

Research Letters

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Mitochondrial toxicity hypothesis for lipodystrophy: a refutation

Only through understanding the aetiology of the metabolic and morphological changes in individuals receiving highly active antiretroviral therapy (HAART) can the optimal management of these problems be established. Evidence from cross-sectional surveys, such as the HOPS cohort [1] have pointed to an interaction between disease or immune recovery and drugs. Although both nucleoside and protease inhibitor (PI) use were associated with the presence of lipodystrophy, additional risk factors were necessary for lipodystrophy to be present [1]. Patients with the syndrome who have never received PI or nucleoside analogues have been reported [2–6], suggesting that although these drug classes or specific members of these classes may influence the time to the onset of clinical manifestations, they are not sufficient alone to cause problems. It is therefore not surprising that switching therapy away from whichever drug or drug class it is the current fashion to blame has not led to the resolution of the syndrome [7]. In considering information from surveys or 'cross-sectional studies' it is reasonable to consider that associations found in such analyses do not necessarily point to the causation of the problem. For example, age over 40 years has been associated with an increased risk of lipodystrophy [1] but clearly being over 40 is not the cause of lipodystrophy.

Mitochondrial toxicity of nucleoside analogues through the inhibition of DNA-polymerase gamma as been suggested to be a key factor in the pathogenesis of lipodystrophy and visceral fat accumulation [8,9]. However, there is little evidence to support mitochondrial toxicity as the mechanism of lipodystrophy, and indeed considerable data has accumulated to refute this hypothesis.

Two fat biopsy studies recently reported [10,11] demonstrated only modest reductions (mean 44% [10]) in mitochondrial DNA, even in samples from lipodystrophic patients. In inherited mtDNA disorders, reductions in mtDNA to less than 20% are generally required for disease to occur [12,13]. Importantly, samples from individuals with lipodystrophy in some cases (13% of samples in one study [11]) had normal levels of mtDNA and some control samples (from HIV-negative individuals) showed diminished mtDNA [10,11]. This underlines that reductions in mtDNA are not necessary for, characteristic of, or diagnostic of lipodystrophy in individuals with HIV. In addition, the mtDNA mutation associated with inherited Made-

lung's syndrome was absent in all samples in one of the studies [10].

Furthermore, two separate groups [14,15] demonstrated that fat oxidation, a mitochondrial function, is normal or potentially increased in persons with metabolic disturbances on PI plus nucleoside reverse transcriptase inhibitor (NRTI)-based regimens. An additional study of muscle biopsies pre- and post-exercise and lactate levels found normal oxidative phosphorylation, with a similar recovery rate in lactate and pyruvate levels after exercise to healthy controls, and no significant abnormalities of muscle mitochondria ultrastructure. The authors of the study [16] suggested that the hyperlactatemia seen in some patients, often suggested to be a marker of mitochondrial toxicity, may in fact be related to a decreased clearance of lactate. Many of the patients studied, all of whom clinically had lipodystrophy, had hypertriglyceridaemia. One potential explanation for these findings is that hypertriglyceridaemia (or type 2 diabetes) can lead to hepatic steatosis [17], hence diminished hepatic function and impaired elimination of lactate. The association of lipodystrophy and hyperlactatemia observed in one cohort analysis [18] may be explained by this mechanism, and enables an understanding of why isolated mild hyperlactatemia may be relatively common but lactic acidosis remains rare. Finding hepatic steatosis on a liver ultrasound or biopsy may simply reflect metabolic disturbances in individuals with HIV infection, not mitochondrial injury by nucleoside analogues.

The results of culturing adipocytes with nucleoside analogues or PI yielded different effects on triglyceride accumulation, fat release (lipolysis) and adenosine triphosphate (cellular energy) levels. Although PI had effects at physiological concentrations on triglyceride accumulation and at higher concentrations on lipolysis and adenosine triphosphate production, effects were not observed at concentrations normally seen in treated persons for either zidovudine or stavudine (< 30 μ M) [19]. Interestingly, when PI and NRTI were given together to the adipocyte culture, a marked synergy was observed on a range of adipocyte functions [19]. This may be an important observation, suggesting that the combination of PI and NRTI may have a greater impact on fat cell function than either alone. This is in keeping with clinical observations [20]. In a further study of stavudine in mice given very high doses of 100 mg/kg per day of the drug (human dose \sim 1.0 mg/

kg per day) over 6 weeks [21], no effects on skeletal muscle or adipose tissue mtDNA were observed. NRTI alone at current doses thus do not appear to affect adipocyte mitochondrial function. Changes in liver mtDNA were observed only in week 1 samples but not at week 6, suggesting a compensatory recovery in mtDNA [21]. This may also explain why mitochondrial toxicity is reported with nucleoside analogues in short-term in-vitro studies [22]. Nucleoside mitochondrial toxicity may, to some extent, be only an acute effect, and could be compensated for by the mechanisms that normally regulate mitochondrial numbers in the cell such as an increased expression of mitochondrial transcription factors [23]. The findings of such studies are perhaps not surprising, for the thymidine analogues zidovudine and stavudine as adipocytes, and other resting cells, are unlikely to express thymidine kinase type 1, the enzyme involved in the first step of activation of these drugs, and these drugs are poor substrates for the mitochondrially located thymidine kinase type 2 [24,25]. Adipocytes are thus unlikely to have active (i.e. potentially toxic) concentrations of phosphorylated thymidine analogues [26].

The end of the era of blaming PI exclusively for lipodystrophy came with the recognition that lipoatrophy and metabolic disturbances could occur in PI-naïve individuals. However, NRTI-naïve but antiretroviral-treated individuals are relatively rare, making and evaluation of NRTI sparing more difficult. A survey from the DP-006 study [2] indicated that nine out of 429 (2.1%) patients in the efavirenz–indinavir arm of the study had been diagnosed with ‘probable lipodystrophy’ despite having never received nucleoside analogues over a maximal follow-up of 88 weeks. Two further recent reports [3,4], evaluating patients on dual PI regimens (both ritonavir–saquinavir) observed lipodystrophy in NRTI-spared individuals but higher rates when NRTI were added to the regimen. In the Prometheus study [3], after 96 weeks follow-up, 22 out of 88 (25%) of stavudine/ritonavir/saquinavir patients but also seven out of 87 (8%) ritonavir/saquinavir alone treated patients were diagnosed with lipodystrophy, including two out of 44 (5%) ritonavir/saquinavir treated patients who had never received NRTI. A second study with 144 weeks of follow-up [4], mostly in persons who discontinued previous NRTI therapy and then received ritonavir/saquinavir found that 6% of NRTI-spared individuals had both buttock and facial wasting, with 9% having an increase in waist size. Patients who added NRTI during the study were more likely to have lipoatrophy [4]. These studies provide several important observations. Most importantly that lipodystrophy/lipoatrophy can occur in the absence of NRTI. A previous study [20] suggested that use of PI can accelerate the onset of or increase the incidence of lipoatrophy in NRTI-treated individuals. These data suggest that this interaction works both ways, that

NRTI also accelerate the onset or increase the incidence of lipoatrophy in PI-treated individuals.

Available data suggest that we should focus our efforts on understanding lipoatrophy during HAART away from drugs and onto drug–disease and immune recovery interactions [1,27]. The recent observation that the metabolic syndrome may occur in individuals several years after bone marrow transplant [28], another group of individuals undergoing immune restoration, suggests that this line of investigation may have potential benefits beyond HIV medicine.

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Low lipolytic enzyme activity in patients with severe hypertriglyceridemia on highly active antiretroviral therapy

Hypertriglyceridemia was reported early in the use of highly active antiretroviral therapy (HAART) [1]. We assayed lipoprotein and hepatic triglyceride lipases, together with the apolipoprotein C and E phenotypes, in 12 HIV 1-infected men with severe hypertriglyceridemia (400–4500 mg/dl).

After a 12 h overnight fast, patients received an intravenous bolus of heparin (100 IU/kg). Pre-heparin and 10 min post-heparin plasma samples were immediately centrifuged and stored at -80°C . Lipoprotein and hepatic lipase activities were measured separately, twice in triplicate, using the Nilsson–Ehle method [2]. Activity was expressed in micromoles of free fatty acids (FFA) released from the radiolabelled emulsion per millilitre of plasma and per hour ($\mu\text{mol}/\text{ml}/\text{h}$). Within-run and between-run coefficients of variation were 6.8 and 7.9% for hepatic lipase, and 7.5 and 8.4% for lipoprotein, respectively.

The apolipoprotein C-II to C-III₁ plus C-III₂ ratio (hereafter referred to as the apo C-II : C-III ratio) [3] and apolipoprotein E phenotypes were determined in fasting samples by means of isoelectric focusing, according to Bouthillier *et al.* [4].

The results are summarized in Table 1. The body mass

index was normal in the 10 patients in whom it was measured. Two patients (nos. 5 and 9) had abdominal lipodystrophy and one (no. 3) had facial fat wasting. Mean lipoprotein and hepatic lipase activities were 9.1 (± 4.0) and 8.6 (± 3.1) μmol FFA/ml per hour, respectively. The apo C-II : C-III ratio was normal in all but one of the patients (no. 1). Apolipoprotein E phenotypes were E3/E3 in eight patients, E2/E3 in two and E3/E4 in two.

All 12 patients had a marked decrease in hepatic lipase activity, and seven also had low lipoprotein activity. It was noticed that fasting levels of FFA were normal (not shown). Neither a lack of apolipoprotein C-II (lipoprotein-activating factor) nor an excess of apolipoprotein C-III (lipoprotein-inhibiting factor) explained the impaired lipoprotein activity. At this time, no activating or inhibiting factor for hepatic lipase modulation has been shown. Purnell *et al.* [6] recently reported a rapid decrease in hepatic lipase activity in HIV 1-seronegative volunteers receiving 15 day HAART, whereas lipoprotein activity remained normal. Tsiodras *et al.* [7] reported that severe hypertriglyceridemia appeared to be mainly linked to protease inhibitors (PI).

In our patients (data not shown), hypertriglyceridemia appeared to be rather relevant to the accumulation of

Table 1. History of HIV 1 infection in 12 patients on highly active antiretroviral therapy.

| Patient no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
|--|---------------------|----------------|----------------|---------------------------------|----------------|----------------|----------------|-------------------------------------|----------------|--------------------|----------------|----------------|----------------|
| Time since AIDS diagnosis (years) | 10 | 1 | 1 | – | – | – | 2 | 5 | 10 | – | – | – | |
| Time since starting first-line HAART (months) | 20 | 20 | 7 | 3 | 8 | 5 | 22 | 32 | 24 | 19 | 32 | 31 | |
| Current HIV therapy | NRTI NNRTI PI | D4T+3TC NFV | D4T+3TC NFV | D4T+DDI RTN–SQV ^a | D4T+3TC IDV | D4T+3TC IDV | D4T+3TC RTN | ZDV+DDI+ABC RTN–SQV ^b | D4T+3TC EFV | D4T+DDI+3TC NVP | ABC+3TC EFV | D4T+3TC IDV | ZDV+3TC RTN |
| CD4 lymphocyte count (/μL) | 215 | 163 | 94 | 893 | 400 | 430 | 124 | 162 | 393 | 923 | 481 | 554 | |
| HIV viral load (log ₁₀ /ml) | 4.9 | 3.6 | 5.9 | < 2.3 | 3.7 | < 2.3 | 5.5 | 4.2 | 2.8 | < 2.3 | < 2.3 | < 2.3 | |
| TG (N ^d < 160 mg/dl) | 510 | 4500 | 1120 | 1420 | 3900 | 400 | 400 | 2300 | 1800 | 560 | 880 | 400 | |
| TC (N ^d < 200 mg/dl) | 106 | 660 | 400 | 437 | 600 | 200 | 270 | 520 | 250 | 210 | 280 | 312 | |
| LPL activity (N ^e = 16.5 + 3 μmol FFA/ ml/h) | 5.8 | 6.9 | 4.1 | 5.1 | 3.1 | 9.4 | 9.8 | 12.7 | 15.1 | 11.8 | 12.9 | 12.5 | |
| HL activity (N ^e = 19.8 + 3.7 μmol FFA/ ml/h) | 12.1 | 4.9 | 5.1 | 4.1 | 4.8 | 8.7 | 12 | 11.1 | 9.0 | 9.3 | 10.5 | 11.4 | |
| Apolipoprotein C-II : C-III ratio (N = 0.10–0.30) | 0.06 | 0.23 | 0.28 | 0.22 | 0.19 | 0.15 | 0.17 | 0.29 | 0.32 | 0.36 | 0.26 | 0.32 | |

Triglyceride (TG) levels, total cholesterol (TC) values, lipoprotein lipase (LPL) and hepatic lipase (HL) activity, and the apo C-II : C-III ratio are shown.

Nucleoside reverse transcriptase inhibitors (NRTI): ABC, abacavir; DDI, dideoxynucleoside; 3TC, lamivudine; ZDV, zidovudine.

Non-nucleoside reverse transcriptase inhibitors (NNRTI); EFV, efavirenz; NVP, nevirapine.

Protease inhibitors (PI): IDV, indinavir; NFV, nelfinavir; RTN, ritonavir; SQV, saquinavir.

FFA, Free fatty acids; HAART, highly active antiretroviral therapy.

^aRTN (100 mg twice a day) + SQV (800 mg twice a day).

^bRTN (200 mg twice a day) + SQV (800 mg twice a day).

^cRTN (400 mg twice a day) + IDV (800 mg twice a day).

^dNational Cholesterol Education Program [5].

^eResults from 10 normolipemic, HIV-seronegative volunteers matched for age and sex.

all triglyceride-rich lipoprotein (TRL) species but not to the sole VLDL hypersecretion, as suggested by Purnell *et al.* The increased levels of chylomicrons and VLDL were probably caused by the decrease in lipoprotein activity, whereas the accumulation of cholesterol- and apolipoprotein-E-rich remnants (chylomicron-remnant and intermediate density lipoprotein) would rather be caused by decreased hepatic lipase activity.

The clearance of the TRL remnant is also dependent on the hepatic apolipoprotein-E receptor pathway. Because the distribution of the apolipoprotein-E phenotype was normal in our patients, the defective TRL metabolism may be caused by a direct interaction of PI with the LDL receptor-related protein, as suggested by Carr *et al.* [8].

Further metabolic investigations are required to explain the mechanisms of lipid disorders in this setting. To survey the occurrence of severe hypertriglyceridemia, we consider that fasting triglyceride and total cholesterol values should be frequently measured in HIV 1-infected patients on HAART.

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An argument for routine therapeutic drug monitoring of HIV-1 protease inhibitors during pregnancy

Inadequate HIV protease inhibitor (PI) exposure is associated with acquired drug resistance and virological failure of therapy [1]. Certain disease states, such as chronic liver disease [2], have been shown to alter the pharmacokinetics of PIs. As pregnancy is associated with increases in plasma volume, fat stores, total body mass, cardiac output, glomerular filtration and altered protein binding [3,4], it is a condition in which altered PI pharmacokinetics may occur. If circulating levels of PIs are significantly lower during pregnancy, the potential for virological breakthrough and the development of drug resistance exists, with important implications for the health of the mother and her child.

We describe a woman with HIV infection who developed virological breakthrough while receiving PI-

based antiretroviral therapy, and in whom pregnancy appeared to contribute to suboptimal circulating PI levels.

The patient was a 35-year-old woman originally from Zaire diagnosed with HIV infection in 1992. She initially received zidovudine in 1993, to which zalcitabine was added in 1996. In July 1997 her plasma HIV-RNA level (pVL) was 8564 copies/ml, her CD4 cell count was 243 cells/ μ l (10%), and her regimen was modified to indinavir 800 mg every 8 h, zidovudine 600 mg twice a day, and lamivudine 150 mg twice a day. In February 1999 her pVL was less than 500 copies/ml. Because she developed nephrolithiasis, indinavir was changed to nelfinavir 1250 mg twice a day in May 1999. At that time her pVL was less than 50

Table 1. Plasma pharmacokinetic parameters^a.

| Pregnancy status | 18 Oct 1999 32 weeks gestation ^b | 11 Nov 1999 36 weeks gestation | 10 March 2000 4 months post-partum | 20 March 2000 4 months post-partum | 18 May 2000 6 months post-partum ^c |
|-------------------------------|--|-----------------------------------|---------------------------------------|---------------------------------------|--|
| Nelfinavir dose | 1250 mg bid | 1000 mg tid | 1000 mg tid | 1000 mg tid | 1250 mg bid |
| C ₀ (µg/ml) | 0.14 | 1.02 ^d | 3.79 ^e | | 0.72 |
| C ₁₂ (µg/ml) | 0.47 | | | | 2.24 ^f |
| C _{max} (µg/ml) | 2.87 | 2.23 ^g | | 7.03 ^g | 5.08 |
| t _{max} (h) | 5.0 | | | | 3.0 |
| AUC ₀₋₁₂ (h•µg/ml) | 15.5 | | | | 38.1 |
| CL/F (ml/min) | 1347 | | | | 546 |
| V/F (l) | 315 | | | | 383 |
| t _{1/2} (h) | 2.7 | | | | 8.1 |

AUC₀₋₁₂, Area under the plasma concentration plotted against time curve over the 12 h dosing interval; bid, twice a day; C₀, observed predose plasma concentration; C₁₂, observed hour 12 concentration at the end of the dosing interval; C_{max}, highest observed plasma concentration; CL/F, apparent oral plasma clearance; tid, three times a day; t_{max}, time of highest observed plasma concentration; t_{1/2}, apparent clinically relevant terminal disposition half-life within one 12 h dosing interval; V/F, apparent terminal-phase volume of distribution.

^aFood intake during drug administration on pharmacokinetic days was controlled to minimize interday pharmacokinetic variability from food effects on bioavailability.

^bDose administered within 10 min of finishing a standard breakfast. Blood collection times: predose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8 and 12 h post-dose.

^cDose administered and blood collection times as in (a) except no 12 h sample was collected.

^dConcentration at 11 h after the previous dose.

^eConcentration at 12.5 h after the previous dose.

^fPredicted from half-life because the patient did not return for blood sampling at 12 h.

^gConcentration at 4 h post-dose.

copies/ml and her CD4 cell count was 384 cells/µl (24%). On 27 July 1999 at 20 weeks' gestation, her pVL was 131 copies/ml. Subsequent values were 62, 228 and 2720 copies/ml on 9 August, 12 October and 19 October, respectively. Because of the concern for altered nelfinavir pharmacokinetics during pregnancy resulting in virological breakthrough, a full pharmacokinetic profile was obtained on 18 October, when the patient was at 32 weeks of gestation. The pharmacokinetic parameters are summarized in Table 1. The plasma nelfinavir level 12 h after dosing (0.47 µg/ml) was below the estimated minimum in-vitro concentration to inhibit replication in 50% of wild-type isolates in 50% of human serum (IC₅₀ = 0.52 µg/ml [5]). The dose of nelfinavir was changed to 1000 mg three times a day and 2 weeks later her pre-dose and 4 h post-dose nelfinavir levels were 1.02 and 2.23 µg/ml, respectively. Despite increased nelfinavir concentrations, her pVL did not become suppressed, although it did decrease to 478 copies/ml on 11 November 1999. On 23 November 1999 a scheduled caesarian section was performed, during which time she received intravenous zidovudine and a single oral dose of nevirapine 200 mg. On pharmacokinetic analysis 4 months after delivery, the pre-dose and 4 h post-dose nelfinavir concentrations were 3.79 and 7.03 µg/ml, respectively. Given such high levels and the unknown potential for toxicity, her dose was adjusted back to 1250 mg twice a day. A full pharmacokinetic profile was obtained 2 weeks later (6 months post-partum). Genotyping of the protease and reverse transcriptase sequences from the virus isolated on 11 November 1999 demonstrated mutations associated with decreased susceptibility to nelfinavir (M36I), zidovudine (K70R, D67N) and lamivudine (M184V).

The oral clearance of nelfinavir increased by 2.5-fold and the volume of distribution decreased (18%) during pregnancy. The resulting 67% decrease in half-life and the larger decrease in total (area under the curve) compared with peak (C_{max}) plasma exposures suggest that pregnancy affected the systemic clearance more than bioavailability. The probable significance of the corresponding decrease in drug exposure is illustrated by virological breakthrough and the development of genotypic drug resistance. Because viral replication was not suppressed at the time of delivery, an elective caesarian section and a single dose of nevirapine were recommended for both the patient and her child.

The presence of pre-existing genotypic mutations when nelfinavir was started is possible, such that eventual virological breakthrough would have been likely. Regardless of this, initially adequate nelfinavir plasma levels would have resulted in a greater likelihood of sustained viral suppression. Biological and systematic variation may have contributed to changes in drug exposure, but the changes are probably too large for this to be the sole explanation. The apparent impact of pregnancy on nelfinavir pharmacokinetics that we observed probably also applies to the pharmacokinetics of other PI, although this remains to be established. For this reason, all pregnant women at our site who are taking a PI are requested to have therapeutic drug monitoring performed in the context of a clinical trial. Given the potential morbidity associated with inadequate circulating PI, an evaluation of the routine therapeutic drug monitoring of PI should be considered in all pregnant patients.

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Large hepatic mitochondrial DNA deletions associated with L-lactic acidosis and highly active antiretroviral therapy

Fatal L-lactic acidosis occurred in an AIDS patient during stavudine-containing highly active antiretroviral therapy. L-Lactic acidosis was attributed to mitochondrial dysfunction, and was associated with large deletions (49–83% of the mitochondrial genome) of most of the hepatic mitochondrial DNA, including deletions in genes for nicotinamide adenine dinucleotide (reduced form) dehydrogenase, cytochrome oxidase and adenosine triphosphatase.

This 64-year-old male AIDS patient was admitted with prominent extrapyramidal signs, unsteady gait and recurrent falls. HIV had been diagnosed 2 years previously after a severe episode of herpes zoster. Therapy with stavudine, lamivudine, and saquinavir had been commenced 12 months before the current presentation. Other medications included acyclovir, cotrimoxazole, clarithromycin, itraconazole for secondary cryptococcal disease suppression, sodium valproate, mexiletine, and sertraline. His neurological signs resolved with the cessation of sertraline.

Severe post-herpetic neuralgia worsened on day 14, and was treated with indomethacin 200 mg twice a day. Within 48 h of indomethacin administration the patient developed severe epigastric pain and vomiting. He became obtunded, hypotensive and developed Kussmaul respirations and signs of peritonism. He was acidotic (pH 7.29) and hyperkalaemic (potassium = 7.8 mmol/l). A necrotic pancreas was found at laparotomy.

Continuous venovenous haemodialysis against bicarbonate was required to control hyperkalaemia. The plasma L-lactate concentration increased to 28 mmol/l (normal < 2.5 mmol/l) despite these interventions. Multi-organ dysfunction was not present. The patient maintained elevated cardiac output, low systemic vas-

cular resistance with inappropriately low oxygen consumption, and a respiratory quotient of 1 (carbon dioxide : oxygen consumed). The patient died 72 h postoperatively when treatment was withdrawn. Plasma bicarbonate concentrations were low for at least 2 months before death (12–19 mmol/l, normal 23–32 mmol/l), compatible with subclinical lactic acidosis.

Immediate post-mortem specimens of blood, skeletal muscle and liver were obtained for mtDNA analysis. MtDNA was amplified by polymerase chain reaction [1]. Amplified products were analysed using restriction fragment length polymorphisms and selected sequencing. Depletion analysis was performed using a modification of Arnaudo *et al.* [2]. Specific DNA analysis for mutations associated with myoclonus epilepsy-ragged red fibres, mitochondrial encephalomyopathy-lactic acidosis-stroke-like episodes, and neurogenic muscle weakness, ataxia, retinitis pigmentosa were performed on amplified mtDNA using appropriate polymerase chain reaction primers [3].

There was no evidence of point mutations classically associated with myoclonus epilepsy-ragged red fibres, mitochondrial encephalomyopathy-lactic acidosis-stroke-like episodes, or neurogenic muscle weakness, ataxia, retinitis pigmentosa/Leigh's disease in blood, muscle, liver or pancreas. No evidence of mtDNA depletion was detected in any patient samples or in age-matched controls. No detectable mtDNA deletions were present in peripheral blood leukocytes. However, a small fraction of skeletal muscle mtDNA existed as a 3500 base pair (bp) form (79% deleted).

In contrast to blood and muscle mtDNA, most of the hepatic mtDNA (normal size 16 569 bp) existed as one of six distinct deleted forms. The sizes of mtDNA

forms present (percentage of mtDNA deleted in parenthesis) were: (i) 8500 bp (49%); (ii) 7600 bp (54%); (iii) 6900 bp (59%); (iv) 6100 bp (63%); (v) 3400 bp (80%); and (vi) 2900 bp (83%). Significant functional impairment of oxidative phosphorylation would be expected with such extensive hepatic mitochondrial damage because five of the six forms had deletions including nicotinamide adenine dinucleotide (reduced form) dehydrogenase, cytochrome oxidase and adenosine triphosphatase genes, based on restriction fragment length polymorphism analysis and selected sequencing (data not shown).

We hypothesize that our patient acquired hepatic mtDNA deletions, possibly on a background of pre-existing subclinical hepatic mitochondrial disease, during the course of antiretroviral therapy. The mutagenic effects of nucleoside analogues, such as stavudine, are thought to be mediated via the inhibition of DNA polymerase- γ , which is essential for mitochondrial replication [4]. Indomethacin, an uncoupler of mitochondrial oxidative phosphorylation, may have produced acute decompensation in the setting of marginally functional hepatic mitochondria [5].

Drug interaction between St John's wort and nevirapine

Cases have been reported that suggest that extracts from St John's wort (*Hypericum perforatum*), mainly used as an antidepressant [1], contain potent inducers of hepatic enzymes, raising the possibility of clinically relevant drug interactions.

Recent pharmacokinetic data show substantially decreased exposure to the antiretroviral drug indinavir by the concomitant use of St John's wort, probably because of the induction of the 3A4 isoform of the cytochrome P450 system. St John's wort reduced the area under the plasma concentration versus time curve of indinavir by 57% (SD 19%), and decreased the extrapolated 8 h trough concentration by 81% (SD 16%) in eight healthy volunteers [2]. This interaction has been recognized as being clinically relevant. An interaction study [3] with digoxin suggested induction of the expression of the drug-transporter P-glycoprotein. Compared with placebo, the co-administration of *Hypericum* extracts significantly decreased digoxin concentrations, an effect that increased with the number of days on *Hypericum* extracts [3]. In addition to such studies, several case reports [4] have shown decreased plasma concentrations or decreased activity of cyclosporin, oral contraceptives, theophylline and warfarin with the concomitant use of St John's wort.

As all of the currently marketed protease inhibitors and non-nucleoside reverse transcriptase inhibitors

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(NNRTI) are substrates for the multidrug transporter P-glycoprotein and/or are extensively metabolized via the cytochrome P450 enzyme system, the concomitant use of St John's wort may result in decreased exposure to these classes of antiretroviral drugs, with clinical consequences.

We present five patients (all men, aged 34-53 years) infected with HIV-1, who had been treated with the NNRTI nevirapine and two nucleoside analogue reverse transcriptase inhibitors for over a year, and who concomitantly used St John's wort for several months. At our clinic, nevirapine plasma concentrations from each patient are routinely measured every 3 months. The five patients had at least one nevirapine plasma concentration with and at least one without St John's wort as co-medication. We noticed that the nevirapine plasma concentrations of these patients were consistently lower on some occasions. To determine whether these lower exposures were caused by the use of St John's wort, a non-linear mixed-effect modelling analysis (NONMEM) was performed [5]. A database consisting of 1330 nevirapine plasma concentrations of 176 patients, collected during 789 visits, was available for this analysis. The pharmacokinetics of nevirapine were described with a one-compartment model with first-order absorption and elimination (Table 1). The median oral clearance was 3.2 l/h, which is in accordance with an earlier reported value [6]. Interindividual

Table 1. Median individual estimates of oral clearance of nevirapine in HIV-1-infected patients.

| | 171 Patients, 767 visits Without SJW | 5 Patients, 22 visits | |
|-----------------------------|---|-------------------------------|----------------------------|
| | | Without SJW (11 visits) | With SJW (11 visits) |
| Median oral clearance (l/h) | 3.2 | 3.3 | 4.3 |
| Interquartile range | 2.7–3.9 | 3.2–4.2 | 3.8–4.7 |

SJW, St John's wort.

and interoccasion (intervisit) variability were 29 and 22%, respectively. With the concomitant use of St John's wort included as a co-variate for oral clearance in the population pharmacokinetic model, the oral clearance of nevirapine was significantly increased with 35% (SE 15%) ($P = 0.02$) for patients using St John's wort, thus leading to a decreased exposure. None of the five patients in our population used any co-medication that could have resulted in an increased oral clearance. As nevirapine is extensively metabolized via the CYP450 system, the concomitant use of St John's wort can therefore explain the increase in oral clearance.

In conclusion, we have shown that St John's wort decreases the exposure to the NNRTI nevirapine by increasing its oral clearance. Because a low exposure to nevirapine may eventually result in antiretroviral resistance and treatment failure [7], the concomitant use of nevirapine and St John's wort should be avoided.

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Topoisomerase II inhibitor induced leukemia in a patient with AIDS

Secondary leukemia resulting from the use of topoisomerase II inhibitors was first described in the mid-1980s [1,2], and was reported to occur after adjuvant chemotherapy for breast cancer [3], combination chemotherapy for non-Hodgkin's lymphoma (NHL) [4], and germ cell tumors [5]. The increase in its incidence is a result of the widespread use of DNA damaging drugs, dose intensity, as well as the lengthening of patient survival in various diseases. This is the third described case of topoisomerase-induced secondary acute myelogenous leukemia (AML) complicating the treatment of an AIDS-related NHL. A brief response was achieved after induction therapy.

The patient was a 33-year-old homosexual man with

HIV who presented in November of 1997 with a left neck mass (measuring $4 \times 6 \times 9$ cm on computerized tomography examination). Tissue pathology revealed a diffuse large B cell lymphoma. Metastases were noted in the liver, pancreas, and spleen with high grade histology and translocation 8 : 14. The patient received cytoxan (187.5 mg/m^2 a day for 4 days), adriamycin (12.5 mg/m^2 a day for 4 days) and etoposide (60 mg/m^2 a day for 4 days) every 4–6 weeks for a total of eight cycles. He was deemed to be in clinical remission in April 1999; a computerized tomography examination at that time demonstrated persistent splenomegaly, but no lymphadenopathy.

The patient returned to the clinic in October 1999

with several months of left shoulder pain, headaches and photophobia, along with a 2 week history of easy bruising and 2 days of severe headaches. He had stopped his antiretroviral therapy for an unclear length of time. Peripheral blood leukocytes were increased to $154\,000/\text{mm}^3$ with a platelet count of $16\,000/\text{mm}^3$ and hemoglobin was 6.6 g/dl. Differential examination showed neutrophils 3%, lymphocytes 10%, monocytes 82%, and blasts 5%. The majority of cells were immature monocytes and promyelocytes, consistent with the diagnosis of AML, French–American–British classification M5. Cytogenic analysis showed a translocation 9:11, consistent with his predominantly monocytic leukemia. Flow cytometry revealed myelomonocytic markers: CD11c, CD13, CD14, and CD33. No T cell or B cell markers were noted.

He received induction chemotherapy with idarubicin $12\text{ mg}/\text{m}^2$ a day for 3 days and cytosine arabinoside $150\text{ mg}/\text{m}^2$ a day for 7 days. His cerebrospinal fluid cytology was positive and he also received intrathecal methotrexate. A bone marrow analysis on day 14 of therapy revealed hypocellular marrow with no evidence of leukemia. Renal failure, herpes labialis, and respiratory aspergillus colonization complicated his hospital course. The patient had disease progression and died 2 months after his diagnosis of AML, with sepsis and *Candida glabrata* fungemia.

Therapy-related AML accounts for 10–20% of AML cases [6]. Chemotherapy-induced AML was first described in long-term survivors of Hodgkin's disease approximately 30 years ago [7]. AML related to previous treatment with topoisomerase II inhibitors was first described in the early 1980s [1,2]. It often has a characteristic translocation involving chromosome band 11q23 [8–10]. In contrast to alkylating agent-associated secondary AML, it has a shorter latency period (24–30 months), and an acute rather than gradual presentation with a high blast count, as well as a monocytic phenotype (French–American–British) M4 or M5 [8,11]. Cases of topoisomerase-induced AML were reported in patients receiving adjuvant chemotherapy for breast cancer [3], after treatment of NHL [4], as well as germ cell tumors [5]. This led to monitoring plans established by the National Cancer Institute, which attempted to quantitate the risk of secondary AML in patients receiving topoisomerase II inhibitors [12].

More than 40 million people are currently infected with HIV, with an increased incidence seen in several countries [13]. The development and use of highly active antiretroviral therapy has been associated with increased survival [13], opening the door for the development of more malignancies, including Hodgkin's disease and anal carcinoma. With the incidence of treatable AIDS-related malignancies on the increase,

the incidence of secondary leukemia in this population is bound to increase.

There have been so far two reported cases of HIV-infected patients who first suffered from NHL and subsequently developed a topoisomerase II-induced AML [14]. Our patient, unlike the previously reported cases, had the typical 9:11 translocation and had induction chemotherapy with both cytarabine and an anthracycline, resulting in a short response. A common feature among the three cases is the short survival (3 weeks to 3 months), raising the question of whether secondary leukemia in this setting is best treated with regimens used ordinarily in non-HIV patients.

The acute leukemia in all three patients had typical features of a topoisomerase II-induced AML, and was an unfortunate indirect consequence of the HIV infection. It seemed to respond well (at least in two patients) to chemotherapy. No clear distinguishing feature from a non-AIDS-related topoisomerase II-induced AML was noted. The very short complete remission could be suggestive of a more aggressive disease in HIV-positive patients. As more cases are described, the natural history of these diseases will be better elucidated in patients with AIDS.

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Factors associated with the response to antiretroviral therapy among HIV-infected patients with and without a history of injection drug use

Studies have found that HIV-positive injection drug users appear to have a lower uptake of highly active antiretroviral therapy (HAART) compared with other HIV-positive individuals in Canada, the United States and Europe [1–4]. In July of 1997 the Therapeutic Guidelines of the HIV/AIDS Drug Treatment Program in British Columbia were revised to recommend HAART for all antiretroviral-naïve individuals with plasma viral loads greater than 5000 copies/ml or CD4 cell counts less than 500 cells/mm³. We examined the impact of the resultant enhanced access to HAART on favourable virological response, particularly among participants with a history of injection drug use compared with non-drug users enrolled in this province-wide population-based programme.

We categorized participants into two cohorts based on the update of the therapeutic guidelines in July 1997. Cohort 1 consisted of individuals who were first prescribed antiretroviral therapy from 1 August 1996 to 31 July 1997. During this time, HAART was only available to those with plasma viral loads greater than 100 000 copies/ml and CD4 cell counts less than

500 cells/mm³. Cohort 2 were individuals who met the updated guideline criteria and were eligible for HAART from 1 August 1997 to 31 October 1998.

In this analysis, a history of injection drug use was obtained by self-report or by the physician. Programme enrollment data were used to assign a level of physician HIV-related experience for each subject, estimated via the cumulative number of patients receiving antiretroviral therapy within their practice, by the date of the subject's first known eligibility [1]. Adherence to antiretroviral therapy was defined as the ratio of the number of months patients received antiretroviral therapy refills relative to the total number of months they were actually prescribed [1]. Cox proportional hazard models were used to estimate the hazard of decreasing plasma viral load below 500 copies/ml at least twice consecutively after the start of antiretroviral therapy. The following prognostic variables were placed in the stepwise procedure to select for those meeting the $P < 0.05$ level for entry into the multivariate model: age, sex, previous AIDS-defining illness, cohort, injection drug use, baseline plasma viral load,

Table 1. Relative hazards from univariate and multivariate Cox regression analyses of factors associated with plasma viral load suppression below 500 copies/ml on at least two consecutive measurements.

| Baseline variable | Cohort 1 ^a | | Cohort 2 ^b | |
|--|-----------------------|----------------------|-----------------------|----------------------|
| | Unadjusted RH | Adjusted RH (95% CI) | Unadjusted RH | Adjusted RH (95% CI) |
| Injection drug use (Yes versus No) | 0.59 | 0.76 (0.59–0.98) | 0.75 | 0.97 (0.71–1.31) |
| Baseline plasma viral load (Log ₁₀ copies/ml) | 0.96 | 0.64 (0.53–0.76) | 0.88 | 0.63 (0.52–0.76) |
| Physician experience (Per 100 patients followed) | 1.34 | 1.09 (0.97–1.22) | 1.37 | 1.29 (1.13–1.49) |
| Antiretroviral therapy (Triple versus double) | 1.99 | 2.42 (1.95–3.00) | 1.58 | 2.50 (1.65–3.79) |
| Adherence (Per 10% increase) | 1.43 | 1.41 (1.33–1.50) | 1.72 | 1.73 (1.57–1.91) |

Multivariate models are adjusted for sex, age, CD4 cell count and AIDS diagnoses (Yes or No) at baseline.

^aBased on 808 participants with non-missing data for all variables.

^bBased on 564 participants with non-missing data for all variables.

CD4 cell count, adherence, physician HIV-related experience and the use of HAART.

There were 826 participants in cohort 1 and 580 participants in cohort 2 who were first prescribed antiretroviral therapy between 1 August 1996 and 31 October 1998. No difference was observed between the two cohorts with respect to age, injection drug use history, CD4 cell count, AIDS diagnosis, adherence or physician experience ($P > 0.05$). Cohort 2 had a lower median plasma viral load (78 500 versus 97 500, $P = 0.03$), and in keeping with the contemporary guidelines, were more likely to be receiving HAART (89.1 versus 44.7%, $P = 0.001$) than cohort 1. As shown in Table 1, after adjusting for sex, CD4 cell count, AIDS diagnosis, baseline viral load, physician HIV-related experience, adherence, and the use of HAART in cohort 1, we found a history of injection drug use to be independently associated with not having achieved viral suppression below 500 copies/ml compared with non-injection drug users [relative hazard (RH) = 0.76; 95% confidence interval (CI) 0.59–0.98]. In cohort 2, after adjusting for the same factors, injection drug use was not significantly associated with the time to achieving plasma viral load suppression (RH = 0.97; 95% CI 0.71–1.31). Furthermore, the receipt of HAART among participants with a history of injection drug use in cohort 2 was 89.2% (116/130), whereas in cohort 1 it was only 39.6% (84/212).

We concluded that enhanced access to HAART via updated therapeutic guidelines promoted the use of

HAART, even among participants with a history of injection drug use, and that they are as likely to suppress their viral load as non-injection drug users.

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Dynamics of seminal plasma HIV-1 decline after antiretroviral treatment

High levels of extracellular and cellular HIV-1 can be detected in the semen of infected men, and are implicated in the sexual transmission of this virus. Cross-sectional studies have demonstrated the presence of semen virus at all stages of infection, although the level of extracellular viral RNA is generally lower (approximately 1 log₁₀) than blood plasma levels [1,2]. The semen viral load, like plasma virus, is reduced by highly active antiretroviral therapy (HAART). Antiviral therapy appears to be the most significant determinant of the presence of detectable extracellular virus in the male genital tract [1,3–5]. This is likely to be the key mechanism by which HAART reduces HIV-1 transmission, although proviral DNA may continue to be detectable in both the semen and blood during HAART [6,7], despite cell-free virus being below the level of detection.

Analysis of the dynamics of viral suppression after the initiation of antiviral therapy has yielded important quantitative measures of the parameters determining

viral production in the blood compartment. However, there are no data available on the dynamics of HIV-1 in the male genital tract. This is partly due to the difficulties in obtaining multiple sequential samples from appropriate volunteers. In order to explore the potential of this approach, we undertook frequent sampling of semen from two HIV-1-infected patients initiating HAART, both of whom had high levels of seminal plasma viral RNA at baseline, in order to determine the short-term kinetics of virus suppression in semen compared with blood. One patient had taken a 16 month drug holiday after the failure of previous therapy with multiple resistance-associated mutations, also allowing an assessment of the efficacy of salvage therapy on semen virus in this context.

HIV-1-infected patients attending Birmingham Heartlands Hospital Department of Sexual Medicine were assessed for seminal plasma viral load in parallel with routine blood viral load monitoring. Two patients were identified with high viral loads in both compartments,

in which the initiation of antiretroviral therapy was indicated. Patient 1 had previously received multiple antiviral therapies, but had stopped all medication 16 months previously. The therapy chosen for re-initiation was guided by the genotypic drug resistance results available from the time of previous antiretroviral failure. He started on didanosine (400 mg a day), lamivudine (150 mg twice a day), adefovir (60 mg a day), efavirenz (600 mg a day) and hydroxyurea (500 mg twice a day). Patient 2 was antiretroviral drug naive (less than 7 days therapy previously) at entry into the study, and was initiated on zidovudine (300 mg twice a day), lamivudine (150 mg twice a day) and nevirapine (200 mg a day – increasing after 2 weeks).

Blood and semen samples were obtained just before starting therapy and then at 2–3 day intervals for the first 14 days. Approximately 1–2 weekly samples were obtained thereafter for patient 1, whereas patient 2 did not provide any further samples. HIV-1-RNA quantitation was undertaken by nucleic acid sequence-based amplification, previously shown to be most appropriate for assessing viral load in semen samples [8]. Taking into account the volume of sample required in the assay, the limit of detection was 400 copies/ml for blood plasma and 800 copies/ml for seminal plasma. The patients enrolled in this study provided written, informed consent, and the study protocol was approved by the Ethics Committee of Birmingham Heartlands Hospital.

A simple biphasic exponential decay model was fitted to the plasma and semen viral load data, of the following form:

$$v = 10^{A-at} + 10^{B-bt}$$

Therefore A and B represent the approximate \log_{10} viral load from which the first and second phases of the decay begin (the starting conditions), whereas a and b are the slopes of these decay phases (in units of \log_{10} virions/ml per day). These slopes can be interpreted as reflecting the average half-lives of the actively infected and persistently/latently infected CD4 cell reservoirs, which can be calculated from the slope parameters thus: $t_{1/2} = \ln 2 / (a \ln 10)$. A non-mechanistic two-phase model was used because the limited volume of data available makes robust parameter estimation of more complex dynamical models problematical [9].

Maximum likelihood methods were used to estimate parameters, assuming viral load measurements are log-normally distributed with a $\pm 0.5 \log_{10}$ measurement error.

The most parsimonious biphasic decay model describing all the data for both patients is the five parameter form given below:

$$v_{plasma1} = 10^{A-at} + 10^{B-bt}$$

$$v_{semen1} = 10^{A-at} + 10^{B-bt}$$

$$v_{plasma2} = 10^{A-at} + 10^{B-bt}$$

$$v_{semen2} = 10^{-C}(10^{A-at} + 10^{B-bt})$$

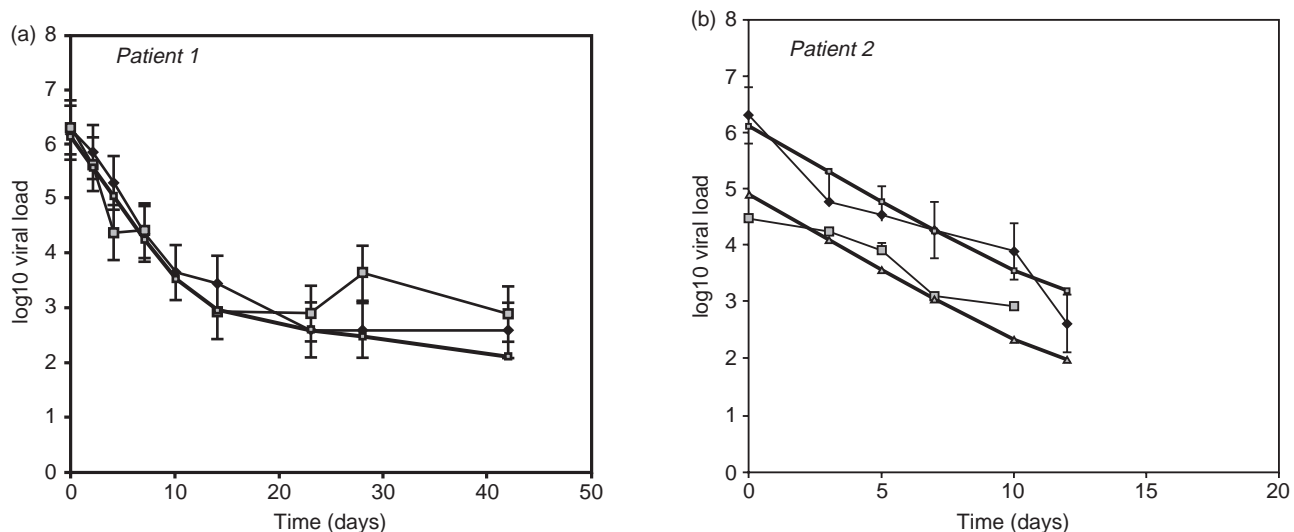


Fig. 1. Blood plasma and seminal plasma viral load reductions after the initiation of antiretroviral therapy for patients 1 (a) and 2 (b). Viral load at each time point is given as copies/ml with a variation of $0.5 \log_{10}$. The model described in the methods was also applied to these data. (a) —◆— Plasma; —□— semen; —□— model. (b) —◆— Plasma; —□— semen; —□— plasma model; —△— semen model.

The parameter C represents the mean number of logs semen viral load is below plasma viral load in patient 2.

Viral decay in patient 1 was distinctly biphasic in both blood and semen (Fig. 1a), whereas the shorter period of sampling for patient 2 only allowed the demonstration of the first phase of decay (Fig. 1b). No significant differences between viral decay in blood and blood and semen for either patient were observed, and a first phase decay of $0.27 \log_{10}/\text{ml}$ per day and second phase decay (only for patient 1) of $0.027 \log_{10}/\text{ml}$ per day were determined. These parameters translate into virus half-lives of 1.1 and 12.1 days for first and second phase decay, respectively. The fit of this model to the four viral load time series is illustrated in Fig. 1, which shows the parallel decay profiles of plasma and semen viral load in both patients.

We have demonstrated the feasibility of the frequent sampling of semen in order to investigate the dynamics of HIV replication in the male genital tract. We show potent inhibition of extracellular viral shedding in the semen in both a drug-naïve patient, and an antiretroviral-experienced patient. This further illustrates the potential of HAART to reduce the sexual transmission of HIV infection. However, this observation must be tempered by the possibility that cellular provirus represents the major source of transmitted virus. The estimates of first and second phases of virus decline in blood plasma and semen plasma suggest that similar dynamics of virus replication are at play within these two compartments, at least for the two patients investigated. Future studies on viral kinetics in the genital tract should assess the impact of different antiretroviral regimens, and monitor changes in cellular proviral DNA and replication-competent virus over a longer period of time.

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On the recent sharp increase in HIV detections in Cuba

Ever since the emergence of the global HIV pandemic, contact tracing has been debated as a control measure for HIV/AIDS. In Cuba, one of the measures of the National Programme for HIV/AIDS is the 'Partner Notification Programme', which traces the sexual contacts of known HIV-seropositive individuals [1,2]. The programme, which began in 1986, is carried out by epidemiology departments at all levels of the Cuban health system through partner notification and interviews of sexual contacts, who are tested for HIV every 3 months for a period of one year after the last sexual contact with the HIV-positive individual, and are observed as long they remain in contact. Recent

growth in tourism in Cuba has led to a re-emergence of prostitutes in recent years [3]. Perhaps not coincidentally, recent data have shown an increase in the number of HIV-positive contacts detected since 1996.

Table 1 shows the accumulated number of known HIV-positive individuals living in Cuba as well as the seroprevalence data from 1991 to 1999, with the number of contacts tested and the number of HIV-positive individuals detected for each calendar year. Any individual tested negative at the end of one calendar year (and counted as such in that year) will be tested again in the next calendar year, as long as it is

Table 1. Number of Cubans known to be living with HIV, Partner Notification Programme seroprevalence data, and the estimated number of HIV-infected Cubans by sexual contact during 1991–1999.

| Year | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| No. known HIV ⁺ ^a | 600 | 699 | 717 | 736 | 741 | 871 | 1099 | 1306 | 1507 |
| No. tested ^b | 905 | 1104 | 1121 | 811 | 773 | 1204 | 2037 | 2172 | 2241 |
| No. tested HIV+ | 81 | 76 | 44 | 44 | 45 | 80 | 113 | 117 | 113 |
| (% tested HIV+) | (8.95) | (6.88) | (3.93) | (5.43) | (5.82) | (6.64) | (5.55) | (5.39) | (5.04) |
| Median estimate ^c | | | | | | | | | |
| No. HIV+ | 685 | 787 | 897 | 1020 | 1182 | 1398 | 1647 | 1854 | 1998 |
| No. unknown HIV+ | 145 | 158 | 252 | 358 | 515 | 614 | 658 | 679 | 642 |

^aNumber of living HIV-positive individuals known to the Cuban health authority.

^bNumber of contacts tested in the Partner Notification Programme.

^cMedian estimates of number of HIV-positive Cubans by sexual contacts using the generalized removal model.

within one year of his or her last known contact. As is the case with all data of this nature, there are obvious limitations with this contact-tracing data. For example, there might be sexual contacts by the traced individuals during the one year period of testing after the last known contact, possibly with unknown HIV-positive individuals, leading to uncertainty in the data.

The number of known HIV-positive individuals includes all modes of transmission, of which approximately 90% are infected sexually [4]. Therefore we will assume that the number of known HIV-positive individuals by sexual contact is approximately 90% of the number of known HIV-positive individuals in Table 1. Also note the sharp increase starting in 1996 in all three sets of numbers. The increase is caused by the large number of homosexual and bisexual HIV-positive individuals detected from 1996 on. These individuals tend to be more sexually active and have a larger number of sexual contacts. Subsequently more of their contacts have been traced and tested positive.

To see whether the sharp increase since 1996 indicates an actual increase in the HIV-positive population, we estimated the number of HIV-infected individuals infected through sexual contact using the HIV seroprevalence data of the Partner Notification Programme as a seroprevalence result from a random sampling of the sexually active population in Cuba. Recently, a ‘generalized removal model for open populations’ was proposed by Hsieh *et al.* [5] using an empirical Bayes approach to estimate the number of HIV-infected individuals in a hidden, hard-to-count population directly from seroprevalence data without any knowledge of the population size. We used this method to estimate the total (both known and unknown) number of HIV-positive individuals among the sexually active population in Cuba. Differing from the procedure used in Hsieh *et al.* [5], there is additional information in this Cuban dataset, in which the number of known HIV-positive individuals at each sampling time is avail-

able (see Table 1). We employed this additional knowledge in our priors to improve our estimates.

Recently, de Arazoza *et al.* [2] gave a mean estimate of the HIV incubation time of the HIV-positive individuals in Cuba as 9.2 years with a standard deviation of 0.273, and a median of 8.42 years with 95% confidence intervals (7.5, 8.83). For our work, we chose the yearly survival rate ϕ for HIV-infected individuals (i.e. the mean probability that an infected individual will not develop AIDS during the 12 months between samples) to be 92%, which would result in an incubation time of approximately 8.3 years. Table 1 lists the median estimates for the number of HIV-positive individuals by sexual contact obtained. We also give the estimated number of HIV-positive individuals unknown to the health authority, which is obtained by subtracting 90% of the known HIV-positive individuals from our estimated number of sexually infected HIV-positive individuals.

Our results show that, in agreement with the seroprevalence data, there is an increase in the number of HIV-positive individuals in Cuba from 1996 to the present. However, the increase is consistent with the previous increase in the first half of the decade. Among the many possible underlying causes of the sharp increase in the number of known HIV-positive individuals is the fact that many more homosexual/bisexual contacts have been traced, and subsequently more HIV-positive individuals have been detected from 1996 on. As this trend continues, the number of unknown HIV-positive individuals actually start to decrease, which is also indicated in our estimates for the unknown HIV-positive individuals.

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