0], CK-18 0.2 [-9.2–5.1], FIB-4 0.02 [-0.1–0.1] and for APRI 0.01 [-0.1–0.1]). In 14 individuals, changes of biomarkers could be assessed both before and during cART. Changes were very similar before and during cART, with a median change of <1% per year for all biomarkers ($P>0.2$ for all comparisons; Additional file 2).

Discussion

We investigated the correlation of non-invasive biomarkers with liver histology in HIV–HCV-coinfected individuals, and assessed longitudinally the progression of liver disease during treated HIV infection compared with untreated HIV infection and to HIV-monoinfected patients. First, we confirmed that biomarkers of liver disease correlate with liver histology in HIV–HCV-coinfected individuals. HYA levels significantly correlated with the stage of fibrosis. Median HYA levels were similar to the values observed in another cohort of HIV–HCV-coinfected individuals (33.6 ng/ml versus 30.2 ng/ml) with comparable proportions of patients with levels above the cutoff for advanced fibrosis (20.1% versus 18.2%) [25]. HYA levels were imperfect predictors of liver fibrosis at the individual level,

Figure 2. Change in non-invasive biomarkers of liver disease within individuals during successful cART, during untreated HIV infection and in HIV-monoinfected individuals



APRI, aspartate aminotransferase-to-platelet ratio index; cART, combination antiretroviral therapy; FIB-4, Fibrosis-4.

in accordance with previous reports [26,27]. However, the clear correlation between HYA levels and METAVIR stage allowed us to estimate the average changes of liver fibrosis in an HIV–HCV-coinfected population. Median APRI-scores at baseline were comparable to previous observations in HIV–HCV-coinfected individuals (0.56 versus 0.59) [28]. Furthermore, median FIB-4 scores were similar in this study compared to a recent report (1.3 versus 1.14) [29]. Similar to HYA levels, APRI and FIB-4 scores were insufficient predictors

Table 3. Change per year in non-invasive biomarkers of liver disease during on-cART follow-up by time-period and clinical characteristics

	HYA		CK-18		FIB-4		APRI	
Variable	Median change/year (IQR)	*P*-value[a]	Median change/year (IQR)	*P*-value[a]	Median change/year (IQR)	*P*-value[a]	Median change/year (IQR)	*P*-value[a]
Time period								
Year 1 on cART	-5.4 (-17.2–6.0)	Ref	5.3 (-9.9–26.6)	Ref	-0.5 (-2.2–-0.1)	Ref	-0.20 (-1.2–0.01)	Ref
Years 1 to 3 on cART	0.1 (-3.9–6.9)	0.98	0.6 (-9.6–18.7)	0.59	0.03 (-0.2–0.2)	0.12	0.01 (-0.1–0.1)	0.13
Years 3 to 6 on cART	1.6 (-0.2–12.0)	0.15	3.8 (-3.7–12.5)	0.91	0.03 (-0.01–0.1)	0.12	0.01 (-0.1–0.1)	0.12
Gender								
Male	0.3 (-5.1–13.7)	Ref	2.4 (-10.7–22.5)	Ref	-0.1 (-1.0–0.1)	Ref	-0.05 (-0.3–0.1)	Ref
Female	-0.2 (-5.0–3.2)	0.4	-1.0 (-8.7–18.1)	0.4	-0.05 (-0.4–0.2)	0.17	-0.03 (-0.2–0.1)	0.21
Mode of HIV infection								
IDU	0.4 (-3.9–12.4)	Ref	1.5 (-8.4–15.8)	Ref	-0.1 (-0.7–0.15)	Ref	-0.01 (-0.2–0.2)	Ref
Non-IDU	-0.9 (-6.4–3.2)	0.06	5.1 (-11.6–27.7)	0.1	0.01 (-0.3–0.2)	0.17	-0.04 (-0.3–0.1)	0.18
HCV genotype								
1	-0.2 (-6.3–4.0)	Ref	0.5 (-10.8–17.6)	Ref	-0.1 (-0.4–0.1)	Ref	-0.04 (-0.2–0.1)	Ref
3	2.7 (-3.3–13.7)	0.42	2.9 (-4.2–26.4)	0.5	-0.1 (-1.0–0.2)	0.75	0.03 (-0.4–0.2)	0.71
HCV RNA levels								
≤6.3 $\log_{10}$ IU/ml[b]	0.1 (-10.5–8.9)	Ref	3.5 (-7.5–18.1)	Ref	-0.1 (-0.7–0.2)	Ref	-0.01 (-0.3–0.1)	Ref
>6.3 $\log_{10}$ IU/ml[b]	-0.01 (-3.9–6.9)	0.42	1.7 (-11.1–22.7)	0.22	-0.1 (-0.4–0.1)	0.17	-0.04 (-0.2–0.1)	0.19
Ethnicity								
Caucasian	0.2 (-5.4–7.6)	Ref	2.3 (-9.6–21.7)	Ref	-0.1 (-0.5–0.2)	Ref	-0.04 (-0.2–0.1)	Ref
Non-Caucasian	2.0 (-8.8–13.9)	0.53	-2.7 (-7.5–17.1)	0.35	-0.3 (-0.6–0.1)	0.2	-0.01 (-0.1–0.05)	0.32
IL28B genotype								
CC	0.1 (-7.0–6.1)	Ref	4.9 (-3.5–27.7)	Ref	-0.2 (-0.8–0.1)	Ref	-0.05 (-0.4–0.03)	Ref
Non-CC	0.04 (-3.7–11.9)	0.07	-0.1 (-15.2–11.8)	0.19	0.03 (-0.4–0.2)	0.33	-0.02 (-0.2–0.1)	0.36

Median values at baseline (before starting combination antiretroviral therapy [cART]) for hyaluronic acid (HYA), cytokeratin-18 (CK-18), Fibrosis-4 (FIB-4) and aspartate aminotransferase-to-platelet ratio index (APRI) were 39 ng/ml, 83 U/l, 1.5 and 0.6, respectively. [a]Linear regression model for cluster correlated data to consider repeated measurements within individuals [24]. [b]Median HCV RNA value at baseline. IDU, intravenous drug use; Ref, reference.

of liver fibrosis in individual patients, but highly correlated with advanced liver fibrosis at the population level. In addition, we measured CK-18 fragment levels as a marker of hepatocellular apoptosis, a strong trigger of hepatic fibrogenesis, which has not been assessed in HIV–HCV-coinfected subjects before. CK-18 positively correlated with liver fibrosis and with aminotransferase levels. Similar to previous studies [17,30], the correlation was stronger for AST compared to ALT. This might be due to the different distribution of the two enzymes in hepatocytes; AST is predominantly a mitochondrial enzyme, while ALT is located in the cytoplasm [21,31]. Because apoptosis is associated with mitochondrial membrane destruction, the apoptotic marker CK-18 might be more strongly associated with the mitochondrial enzyme AST compared to the cytosolic ALT. Bantel *et al.* [15] found increased CK-18 levels in HCV-monoinfected individuals with progressive liver disease even in those with normal aminotransferase levels. HYA and CK-18 levels, and APRI scores were significantly higher in HIV–HCV-coinfected compared to HIV-monoinfected individuals, while FIB-4 scores did not differ significantly. The formula to estimate FIB-4 levels includes ALT values in the denominator. Because median ALT levels were >3× as high in HIV–HCV-coinfected compared to HIV-monoinfected patients (73 U/l versus 24 U/l), baseline differences in FIB-4 levels between HIV–HCV-coinfected and HIV-monoinfected individuals might have been diminished.

In accordance with previous studies [32,33], all assessed biomarkers strongly correlated with hepatic fibrosis in our HIV–HCV-coinfected cohort. Therefore these biomarkers can be considered as specific surrogates of liver fibrosis. In longitudinal analyses, we observed only minor median changes in non-invasive biomarkers both during untreated and treated HIV infection. During approximately 2 years of untreated HIV infection, the median change in markers of liver fibrosis was <3%. Median HYA and CK-18 levels changed <5% per year during successful cART. APRI and FIB-4 scores improved during the first year on cART. This finding could either be due to a remission of liver fibrosis or due to normalization of HIV-induced thrombocytopaenia because platelet counts are

included in both scores (median [IQR] change 40 g/l [23–61]; Additional file 2). Given the rapid decline of APRI and FIB-4 scores during the first year on cART, along with a similar improvement of platelet counts, we assume that reversion of HIV-induced thrombocytopaenia, rather than regression of liver fibrosis explains this finding. After the first year of cART, there were no further relevant changes in APRI and FIB-4 scores.

It is important to note that the lack of a median change in biomarker levels at the population level does not exclude a fast liver fibrosis progression in some individuals, as described before [34,35]. Previous reports based on paired liver biopsies suggested that significant (≥2 stages) fibrosis progression occurs in about 20% of patients within approximately 3 years [34,35]. However, the median changes in fibrosis progression observed in our study are in line with previous estimates. Thein *et al*. [36] estimated a median fibrosis progression rate of approximately 0.1 per year across all fibrosis stages among HIV–HCV-coinfected individuals. Similar progression rates (0.1 to 0.15) were observed in other cohorts of HIV–HCV-coinfected individuals [4,37]. Based on these observations, liver fibrosis would progress on average one stage every 10 years. In the SLAM-C trial, liver fibrosis did not progress (median change 0 METAVIR units/year) over 72 weeks [38]. An average progression of liver disease of >1 stage would have been surprising in our study population. Given that all assessed biomarkers are relatively insensitive for changes in liver fibrosis of <2 stages, the small changes observed in our study are expected. Furthermore, because only 26% of our study population had advanced liver fibrosis, and because of the minimal changes in biomarkers of liver disease, the low frequency of liver decompensation is also expected. This finding is in line with a recent study that reported a <1% risk to develop a liver-related event during a 5-year interval when baseline HYA was <75 ng/ml [39]. Liver fibrosis might progress considerably faster in the first months after HCV transmission in HIV-infected patients, as recently demonstrated [40,41]. Of note, these patients were infected with HCV after HIV infection, whereas in most other studies including our cohort, HCV infection occurred before HIV infection [42]. This suggests that the time point and the chronology of HCV and HIV infections might influence liver fibrosis progression rates.

Some studies reported a beneficial effect of cART on liver disease progression [6,43]. In our study, the progression rates assessed by non-invasive biomarkers did not differ before and during cART. It is possible that the relatively short follow-up pre-cART was insufficient to detect significant differences compared to the cART period.

Our study has strengths and limitations. A major strength is the longitudinal design that inherently controls for demographic and clinical confounders. Furthermore, by limiting the analyses to patients with uninterrupted and successful cART, we eliminated confounding through treatment failure or poor adherence to cART. As the large majority of SHCS participants initiates cART within the first two years after diagnosis, it was unfortunately not possible to include a large patient population with both long-term follow-up before and during cART. Accordingly, follow up time was considerably shorter before compared to during cART. Although the assessment of changes per year should overcome some of the limitations with regard to different follow-up times, it is possible that longer observation pre-cART would have revealed different dynamics in biomarker changes. A further strength is the use of several biomarkers that have been consistently validated in previous studies and that correlated well with liver histology in our cohort. Unfortunately, we could not assess changes of liver histology longitudinally by a follow-up biopsy. However, given the risks and aversion of patients to undergo a second biopsy, such studies are difficult to perform. Some studies found a better accuracy for transient elastography (Fibroscan®) compared to non-invasive serum markers of liver fibrosis in HIV–HCV-coinfected patients [44], but unfortunately this tool was not available to use when this study had been started. Further non-invasive biomarkers based on calculations of several serum markers (for example, Fibrotest, Hepascore or Fibrometer) have similar AUROC values compared to the tests used in this study [13]. Because the AUROC values for advanced fibrosis measured in this study were >0.8 and comparable to other established biomarkers, we surmise that our analysis would have captured a major change in the proportion of patients with advanced fibrosis during follow-up. A further limitation is the imperfect prediction of liver histology by non-invasive biomarkers at the individual level [13]. Despite AUROC values of >0.8, >25% of patients would be misclassified when using the optimal cutoffs for significant fibrosis, as assessed by paired biomarker levels and liver histology (Figure 1). In clinical practice, the judgment of liver fibrosis in an individual will usually rely on a combination of clinical, histological and laboratory characteristics and follow-up screening will make use of the parameter(s) considered best. However, an exact prediction of liver histology with non-invasive biomarkers was not the scope of this study. The main aim of our study was rather to assess the average effects of HIV and cART on liver disease progression, which could be achieved given the significant correlation of biomarkers and liver histology. As we excluded patients treated for HCV infection, it is possible that slow progressors who do not need therapy are overrepresented in our cohort. However, previous studies in the SHCS indicate that

<20% of HIV–HCV-coinfected patients start therapy, and that lack of significant fibrosis is not a major reason for not starting therapy (<5% of cases) [45].

The results of our and other studies suggest that progression of liver fibrosis is relatively slow in many HIV–HCV-coinfected individuals, at least during effective cART. This could deliver important information to clinicians with regard to the urgency of starting HCV therapy. An optimal timing of HCV therapy is particularly important in the current era of direct-acting antiviral agents, where more effective and/or more tolerable drugs will likely become available in the foreseeable future. Our study suggests that in many HIV–HCV-coinfected individuals with non-severe fibrosis, HCV therapy could be safely deferred until conditions for treatment with regard to comorbidities and drug adherence have been optimized, or until new antiviral agents are also available for the treatment of non-1 HCV genotype infection. Nevertheless, the observation that liver fibrosis progression is accelerated in some individuals [34,35] underscores the need to maintain a high clinical vigilance with regard to liver fibrosis progression in HIV–HCV-coinfected patients. Although we did not find evidence for a beneficial effect of cART on liver fibrosis progression, the minimal changes in biomarker levels and the rarity of liver-related events suggest that cART is safe and possibly beneficial with regard to liver disease in HIV–HCV-coinfected patients.

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Disclosure statement

The authors declare no competing interests.

Additional files

Additional file 1: A list of members of the SHCS can be found at http://www.intmedpress.com/uploads/documents/AVT-13-OA-2971_Rohrbach_Additionalfile1.pdf

Additional file 2: Figures illustrating the receiver operating characteristic curve analyses to determine the AUROC and optimal cutoff values for non-invasive biomarkers; correlations between CK-18 and AST levels; change in platelet counts, AST and ALT levels within individuals during successful cART, during untreated HIV-infection and in HIV-monoinfected individuals; and longitudinal change in non-invasive biomarkers of liver disease in individuals with follow-up during untreated and treated HIV infection can be found at http://www.intmedpress.com/uploads/documents/AVT-13-OA-2971_Rohrbach_Additionalfile2.pdf

Additional file 3: A table displaying median non-invasive biomarker levels before and during cART by cause of death can be found at http://www.intmedpress.com/uploads/documents/AVT-13-OA-2971_Rohrbach_Additionalfile3.pdf

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