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HIV, hepatitis B and hepatitis C coinfection in Kenya

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There are few data regarding hepatitis and HIV coinfection in Africa. In 378 HIV seropositive individuals in Nairobi, 23 (6%) were hepatitis B virus (HBV) and HIV coinfected, four (1%) were hepatitis C virus (HCV) and HIV coinfected and one patient was infected with all three viruses. Coinfected individuals were more likely to be men and older; a lack of HBV vaccination was a risk factor for HIV/HBV coinfection ($P = 0.001$) and tenofovir containing regimens appeared most effective at reducing HBV viral load.

Although rates of coinfection with at least two of hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV are well reported in numerous studies from Europe and America, there are few data on the prevalence of coinfection in African populations [1–7]. The 2003 Kenyan Demographic and Health Survey reported that 6.7% of Kenyan adults are infected with HIV [8] and other small studies have suggested that the seroprevalence of HBV or HCV is approximately 5% [9,10]. As both HBV and HCV are transmitted through routes similar to HIV, coinfection would be expected to be common.

To investigate this further, we performed a prospective study of patients attending the Aga Khan University Hospital (AKUH), Nairobi, Kenya. All HIV-positive patients over the age of 13 years (both from the in-patient and out-patient settings) were included, after written informed consent was obtained. Statistical analysis was undertaken using SPSS, version 8.0. Ethical approval was granted by ethical bodies at both the AKUH and collaborators in London.

Two millilitres of blood was obtained from each subject by venepuncture for serological HCV and HBV tests. The samples were collected and processed using standard methods, stored at -20°C and analysed in batches. Routine blood tests such as CD4 (Beckman Coulter Flow cytometry), viral load (Nucleisense technique), and liver function tests (aspartate and alanine aminotransferase) were also performed simultaneously. HepBsAg was assayed using enzyme-linked immunosorbent assay (ELISA) kits (Enzygnost) and individuals found to be HBsAg positive also had an HBeAg test. HCV antibodies were detected using fourth-generation ELISA kits (DRG International, Inc. Mountainside, New Jersey, USA/ Biokit Labsystems, Helsinki, Finland).

As prospective data were not routinely collected, a study questionnaire was completed with the assistance of one of the investigators, with simple questions regarding age, history of intravenous drug use, HBV vaccination and sexual orientation.

A total of 378 consecutive HIV-positive individuals were recruited and, of these, there were 209 men (55%) and 169 women (45%) with a median age of 39.5 years (range 13–65). A total of 351 (92.8%) had HIV infection alone, 23 (6.1%) were infected with both HIV and HBV and a further four (1.1%) had HIV and HCV coinfection. One individual (0.3%) was infected with all three viruses. Of the HIV and HBV coinfected patients, four (17%) were HBeAg positive.

We compared the characteristics of the HIV positive individuals with those who were HIV and HBV coinfected (Table 1). There were no differences with regards to CD4 count, viral load and liver function tests (AST and ALT levels) but coinfected patients were older ($P = 0.05$) and more likely to be male ($P = 0.02$). Of interest, however 57% of individuals had CD4 counts below 200 cells/μl, a fact that may limit the validity of antibody responses measured and increase false negatives.

In the questionnaire study, all individuals were heterosexual apart from a single homosexual male (who had HIV infection alone). No individual admitted to intravenous drug use. As may be expected, previous hepatitis B vaccination appeared in this cohort to protect against HBV infection. No patient with previous hepatitis B vaccination developed subsequent hepatitis B infection whereas 23 of 309 (7.4%) who had not been vaccinated were coinfected with HIV and HBV ($P = 0.001$).

This study also highlighted that HCV infection was uncommon in this high-risk population, which was generally representative of the general population (with a slightly increased number of men which is observed in urban settings such as this). HIV viral load had no effect on the presence of both HBV/HIV and HIV/HCV co infections.

Of those who were infected with both HIV and HBV, nine received zidovudine, lamivudine and efavirenz, six received tenofovir and emtricitabine and one received zidovudine, lamivudine and nevirapine. The group that received tenofovir had a greater reduction in HBV DNA viral load than the group that received lamivudine

Table 1. Characteristics of HIV positive and HIV/HBV coinfected individuals.

	HIV only	HBV coinfection	*P*
No. of patients	351	23 (6)	<0.0001
Age[a] (years), mean ± SD	39.2 ± 8.15	42.7 ± 9.13)	0.05
Age (years)			
<25	15 (4.29)	1 (4.35)	0.405
25–30	30 (8.57)	1 (4.35)	
30–35	74 (21.14)	2 (8.70)	
35–40	85 (24.29)	5 (21.74)	
>40	146 (41.71)	14 (60.87)	
CD4 counts (cells/μl)			
<50	93 (26.57)	6 (26.09)	0.405
50–100	39 (11.14)	4 (17.39)	
100–200	67 (19.14)	4 (17.39)	
200–300	48 (13.71)	6 (26.09)	
300–400	37 (10.57)	1 (4.35)	
>400	66 (18.86)	2 (8.70)	
HIV viral load			
<50	63 (17.95)	2 (9.09)	0.245
50–1000	26 (7.41)	0 (0.00)	
1000–100 000	158 (45.01)	14 (63.64)	
>100 000	104 (29.63)	6 (27.27)	
Sex			
Women	161 (45.87)	5 (21.74)	0.024
Men	190 (54.13)	18 (78.26)	
Liver function			
Normal	137 (39.03)	12 (52.17)	0.212
Abnormal	214 (60.97)	11 (47.83)	

HBV, hepatitis B virus. Percentage values are given in parenthesis.
[a]Age was treated as a continuous variable.

($P = 0.0031$). The effects of treatment on a more widespread scale, and the effects of hepatitis on HIV and HIV on hepatitis in this setting remain to be established.

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HIV Gag-specific immune responses predict the rate of CD4 decline

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In the present study, we assessed whether Gag-specific interferon (IFN)-γ secreting responses correlate with the rate of disease progression as defined by the annual rate of CD4 decline. Although neither the breadth nor the magnitude of the proteome-wide HIV-specific IFN-γ response correlated with viral load or rate of CD4 decline, the preferential targeting of Gag is associated with slower annual $CD4^+$ T cell decline.

There is compelling evidence that $CD8^+$ T cells play an important part in the control over viral replication. Observations linking the expression of major histocompatibility complex class I alleles with disease progression rates and the appearance in early infection of viral variants bearing recognition escape mutations within sequences targeted by virus specific $CD8^+$ T cells is consistent with these cells playing an active role in controlling viral replication and exerting selective pressure on the virus [1–3]. The most direct evidence supporting a role for $CD8^+$ CTL in viral control comes from an animal model for HIV infection. Depletion of $CD8^+$ T cells from macaques infected with the simian immunodeficiency virus (SIV) results in either increased viremia that

remains high until the $CD8^+$ T cells are reconstituted or uncontrolled viremia and rapid disease progression [4].

Previous studies analyzed HIV-specific interferon (IFN)-γ secreting $CD4^+$ and $CD8^+$ T cell responses directed against the entire HIV proteome and found that neither the breadth nor the magnitude of IFN-γ secreting lymphocytes correlated with viral load, CD4 counts or rate of CD4 decline [5–7]. These studies supported the view that IFN-γ secretion is a poor correlate of in-vivo protection against HIV disease progression and other functional markers such as IL-2 secretion and proliferation should be assessed when monitoring immune competence. More recently, it has been reported that the preferential targeting of Gag p24 was associated with viral control [8,9]. A large cohort study confirmed and extended these findings by showing that an increasing breadth of responsiveness to Gag epitopes was associated with lower viral load in chronic HIV infection [10]. Mechanisms underlying the association between Gag recognition and viral load may be related to the observation that targeting certain Gag epitopes exerts immune pressure that favors the outgrowth of viral variants that, although able to escape immune recognition, have a diminished fitness and replicative capacity [11] and that Gag-specific $CD8^+$ T cells recognize and eliminate infected cells within 2 h postinfection before proviral integration [12] and de-novo protein synthesis [13].

In light of these findings, we analyzed data from 31 treatment-naïve HIV-1 clade B infected subjects in the chronic phase of infection to determine whether targeting Gag was associated with slower rates of CD4 decline [7]. At the time point used for screening HIV-specific IFN-γ secretion, the study population with a median of 34 years (18–51) had $CD4^+$ T cell counts of 572 (374–999) cells/μl, $CD8^+$ T cells count of 1144 (720–2412) cells/μl and viral loads of 1452 (72–22 065) HIV-1 RNA copies/ml. Monthly absolute $CD4^+$ T cell count values taken over a 24 (12–24)-month period was used to determine the annual rate of $CD4^+$ T cell loss for this population, which was 57 (0–177) cells/μl/year.

Our initial findings showed that neither the breadth nor magnitude of absolute HIV-specific responses to the entire proteome or to individual gene products correlated with viral load or predicted the rate of CD4 decline [7]. In light of the studies mentioned above, subsequent analysis of the data revealed that the proportion of Gag recognition relative to the total HIV-specific response within an individual was associated with the rate of CD4 decline ($r = -0.44$, $P = 0.013$ and $r = -0.47$, $P = 0.008$ for correlations between rate of CD4 decline and the breadth and magnitude of Gag specific responses, Spearman's and Pearson's correlation, respectively) suggesting that the preferential targeting of Gag determinants within an individual is associated with a slower decline in $CD4^+$ T cell count (Fig. 1a, b). Additionally, time to loss of 60 $CD4^+$

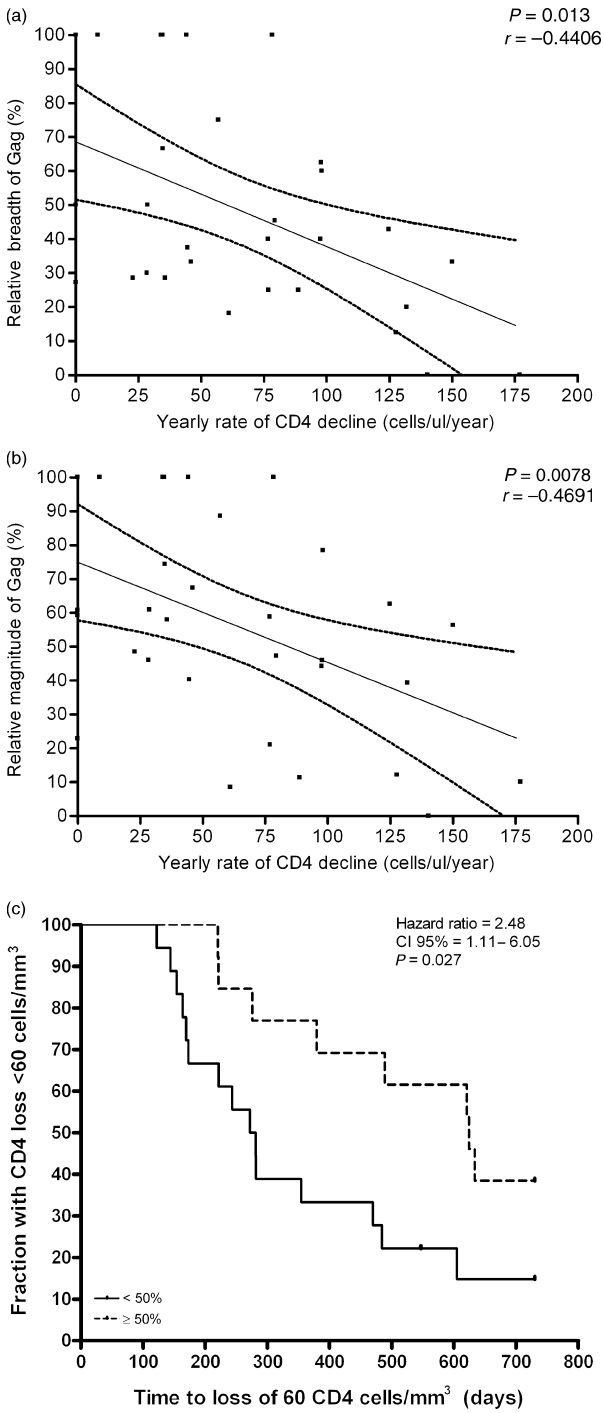

Fig. 1. Association between the percent contribution of the Gag-specific response and rate of CD4 decline. Correlation between the relative breadth (a) and relative magnitude (b) of Gag-specific interferon-γ secreting responses with the annual rate of $CD4^+$ T cell decline in 31 untreated HIV-infected individuals in the chronic phase of infection. Relative breadth or magnitude of Gag recognition is defined as the percentage of the HIV-specific response to the entire proteome attributed to HIV Gag-specific responses. (c) Results of a Kaplan–Meier analysis of time to loss of 60 CD4 cells/mm^3 in the study population whose relative recognition of Gag was less than 50% versus at least 50% of the entire HIV-specific response.

T cells/μl was a median of 276 versus 624 days for individuals whose HIV Gag-specific response represented less than 50% and at least 50% of the entire HIV-specific response (hazard ratio = 2.48; 95% confidence intervals = 1.11–6.05). No significant correlation was found between the relative recognition of Gag and plasma viral load ($r = -0.28$, $P = 0.13$) or between the relative recognition of other gene products such as Pol, Nef or Env and either viral load ($r = -0.35$, $r = 0.32$ and $r = 0.06$; $P > 0.05$ for all analyses) or rate of CD4 decline ($r = 0.03$, $r = 0.29$ and $r = 0.35$; $P > 0.05$ for all analyses). The absence of a significant association between the relative recognition of Gag and viral load may be related to the population size not being large enough to detect such a difference or to viral load contributing approximately 5% of the variability in HIV disease progression [14] or both.

These data support previous observations and suggest that monitoring relative Gag responses in infected individuals might prove beneficial for predicting subsequent disease course. Additionally, these findings support using Gag immunogens in the development of T cell based vaccines.

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Outcomes of multidrug-resistant patients switched from enfuvirtide to raltegravir within a virologically suppressive regimen

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Enfuvirtide has been a cornerstone of salvage therapy for multidrug-resistant HIV. Raltegravir provides another novel class option, with the advantages of easier administration and improved tolerability. Thirty-five adults electively replaced

enfuvirtide with raltegravir while the rest of their regimen was unchanged. All maintained virologic suppression after a median of 7 months except one who experienced a transiently detectable viral load after 5 months. The new regimen was well tolerated with no apparent new drug-related adverse clinical or laboratory events.

The fusion inhibitor enfuvirtide and the integrase inhibitor raltegravir each have demonstrated antiviral activity in treatment-experienced patients with HIV resistant to nucleosides (NRTI), nonnucleosides (NNRTI), and protease inhibitors [1–4]. While enfuvirtide has been a cornerstone of salvage therapy in this setting, its long-term tolerability has often been hampered by the need of twice-daily subcutaneous injections and persistent injection site reactions [5]. Recently, raltegravir has become an alternative to enfuvirtide in this setting. Raltegravir is a well tolerated oral agent in a new class without crossresistance to the NRTI, NNRTI, and protease inhibitor classes [6].

We offered an elective switch from enfuvirtide to raltegravir to all HIV-positive adults who had plasma HIV RNA less than 50 copies/ml (Roche HIV-1 RNA Ultrasensitive PCR assay) and who had ongoing injection site reactions and/or injection fatigue while receiving an enfuvirtide-containing regimen within a single tertiary care HIV clinic (John Ruedy Immunodeficiency Clinic, St Paul's Hospital, Vancouver, British Columbia). The switch was accomplished in a single day without overlapping dosing. The remainder of the salvage regimen was left unchanged. Raltegravir was obtained through the Special Access Programme of Health Canada, and was administered in doses of 400 mg orally twice daily. Patients received clinical and laboratory follow-up according to standard clinical practice after the change in regimen.

Thirty-five patients (34 male) switched from enfuvirtide to raltegravir between 20 November 2006 and 19 October 2007. At the time of the switch, patients had received enfuvirtide for a median of 25 months (range 5–75 months) and had sustained HIV RNA less than 50 copies/ml for a median of 24 months (range 1–72 months) (Table 1). Concomitant antiretrovirals included one to four nucleoside or nucleotide RTI in all patients, one NNRTI in six patients, and one or two protease inhibitors in all patients. Twenty-six patients received a single protease inhibitor: lopinavir/ritonavir ($n=9$), darunavir/ritonavir ($n=8$), or atazanavir ($n=7$ ritonavir-boosted, $n=1$ unboosted). Of note, one patient was receiving tipranavir/ritonavir, which is known to reduce raltegravir trough levels by 55% [7]. Eight patients received dual boosted protease inhibitors: lopinavir/saquinavir ($n=5$), lopinavir/atazanavir ($n=2$), or atazanavir/saquinavir ($n=1$). One patient received atazanavir/saquinavir without ritonavir boosting.

Table 1. Baseline characteristics of 35 patients at time of enfuvirtide discontinuation and raltegravir initiation.

	Median	Range
Age, years	49	34–69
Time on enfuvirtide before switch, months	25	5–75
Time HIV RNA <50 copies/ml before switch, months	24	1–72
CD4 count, cells/mm^3	350	90–770
CD4 fraction, (%)	16	4–43
Concomitant ARVs, (N)		
Nucleoside/nucleotide RTIs	3	1–4
Nonnucleoside RTIs[a]	0	0–1
Protease inhibitors[b]	1	1–2

ARVs, antiretrovirals; RTIs, reverse transcriptase inhibitors.
[a]etravirine $n=3$; efavirenz $n=2$; nevirapine $n=1$.
[b]Ritonavir-boosted in 33/35.

As of 10 January 2008, all 35 patients remain on raltegravir after a median follow-up time of 7 months (range 1–13 months), and none have resumed enfuvirtide. Thirty-four patients have HIV RNA less than 50 copies/ml. The remaining patient had HIV RNA less than 50 copies/ml at 1 and 2 months and 60 copies/ml after 5 months on raltegravir. His concurrent regimen consists of ritonavir-boosted atazanavir and lamivudine, and he had previously experienced intermittent viral load blips less than 100 copies/ml while taking enfuvirtide. The only patient who received concomitant tipranavir/ritonavir (in addition to tenofovir, zidovudine, lamivudine, and abacavir) has sustained a plasma HIV RNA less than 50 copies/ml for 6 months after the switch to raltegravir. Plasma raltegravir levels have not been measured.

Injection site reactions resolved in all patients after enfuvirtide was discontinued. Five patients have experienced new clinical events, but no new laboratory events were observed. Early events occurring within 1 month after the switch to raltegravir were mild peripheral neuropathy and diarrhea; exacerbation of depression; and prostate cancer (in a 56-year-old man). One patient experienced two episodes of pneumonia after 1 and 6 months on raltegravir. In addition, a B-cell lymphoma was diagnosed in a 52-year-old man after 9 months on raltegravir. It is unlikely that any of these events can be directly attributed to raltegravir. Whereas excessive rates of depression with raltegravir as compared with placebo have not been reported in phase II and III clinical trials [3,4,6], anecdotal reports are emerging of psychiatric disorders, including depression, temporally related to the onset of raltegravir therapy [J Gatell, P Ruane, personal communication]. These observations require further study as the clinical use of raltegravir expands into broader patient populations.

On the basis of these results, changing from enfuvirtide to raltegravir within a virologically suppressive regimen appears to be well tolerated and effective, at least over the

short term, in patients with multidrug-resistant HIV. The switch from an injectable to an oral medication is highly acceptable to patients and may facilitate long-term adherence. In addition, this strategy is economically attractive based on the higher price of enfuvirtide.

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Liver ultrastructural morphology and mitochondrial DNA levels in HIV/hepatitis C virus coinfection: no evidence of mitochondrial damage with highly active antiretroviral therapy

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Liver mitochondrial toxicity is a concern, particularly in HIV/hepatitis C virus (HCV) coinfection. Liver biopsies from HIV/HCV co-infected patients, 14 ON-highly active antiretroviral therapy (HAART) and nine OFF-HAART, were assessed by electron microscopy quantitative morphometric analyses. Hepatocytes tended to be larger ON-HAART than OFF-HAART ($P = 0.05$), but mitochondrial volume, cristae density, lipid volume, mitochondrial DNA and RNA levels were similar. We found no evidence of increased mitochondrial toxicity in individuals currently on HAART, suggesting that concomitant HAART should not delay HCV therapy.

Today, up to 50% of HIV patients die from end-stage liver diseases [1]. Possible reasons for this include longer life expectancy and a decline in opportunistic infections, accompanied by an increase in underlying comorbid conditions, such as liver diseases and idiosyncratic hepatotoxic reactions [1–3]. This is especially relevant to the HIV/hepatitis C virus (HCV) coinfected population in whom liver injury is more rapid and prevalent [2,4], poor liver conditions decrease tolerance for highly active antiretroviral therapy (HAART) and end-stage liver diseases are the primary cause of death [5,6].

Nucleoside reverse transcriptase inhibitors (NRTIs) can cause mitochondrial DNA (mtDNA) depletion and possibly deletion and mutation, which may affect mitochondrial structural integrity and functions [7,8]. Such HAART-related mitochondrial toxicity may lead to hepatic steatosis, hyperlactatemia/lactic acidosis and liver failure. Although abnormal liver mitochondrial ultrastructure has been demonstrated in HIV/HCV coinfection [9,10], it is unclear what damage is caused by HIV or HCV infection versus NRTI-related mitochondrial toxicity. We hypothesized that HIV/HCV coinfected individuals currently receiving HAART would show greater liver mitochondrial damage than those not on

HAART. Liver mitochondrial damage was assessed through mtDNA and gene expression levels, as well as electron microscopic ultrastructural analyses.

In this prospective cohort study, HIV/HCV coinfected men were either HAART-naïve or off HAART more than 6 months (OFF-HAART), or on stable HAART for longer than 6 months (ON-HAART). At the time of liver biopsy, all were HCV therapy naïve, free from chronic liver diseases and had no opportunistic infections within the last month. Demographic and clinical characteristics such as age, HCV genotype, CD4 cell count, albumin, bilirubin, aspartate aminotransferase, alanine aminotransferase, platelet count and lactate were comparable between the ON-HAART and OFF-HAART groups.

Two ultrasound-guided liver tissue biopsies were collected between 2003 and 2006 from each subject. The first one was used for pathology using modified Ishak–Knodell scale scoring whereas the second was used for mtDNA and mtRNA quantifications and electron microscopic stereological morphometry. The mtDNA/nuclear DNA (nDNA) ratio was determined as described previously [11,12]. Mitochondrial mRNA was quantified similarly for both a mtDNA-encoded (COX1) and a nDNA-encoded (COX8) gene, and normalized to the housekeeping gene β-actin mRNA level. Tissue for electron microscopic analysis was fixed in 2.5% glutaraldehyde and postfixed in a mixture of 2% osmium tetraoxide and 2% potassium ferrocyanate. The following hepatocyte ultrastructural characteristics were quantitatively and stereologically analyzed: hepatocyte volume using the star volume method [13]; hepatocyte mitochondria, glycogen and lipid volume fractions using the point counting method [14]; and mitochondria cristae surface density, using the line intercept method [15]. To eliminate potential bias, a standardized random sampling protocol was established by a single examiner, and the number of images analyzed for each parameter was determined as that providing a stable and low coefficient of error.

Overall, no statistically significant difference was observed between ON-HAART and OFF-HAART samples, for any of the studied parameters (Table 1). No differences were seen in mtDNA and mtRNA levels, but there was an overall positive correlation between COX1 and COX8

Table 1. Characteristics of the study population and study results.

	Median [IQR]		
	On-HAART ($n=14$)	Off-HAART[a] ($n=9$)	P[g]
Patient characteristics			
Age (years)	43 (39–47)	48 (46–53)	0.09
HCV genotype (1/2/3/4)	10/0/4/0	7/0/2/0	
CD4 cell count (cells/μl)	390 (320–580)	400 (340–410)	0.87
Plasma HIV RNA (copies/ml)	<50 (<50–<50)	48200 (20500–88400)	<0.05
Albumin (g/l)[b]	42.0 (40.5–44.0)	41.0 (40.0–42.0)	0.86
Bilirubin (μmol/l)[b]	12.0 (8.5–17.5)	9.0 (5.8–13.0)	0.15
Platelet count (10^9 cells/l)[b]	174 (150–203)	210 (149–224)	0.58
AST (U/l)[b]	71.0 (45.5–89.8)	53.0 (49.0–79.0)	0.64
ALT (U/l)[b]	74.0 (60.0–176.0)	58.0 (50.0–88.0)	0.68
Lactate (mmol/l)[b]	1.50 (1.23–2.05)	1.70 (1.35–1.75)	0.88
Duration of HAART at the time of biopsy			
Total ($n=14$) (months)	28 (11–94)		
D-drugs ($n=2$) (months)[c]	22 (–)		
PI ($n=10$) (months)[d]	23 (15–40)		
NNRTI ($n=6$) (months)[d]	22 (10–35)		
qPCR and EM results			
mtDNA/nDNA	506 (381–804)	508 (394–823)	0.90
COX1 mtRNA/β-actin mRNA[e]	25.7 (17.0–41.6)	34.6 (22.9–37.9)	0.80
COX8 mtRNA/β-actin mRNA[e]	1.0 (0.8–1.4)	1.1 (0.6–1.6)	0.83
Mitochondria volume fraction (%)	17.3 (15.8–20.0)	18.4 (16.3–22.2)	0.49
Cristae density ($\mu m^2/\mu m^3$)	4.7 (4.5–6.0)	6.5 (5.0–7.1)	0.23
Glycogen (%)[f]	24.7 (22.5–30.0)	22.2 (18.7–30.8)	0.67
Lipid (%)	2.0 (0.8–5.0)	2.1 (0.8–7.0)	0.87
Cell size (μm^3)	4425 (3369–5317)	3369 (2721–3596)	0.05
Ishak–Knodell score	7.0 (5.0–9.0)	7.0 (6.0–12.3)	0.44

[a]HAART naïve ($n=5$) or longer than 6 months off HAART ($n=4$) at the time of biopsy.
[b]Normal range: albumin 35–48 g/l, bilirubin total <20 μmol/l, platelet 150–400 × 10^9 cells/l, AST <40 U/l, ALT 7–56 U/l, lactate 0.5–2.1 mmol/l.
[c]D-drugs = stavudine (d4T), didanosine (ddI) or zalcitabine (ddC). D4T in both cases.
[d]Two patients were taking both PI and NNRTI at the same time (i.e. eight on a protease inhibitor, 4 on a NNRTI and 2 on both protease inhibitor and NNRTI).
[e]Total 17 samples RNAlater treated were analyzed ($n=10$ for ON-HAART and $n=7$ for OFF-HAART).
[f]Two samples were not analyzed as they were not fixed with potassium ferrocyanate.
[g]Between-groups comparisons were done using the Krusskal–Wallis test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; EM, electron microscope; HAART, highly active antiretroviral therapy; HCV, Hepatitis C virus; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitors; PI, protease inhibitors.

expression levels ($R = 0.63$, $P = 0.006$). Volume fractions of hepatocyte metabolic constituents did not significantly differ between ON-HAART and OFF-HAART samples, however, hepatocytes were weakly but significantly larger in the ON-HAART group compared with the OFF-HAART one ($P = 0.05$), and median cristae surface density was 28% lower ON-HAART (Table 1). All study participants were infected with either HCV genotype 1 or 3a, and the latter was associated with significantly higher intrahepatocyte lipid accumulation [median (interquartile range = 1.3 (0.6–2.3)% vs. 12.0 (8.0–14.1)%, $P = 0.002$].

HIV/HCV coinfected individuals are more at risk of hepatotoxicity than HIV or HCV monoinfected ones [2,4,6,16]. It has been hypothesized that NRTI-induced liver mitochondrial toxicity may further exacerbate liver damage and contribute to lower tolerance of HCV therapy [5,17]. NRTIs of the dideoxynucleotide type [D-drugs: d4T, ddI and zalcitabine (ddC)] are blamed for mitochondrial toxicity in both clinical and in-vitro studies [11,18]. Decreased liver mtDNA levels in HIV/HCV coinfected patients on D-drugs, but not in those on non-D-drug-containing HAART have been reported [19]. We found no evidence of increased liver mitochondrial damage in association with current HAART. As the majority of ON-HAART subjects were receiving non-D-drug HAART regimens, our results are consistent with the previous finding [19] and suggest low liver mitochondrial toxicity for non-D-drug HAART. Mitochondrial gene expression of both nDNA and a mtDNA-encoded genes did not differ between the two groups, yet showed a significant correlation between them, suggesting the absence of mtDNA-specific alterations in expression patterns.

We rigorously and objectively evaluated hepatocyte cell volume, mitochondria volume fraction, cristae surface density, lipid and glycogen volume fraction by quantitative electron microscopy. No difference in any of the studied parameters was detected between ON-HAART and OFF-HAART liver samples, except for the cell size, which was marginally larger in ON-HAART samples. This appears to be inconsistent with two studies in HIV/HCV coinfected individuals that suggested an association between HAART and increased liver mitochondrial ultrastructural alterations [9,10]. Only two of our participants received d4T and none was on ddI or ddC. Exposure to different NRTIs in these small studies may partially explain this difference. Hepatomegaly has been observed in HIV patients [20] and coinfection with HCV increases the risk of steatosis or steatohepatitis, which may enlarge hepatocytes. However, the relationship between hepatomegaly and HAART remains unclear since mitochondrial dysfunction can also lead to steatosis and steatohepatitis [21,22]. Although our data do not suggest increased mitochondrial damage in ON-HAART individuals, it is possible that enlarged hepatocytes reflect weak hepatotoxicity. As noted by others [23], HCV genotype 3 was statistically significantly associated with higher lipid volume fraction (Table 1), validating the stereological methodology used in this study.

In conclusion, although the sample size was limited, this study provided no evidence that patients coinfected with HIV and HCV show increased liver mitochondrial damage if on concomitant HAART. These results suggest that HAART with predominantly non-D-drug-containing regimen may not be a reason to delay or avoid the start of HCV antiviral therapy in coinfected individuals. This is especially true as liver disease progression and not drug-induced hepatotoxicity is the most common cause of mortality in this population [24].

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The University of British Columbia has a patent on the mtDNA/nDNA real-time PCR assay used in this study, on which H.C.F.C. and J.S.M are inventors.

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