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Is long postpartum sexual abstinence a risk factor for HIV?

Prolonged postpartum sexual abstinence may increase the risk of HIV through an associated increase in male extramarital sexual contacts, but this has never been demonstrated. As part of a study of antenatal clinic HIV surveillance, we collected information on HIV status and duration of postpartum abstinence in women in three African cities. In Yaoundé, Cameroon, prolonged abstinence was common and was associated with a higher prevalence of HIV. In Kisumu, Kenya, and Ndola, Zambia, the practice was much less common and no such association was found.

Prolonged postpartum sexual abstinence could be a risk factor for HIV in women as it encourages husbands to seek extramarital partners. On the other hand it could be protective because it reduces the number of episodes of sexual intercourse for the wife, and may therefore reduce the risk of transmission if the husband is HIV positive [1]. An increase in extramarital sexual contacts associated with postpartum sexual abstinence has been reported among men in Benin [1] and Nigeria [2], but no studies have shown associations between postpartum sexual abstinence and HIV status. As part of a multisite study on antenatal clinic HIV surveillance, we collected information on the duration of postpartum abstinence since the last birth, as one of several factors that may affect the birth interval.

The study was conducted in six antenatal clinics in Yaoundé, Cameroon, two in Kisumu, Kenya, and five in Ndola, Zambia, from October to December 1998 [3]. In each city procedures followed routine sentinel surveillance procedures but used a longer questionnaire. HIV status was assessed on anonymized blood specimens remaining from syphilis testing at the first antenatal clinic visit. Initial screening used an enzyme-linked immunosorbent assay, with confirmation of positive results using a rapid test. Ethical permission for the study was received from appropriate authorities in each country, the London School of Hygiene and Tropical Medicine, UK, and the Institute of Tropical Medicine, Antwerp, Belgium.

The prevalence of HIV among antenatal women was 5.5% (85/1532) in Yaoundé, 30.6% (453/1480) in Kisumu, and 27.3% (279/1021) in Ndola. Half the women in Yaoundé reported postpartum abstinence after the last birth of more than 6 months, and 23% more than 12 months, compared with 17 and 8% in Kisumu, and 13 and 4% in Ndola, respectively. In all

Table 1. HIV status by duration of postpartum abstinence after the last birth among women attending antenatal clinics in Yaoundé, Kisumu and Ndola.

Duration (months)	All			Currently married			Married to current husband at time of last birth		
	HIV+/N	%	*P* *	HIV+/N	%	*P* *	HIV+/N	%	*P* *
Yaoundé									
< 3	4/160	2.5		4/146	2.7		3/126	2.4	
3–6	13/301	4.3		7/276	2.5		5/234	2.1	
7–12	13/249	5.2		10/224	4.5		8/181	4.4	
> 12	21/207	10.1		16/169	9.5		7/111	6.3	
Total	51/917	5.6	0.001	37/815	4.5	0.001	23/652	3.7	0.05
Kisumu									
< 3	173/495	35.0		163/473	34.5		136/405	33.6	
3–6	80/268	29.9		67/244	27.5		50/195	25.6	
7–12	35/89	39.3		30/76	39.5		6/34	17.7	
> 12	27/68	39.7		21/52	40.4		3/9	33.3	
Total	315/920	34.2	0.5	281/854	33.3	0.6	195/643	30.3	0.03
Ndola									
< 3	55/215	25.6		53/211	25.1		44/188	23.4	
3–6	120/404	29.7		115/391	29.4		90/341	26.4	
7–12	20/64	31.3		18/58	31.0		13/47	27.7	
> 12	13/32	40.6		9/27	33.3		1/10	10.0	
Total	208/715	29.1	0.07	195/687	28.4	0.2	148/586	25.3	0.8

* *P* from χ^2 test for trend.

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sites, abstinence was shorter in currently married women than in never married or divorced or widowed women, but was similar for those in monogamous and polygamous marriages.

In Yaoundé, HIV seropositivity was strongly associated with the duration of postpartum abstinence overall and after restricting to currently married women, but the association was of borderline significance after additionally restricting to those who were married to their current husband at the time of the last birth (Table 1). The trend persisted after adjusting for age, monogamy/polygamy, the number of previous births, schooling, occupation, ethnic group, religion, clinic, and the duration of postpartum amenorrhoea, but remained of borderline statistical significance. Of these factors, only postpartum amenorrhoea and ethnic group were associated with the duration of postpartum abstinence: abstinence was longer in those with prolonged postpartum amenorrhoea and among the Pahouin ethnic groups.

In Kisumu, there was no evidence of an association between the duration of postpartum abstinence and HIV status overall or after restricting to married women. Among those who were married to their current husband at the time of the last birth there was a trend towards decreased risk with longer abstinence. The highest prevalence of HIV infection (15/38, 39.5%) was in those who had resumed sexual intercourse within 2 weeks, but this was not significantly different from the prevalence in those resuming later (180/605, 29.8%). The trend was no longer significant after adjusting for ethnic group. In Ndola, there was a slight increase in the proportion of HIV positivity with an increased duration of abstinence overall, but this was lost after restricting to married women.

Prolonged postpartum abstinence in west Africa is well recognized [4]. It was frequently reported by the antenatal clinic attenders in Yaoundé, and the proportion with prolonged abstinence in the population will be even higher, because women with prolonged abstinence are under-represented in antenatal clinics. The duration was similar in women of different ages, suggesting little change in practice over time.

Postpartum abstinence was much shorter in Kisumu and Ndola than in Yaoundé. In both Kisumu and Ndola, there were very few women with prolonged postpartum abstinence among those who were married to their current husband at the time of the last birth. This suggests that the major reason for prolonged abstinence was partner change. In Kisumu, 78% of the women were Luo, and traditionally the Luo practise ritual sex within a few days of birth [4]. This was not asked about specifically in this study, and the women may or may not have counted this as ending postpartum abstinence. Sexual intercourse soon after birth may be a risk factor for HIV transmission [5], and was associated with the highest prevalence of HIV in Kisumu. Very few women in the other cities resumed sexual intercourse before 2 weeks. There was no evidence that prolonged postpartum abstinence was associated with HIV status in Kisumu or Ndola, but given the small numbers of women with prolonged abstinence in these cities, the study had limited power to detect such trends.

In Yaoundé, a strong association between prolonged female postpartum abstinence and HIV status overall was apparently partly caused by the inclusion of women who were not married to their current husband at the time of the previous birth, who might have had longer abstinence (and a higher risk of acquiring HIV) as a result of partner change. Among those with continuing marriages, the trend of increased HIV risk associated with prolonged abstinence persisted, but was of borderline statistical significance. In this cross-sectional study the timing of the acquisition of HIV is unknown. The inclusion of women who were already HIV positive before the previous birth is likely to have led to the underestimation of any association with postpartum abstinence. We only asked about abstinence after the most recent birth. However, culturally determined prolonged abstinence is likely to be similar after each birth, and restricting the analysis to those with only one previous birth gave similar trends.

The results should be interpreted cautiously. The association between prolonged abstinence and HIV in Yaoundé may be a chance finding. The study was not designed to look at this issue, and the association was noticed in the course of other analyses. However, the possibility of such an association had been suggested previously, and this is the first study to demonstrate it directly.

Judith R. Glynn[a], Anne Buvé[b], Michel Caraël[c], Isaac B. Macauley[d], Maina Kahindo[e], Rosemary M. Musonda[f] and Léopold Zekeng[d], *[a]London School of Hygiene and Tropical Medicine, London, UK; [b]Institute of Tropical Medicine, Antwerp, Belgium; [c]UNAIDS, Switzerland; [d]Ministry of Public Health, Cameroon; [e]National AIDS/STD Control Programme, Kenya; and [f]Tropical Diseases Research Centre, Zambia.*

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Sperm mitochondrial DNA deletions as a consequence of long term highly active antiretroviral therapy

Highly active antiretroviral therapy (HAART) has dramatically improved the prognosis for those infected with HIV-1 [1]. However, with the use of long-term HAART, a variety of side-effects may occur, for example lactic acidosis [2], peripheral neuropathy [3], and probably lipo-atrophy [4]. Nucleoside anti-retroviral agents are thought to generate either depleted [5] or mutated mitochondrial DNA either by inhibition of mitochondrial-specific polymerase γ [6] or through the increased generation of free radicals [7]. Different combinations of drug therapy are thought to have varying quantitative toxic effects on these two causes of mtDNA damage [8]. However, research has been limited by the lack of easily obtainable tissue as a result of the invasive nature of the clinical procedure. Importantly, however, human spermatozoa are an easily obtainable clinical material that is susceptible to the generation of multiple mtDNA deletions [9,10]. In order to determine whether spermatozoa could provide clinically relevant data as to the effects of HAART on individual patients, we used Long polymerase chain reaction (PCR) [11] on a series of sperm samples to detect the presence of multiple mtDNA deletions.

We analysed sperm samples produced by masturbation from 10 men attending our clinic. Four of the men had never taken antiretroviral therapy whereas the others ($n = 6$) took a combination of HAART. For one of the men, we analysed sperm samples at three different times points over 14 months (see Table 1). From each of the sperm samples, total DNA was isolated using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). Deletions were detected using Long PCR to amplify 10.6 kb of the mitochondrial genome with primers specific to the region of the genome most prone to mtDNA deletion (D6: 5′–TCT AGA GCC CAC TGT AAA G–3′ and R10: 5′–AGT GCA TAC CGC CAA AAG A–3′) [9]. The resulting PCR products were resolved on 0.8% agarose gels. The results are presented in Table 1.

Patient 1 was initially treated with lamivudine/delavirdine/indinavir, which was then quickly modified to stavudine, didanosine, nevirapine didanosine/stavudine/nevirapine/hydroxyurea. After 24 weeks, treatment was again changed to zidovudine/lamivudine/nevirapine because of side-effects. Before treatment, no mtDNA deletions were detected. However, at 6 months, this patient developed multiple mtDNA deletions, which persisted and became more clearly evident

Table 1. Correlation of multiple sperm mtDNA deletions with clinical status of patients.

Patient	Duration of treatment (months)	Current HAART	mtDNA deletions	Lipodystrophy
1	Nil	No	No	No
1	6	d4T, ddI, nevirapine, hydroxyurea	Yes	No
1	14	ZDV, 3TC, nevirapine	Yes	No
2	Nil	No	No	No
3	Nil	No	No	No
4	Nil	No	No	No
5	Nil	No	Yes	No
6	9	ZDV, 3TC, efavirenz	No	No
7	24	d4T, ddI, abacavir	Yes	Yes
8	24	3TC, ddI, nevirapine	Yes	Yes
9	32	d4T, 3TC, delaviridine	Yes	Yes
10	6	d4T, 3TC, nevirapine	No	No

d4T, Stavudine; ddI, didanosine; 3TC, lamivudine; ZDV, zidovudine.
The duration of treatment indicates the total time in months of taking highly active antiretroviral therapy (HAART). The presence of lipodystrophy was based on patient/physician reporting of unequivocal changes in fat distribution.

at 14 months of treatment. Of the four men who had never taken antiretroviral agents when the samples were analysed, only one (patient 5) of these had observable multiple mtDNA deletions. The two patients on HAART for less than 12 months harboured no mtDNA deletions. However, three men on HAART for more than 12 months possessed multiple mtDNA deletions. Interestingly, these three patients also presented with clinically evident lipodystrophy [4].

The proportion of patients with mtDNA deletions was significantly greater in those patients who had taken HAART for more than 12 months compared with those who had taken HAART for less than 12 months (two-tailed Fisher's exact test $P < 0.05$). Those patients who harboured mtDNA deletions had taken either stavudine [12] or didanosine, but only one patient had taken only zidovudine/stavudine. Furthermore, none of these patients had taken protease inhibitors.

We have demonstrated the propagation of multiple mtDNA deletions as a result of long-term HAART in sperm samples. These deletions may arise through damage to the mitochondrial genome of their precursor spermatogonial cells, resulting in the amplification of these deleted molecules [13]. De novo multiple mtDNA deletions can only occur in cells that frequently replicate mtDNA. Spermatozoa are produced approximately 60 days before ejaculation, and it is common to detect both normal and abnormal spermatozoa in semen samples [14]. It is evident from various studies that abnormal cells will undergo apoptosis [15] and subsequent phagocytosis [16]. However, abnormal spermatozoa may be the consequence of an abortive mitochondrial-mediated apoptotic event [17] at the stem cell level accounting for the presence of both the abnormal and normal populations of mature sperm cells in samples analysed. The results of this study strongly suggest that an analysis of sperm mtDNA should be included in further studies of HAART-mediated mitochondrial disease.

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David J. White[a], Dushyant Mital[a], Steven Taylor[a] and Justin C. St John[b], *[a]Department of Infection, Birmingham Heartlands Hospital, Birmingham, UK; [b]Reproductive Biology and Genetics Group, Department of Medicine, University of Birmingham, Birmingham, UK.*

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Informed consent was obtained from all patients enrolled in this study and the ethical guidelines of Birmingham Heartlands Hospital were followed in the conduct of this clinical research.

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First CRF01_AE/B recombinant of HIV-1 is found in Thailand

In Thailand, the HIV-1 epidemic started abruptly in 1988 with the introduction of subtype B and subtype E, now called the circulating recombinant form (CRF), CRF01_AE. These two strains appeared independently in distinct high-risk populations [1]: subtype B among injecting drug users (IDU) and CRF01_AE among those who were heterosexually exposed [2,3]. HIV-1 subtype B is still common among infected Bangkok IDU, but CRF01_AE was found in 80% of IDU surveyed in 1995–1998 [4]. Dual infection with HIV-1 subtype B and CRF01_AE was observed by 1994 [5], providing the opportunity for recombination between these two subtypes in the Thailand epidemic. Whereas recombination between CRF01_AE and subtype B has occurred in an experimental dual infection of a chimpanzee [6], such a recombinant in humans has not yet been described. Here, we identify an AE/B intersubtype recombinant of HIV-1 found in a multiply exposed individual in Thailand. The full-length genome of this recombinant has been analysed and characterized.

In 1997, screening assays for the subtype of the virus of a 40-year-old Thai man (NP1623) provided evidence

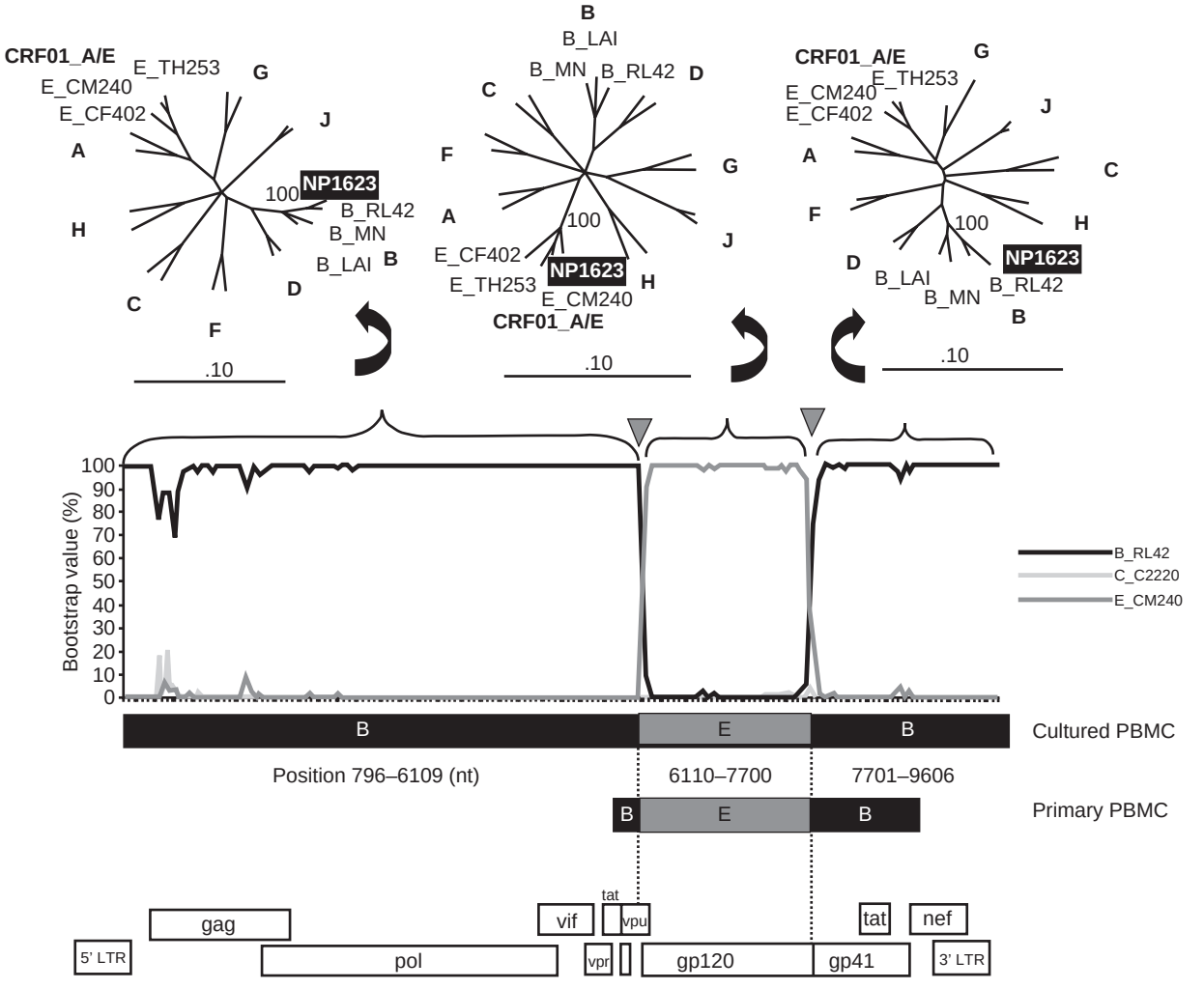

Fig. 1. Recombinant analysis of full-length genome.
Bootscan analysis of the full-length genome of NP1623 isolate using CRF01_AE (CM240), subtype B′ (RL42) as parental subtypes and subtype C (ETH2220) as the outgroup. The arrowheads mark the recombination breakpoints and the numbers refer to the location of the breakpoints corresponding to nt on HXB2 [11]. The upper panel shows the phylogenetic trees of sub-regions, which were constructed by neighbor joining. Parsimony bootstrap values are indicated at the nodes. The panel at the bottom shows the deduced structure of NP1623, both from culture and from primary peripheral blood mononuclear cells (PBMC), with respect to the HIV-1 genome structure. The full-length sequence from virus culture and the envelope sequence from primary PBMC of NP1623 are available under GenBank accession nos. AF362994 and 362995, respectively.

of subtype discordance in different parts of the genome. A V3 loop peptide enzyme immunoassay [7] classified the serum of NP1623 as CRF01_AE and an envelope heteroduplex mobility assay [8] confirmed that designation. A restriction fragment length polymorphism analysis from the gag leader region [9], however, indicated that NP1623 was infected with subtype B, as did a differential polymerase chain reaction (PCR) assay in gp41 [10]. These results suggested discordance between the subtype of gp120 and the subtype of the rest of the virus.

Peripheral blood mononuclear cells (PBMC) were separated by Ficoll gradient and co-cultivated with phytohemagglutinin-stimulated donor PBMC. Full genomes of HIV-1 were amplified from the cultured PBMC DNA by nested PCR, with endpoint dilution of the DNA template in the first round. The DNA template was fully sequenced on both strands using BigDye terminator reaction kits and an ABI 373 DNA sequencer. A multiple alignment of the NP1623 full-length sequence with reference sequences of all HIV subtypes was generated.

Bootscan analysis of the full genome sequence revealed that the virus had a recombinant structure with three segments (Fig. 1). Neighbor-joining phylogenetic analyses with parsimony bootstrap were performed on the segments of the genome and are shown in the upper panel of Fig. 1. The structure of the virus from NP1623 is as follows: subtype B from the beginning of gag until mid-*vpu*, where the subtype shifts to CRF01_AE. It then changes back to subtype B in the C5 region of gp120 and remains subtype B through gp41 and nef. The subtype B segments of the genome cluster most closely with the 'Thai B' sample, RL42. These same breakpoints were also confirmed in an independent amplification and sequencing of envelope directly from patient PBMC (Fig. 1, lower diagram).

The initial separation of CRF01_AE and subtype B in different risk groups in Thailand may have delayed the onset of significant numbers of dual infections, each of which can potentially lead to recombination, for almost a decade. The most significant factor in this respect may not be individuals who are exposed both heterosexually and through injecting drug use, as is the case reported here, but may rather be the growing proportion of CRF01_AE among an IDU population that, initially, was almost exclusively infected with subtype B. Indeed, we cannot discern whether the patient studied here was dually exposed by injecting drug use, was exposed to each strain by a different route, or was singly infected with the recombinant strain itself.

The HIV-1 epidemic in southeast Asia is becoming more complex with respect to HIV-1 diversity. Recent reports include subtypes B, 'Thai B', C, D, and CRF01_AE and a B/C recombinant in southern China. A CRF01_AE/subtype C recombinant has been detected in Thailand. The CRF01_AE/B recombinant reported here may be a harbinger of more recombinants. Intensified monitoring is particularly important in the light of the ongoing and projected HIV-1 vaccine trials. A significant fraction of recombinant strains among incident infections could necessitate an approach such as that used here, with multiple genetic regions analysed and discordances followed up with full genome sequencing, to evaluate the relative effectiveness of vaccines against different HIV-1 subtypes.

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Sodsai Tovanabutra[ab], Victoria Polonis[cd], Mark De Souza[cd], Rapee Trichavaroj[d], Penprapa Chanbancherd[e], Bohye Kim[b], Eric Sanders-Buell[b], Sorachai Nitayaphan[f], Arthur Brown[d], Merlin R. Robb[g], Deborah L. Birx[g], Francine E. McCutchan[b] and Jean K. Carr[b],
[a]Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand; [b]Henry M. Jackson Foundation, Rockville, MD, USA; [c]Henry M. Jackson Foundation, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; [d]US Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; [e]Army Institute of Pathology, Bangkok, Thailand; [f]Thai Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and [g]Walter Reed Army Institute of Research, Rockville, MD, USA.

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MIP-1α promoter polymorphism in humans and monkeys: identification of two polymorphic regions characterized by the insertion of unique sequences in monkeys

HIV-1, HIV-2 and SIV use chemokine receptors to gain entry and initiate infection. Although many chemokine receptors have been identified that act as HIV-1 entry co-factors, CCR5 chemokine receptor is primarily used for establishing the infection, governing transmission and tropism [1,2]. During the course of the disease, variants that can use other co-receptors appear, prominent among them being the X4 viruses that use CXCR4 chemokine receptors mainly present on human T lymphocytes. The promoter regions of the chemokine receptor CCR5 have been found to be highly polymorphic in humans [3] and monkeys [4,5], and mutations affecting the progression of HIV in humans have been described [6]. Beta-chemokines (regulated upon activation: normal T cell expressed/secreted; RANTES, *MIP*-1α and β) are potent inhibitors of infection by R5 viruses and stromal cell-derived factor 1 (SDF-1) (α-chemokine) can block infection by X4 viruses [1]. Recently, mutations in the chemokine RANTES promoter have been described in humans that affect the progression of HIV [7]. We recently reported the presence of novel mutations in the SDF-1 gene [8] and in RANTES promoter [9] regions of monkeys.

We sought to characterize 134 bases from the promoter region of *Mip*-1α that constitutes the basal minimal promoter [10,11], characterized earlier from five normal humans and three species (langur, *Prebytis entelus*, rhesus, *Macaca mulatta* and baboon, *Papio anubis*) of monkeys. The promoter region was amplified by using the following set of primers that spanned the region −18 to −152 [11].

Forward primer: 5'–TCCTGAGCCCCTGTGGTC ACCAGGG and

Reverse primer: 5'–CTCTCCTCTTTATAGGCA GCCCTG

The conditions for carrying out polymerase chain reaction was same as that described earlier [12]. Amplified product was cloned into a T-tailed vector (pGEM-T-Ez, Promega Biotech, WI, USA), and the recombinant clones were sequenced as described earlier [8,9]. Sequences generated for humans and monkeys were aligned with the published sequence [11], and the results are shown in Fig. 1. Two highly polymorpic regions (A and B) were identified in langur and rhesus monkeys only, the baboon shows no polymorphism at these two regions that are identical to published human sequences. Interestingly, at the −126 position, the baboon shows point mutation that is common with the other two species of monkeys (A to G transition) but at positions −120 and −89, it showed sequences identical to humans. An insertion of a tetranucleotide AAGG in place of a G nucleotide (position −22) (region B) was observed downstream of a conserved TATA box. The insertion of four nucleotides so close to the TATA binding protein has the potential to change the secondary structure of the chromosome that is very likely to affect the efficiency of the Pol II-mediated transcription. The other polymorphic region A was present at the −108 and −109 positions, where TT was substituted by ACTC in the same two species of monkeys (rhesus and langur). This region overlaps the ICK-1 element, which plays an important regulatory role in transcription as four nuclear factors bind in this region [11]. There are three single nucleotide polymorphisms (SNP) (−89, −120 and −126) in the monkeys. Most importantly, we observed A to G transition in one out of five normal unrelated healthy Indians and in rhesus and baboon monkeys at position −126. Langur monkeys do not show any polymorphism at this region and are identical to humans. At least two monkeys from each species were analysed to rule out polymerase chain reaction-generated mistakes.

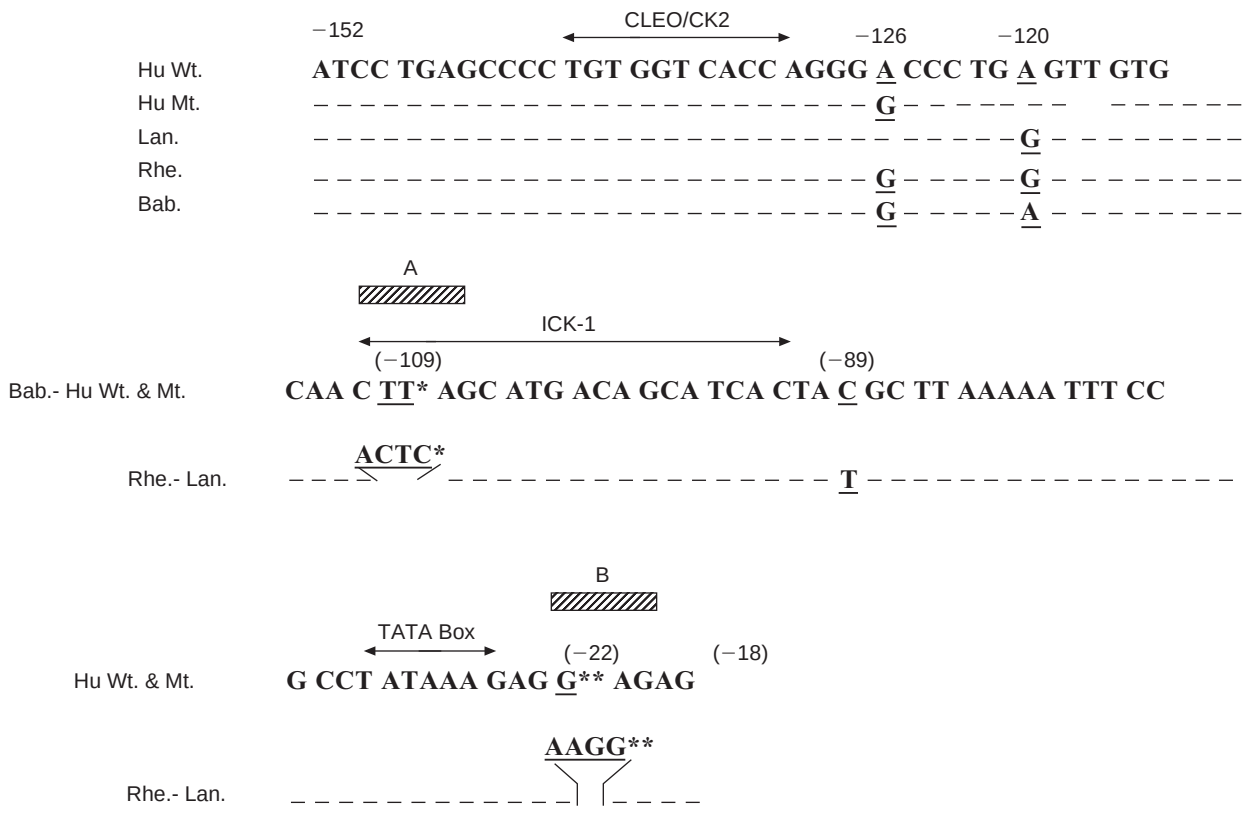

Fig.1. Comparison of the *Mip*-1α promoter sequences in humans and different species of monkeys.
Genomic DNA was isolated from the peripheral blood as described before [12], and approximately 134 bases of the promoter region was amplified by polymerase chain reaction using the primers described in the text. For nucleotide positions refer to Ritter *et al.* [11]. Various transcription factor binding sites are shown at the top of the sequence. Point mutation (A to G) at position −126 was observed in one out of five humans only, the rest showed no mutation. * Shows the substitution of ACTC in place of TT (position −108 and −109) and ** represents the substitution of G with AAGG at position −22, which is just downstream of the conserved TATA box. Sequences from −35 to −74 are not shown as it is identical in humans and all the monkeys. Bab.-Hu.Wt. & Mt. indicate identical sequences among wild-type human (from published sequences), mutant human and baboon monkeys. Rhe.-Lan. indicate identical sequences of rhesus and langur monkeys.

In summary, we have, for the first time, identified a new kind of mutations (insertions) in the chemokine *MIP*-1α gene promoter, both in monkeys and humans, the product of which is a chemokine that is known to inhibit the infection of R5 viruses. This insertion of sequences has the potential to create new or modify existing transcription factor binding sites. It was remarkable that the two polymorphic regions that were observed in langur and rhesus monkeys were not found in baboons. Common mutations in some humans at position −126 and monkeys strongly suggests that similar selective pressures must have acted on this gene. These observations raise interesting evolutionary questions and may also help understand why different species of monkeys produce widely varying outcomes to the disease when infected with HIV-1, HIV-2 or SIV.

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Ritu Goila, Felix Kumar and Akhil C. Banerjea, *Laboratory of Virology, National Institute of Immunology, JNU Campus, New Delhi-110067, India.*

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Prevention of invasive aspergillosis in AIDS by sulfamethoxazole

Because invasive aspergillosis is encountered relatively infrequently in HIV-infected patients, we hypothesized that prophylaxis for *Pneumocystis carinii* pneumonia (PCP) might also prevent invasive *Aspergillus* infections.

Invasive aspergillosis is a significant cause of morbidity and death in immunocompromised patients, including those with AIDS. Although the incidence of this infection is low, the risk is increased in those with low counts of CD4 T lymphocytes, neutropenia and during corticosteroid treatment. Sulfonamides, especially trimethoprim–sulfamethoxazole (TMP–SMZ), are antimicrobial agents that have frequently been employed to prevent PCP, toxoplasmic encephalitis and bacterial respiratory infections in HIV-infected patients. During prophylaxis with TMP–SMZ, 13.9% of patients develop adverse events that necessitate discontinuation [1], and alternative regimens such as dapsone, or aerosolized pentamidine are considered [2]. Because *Pneumocystis* is now believed to belong to the fungal kingdom, we investigated whether these drugs were active against *Aspergillus* spp. This is of relevance because patients at risk of PCP are often also at risk of developing invasive aspergillosis.

The literature was reviewed for cases of invasive aspergillosis in HIV-infected persons or those with AIDS, which had also documented the drug that was used to prevent PCP. Only 18 cases have been described in the literature that met these criteria [3–5]. Among these, 14 patients had received dapsone or pentamidine prophylaxis and four TMP–SMZ. The largest series was published by Denning *et al*. [5], in which 13 patients with AIDS were described who developed invasive aspergillosis, of whom 12 (92%) had received PCP prophylaxis with pentamidine. Taking into account the proportion of patients who receive either TMP–SMZ or alternative regimens, the probability of developing invasive aspergillosis in patients who received alternative PCP prophylaxis is much higher than for those on prophylaxis with TMP–SMZ (relative risk 21.5). This suggests that TMP–SMZ might show activity against *Aspergillus* as opposed to pentamidine or dapsone. Therefore, the in-vitro activity of TMP–SMZ, dapsone and pentamidine was determined against a collection of 20 clinical *Aspergillus fumigatus* isolates and 10 *Aspergillus flavus* isolates. The in-vitro activity was determined according to the proposed guidelines of the National Committee for Clinical Laboratory Standards for the susceptibility testing of conidium-forming fungi (M38-P) using a broth microdilution format [6]. The minimal inhibitory concentration (MIC) was read at 50% inhibition of growth compared with that of the drug-free well. TMP–SMZ was active *in vitro* against *A. fumigatus* (geometric mean MIC 54.6 μg/ml, range 40–160 μg/ml), and trimethoprim alone was inactive (geometric mean MIC > 8 μg/ml) (Table 1). The antifungal activity of TMP–SMZ appeared to be fungistatic based on MIC/MFC ratios. Dapsone and pentamidine were inactive *in vitro* against all *Aspergillus* isolates at therapeutic concentrations. The recommended dose of TMP–SMZ for the prevention of PCP pneumonia in patients with HIV infection is 160/800 mg a day [2], which results in peak blood levels of sulfamethoxazole that range

Table 1. In-vitro activity of trimethoprim–sulfamethoxazole, dapsone and pentamidine against *Aspergillus fumigatus* and *Aspergillus flavus*.

Strain	Number of isolates	Drug					
		TMP–SMZ		Dapsone		Pentamidine	
		MIC	MFC	MIC	MFC	MIC	MFC
A. fumigatus	20	56.5 (40–160)	> 320	> 8	> 8	> 128	> 128
A. flavus	10	184 (160– > 320)	> 320	> 8	> 8	> 128	> 128

MFC, Geometric mean of the minimal fungicidal concentrations (μg/ml); MIC, geometric mean of minimal inhibitory concentration (μg/ml) and range; TMP–SMZ, trimethoprim–sulfamethoxazole.

between 40 and 60 μg/ml. These blood levels are equal to or above the MIC of 19 out of 20 *A. fumigatus* isolates, but are too low to inhibit any of the *A. flavus* isolates.

The results of the literature review and the in-vitro findings suggest that PCP prophylaxis with TMP–SMZ may prevent invasive aspergillosis in HIV-infected patients when it is caused by *A. fumigatus*, although the concentrations achieved in the blood are sub-optimal compared with the MIC of the isolates. Further studies are required to confirm this observation, and to establish whether sulfonamides also show activity against other fungal pathogens such as *Cryptococcus* and *Histoplasma*.

Javier Afeltra[a], Jacques F.G.M. Meis[ab], Johan W. Mouton[b] and Paul E. Verweij[a], *[a]Department of Medical Microbiology, University Medical Center, Nijmegen, the Netherlands; and [b]Department of Medical Microbiology, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands.*

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Long-term outcomes of protease inhibitor-based therapy in antiretroviral treatment-naive HIV-infected injection drug users on methadone maintenance programmes

The effectiveness of protease inhibitor (PI)-based highly active antiretroviral treatment (HAART) relies on a strong commitment to adhere to complex therapies with potentially adverse effects [1,2]. Although methadone maintenance programmes (MMP) reduce drug abuse-related complications and improve the compliance of HIV-positive intravenous drug users (IDU) with the monitoring and treatment of their infection [3,4], the belief that these individuals are poorly adherent, and pharmacokinetic concerns in patients on methadone have led to the suboptimal use of PI-based therapy in this population [5–8]. To date, there are few reports of outcomes in observational cohorts using HAART in IDU [9].

Between March 1996 and May 1998, we prospectively studied all antiretroviral treatment (ART)-naive patients (n = 208) starting therapy at a specialized oupatient clinic from a tertiary centre in Madrid, Spain. The observational nature of this study allowed us to evaluate

the one year effectiveness of PI-based HAART in IDU on methadone (n = 54) in comparison with the overall population of non-methadone individuals (n = 154). Table 1 contains a summary of the univariate analysis of baseline features and outcomes according to methadone use in the cohort. The great majority of methadone patients (85%) were already on a MMP before starting ART (median time 5.5 months; range 1–44). At entry, the only significant difference between the groups was a longer time from AIDS diagnosis in methadone patients (27 versus 2 months, $P = 0.0001$), whereas there were no differences according to age, sex, or prescribed PI, indinavir being the most frequently used (83 versus 80% in MMP patients versus non-MMP patients, $P = 0.61$). After 12 months, both groups obtained similar clinical, virological and immunological outcomes, despite at least 30 days of continued drug interruption being more frequently reported by methadone patients (41 versus 18%, $P = 0.001$). During the follow-up period, four (7%) subjects from the MMP group had a recurrence of intravenous heroin abuse, whereas six (11%) reported other substance abuse, mostly cocaine (83%). Of note was the fact that regardless of the recurrence of drug abuse or the adherence level with ART, 75% of methadone subjects maintained their regular visits to the AIDS clinic, and after a median follow-up time of 546 days [95% confidence interval (CI) 517–574], the overall mortality rate was 0.9%, exclusively among non-methadone patients.

Using multivariate analysis, methadone was not a significant factor predicting a worse long-term virological outcome. The lack of AIDS diagnosis at baseline [relative risk (RR) 0.35; 95% CI 0.14–0.89; $P = 0.02$] and adherence over 90% (RR 16.66; 95% CI 5.5–50; $P = 0.0001$) were the strongest predictors for long-term virological success.

The results of our study support the usefulness of methadone for the adequate management of HIV infection in active IDU, including the use of the most optimal ART [1]. In contrast with previous reports [9,10], we did not find significant differences in the rates of virological or immunological success achieved by methadone users when compared with naive patients with similar baseline features starting a potent PI-based therapy. A remarkable finding was the significantly longer time from AIDS diagnosis in methadone patients in our cohort. This probably reflects a higher rate of progression of HIV infection in IDU before MMP enrolment, with a lifestyle that did not allow timely prophylaxis or treatment for opportunistic diseases [4], and also that physicians may be less likely to recommend ART for these patients because of the belief that they are less adherent to complex regimens and have a high rate of comorbidities, potential drug interactions or poorer tolerability [11–13].

Adherence has arisen as the 'Achilles' heel' of potent

Table 1. Univariate analysis of baseline features and outcomes according to methadone use (208 patients).

	Total (n)	MMP (n = 54) (n) (%)	Non-MMP (n = 154) (n) (%)	*P* value
Baseline features				
Intravenous drug abuse as risk factor for HIV	132	54 (100)	78[a] (51)	0.001
AIDS diagnosis at baseline	70	19 (35)	51 (33)	0.78
(median months, range)		27 (2–94)	2 (1–61)	0.0001
HIV RNA (log_{10}, median, range)	195[b]	5.1 (2.3–6.1)	5.0 (2.3–6.5)	0.54
CD4 cell count (cells $\times 10^6$/l, median, range)	202[c]	148 (1–569)	169 (2–865)	0.34
Effectiveness				
Initial virological response[d]	167	43 (80)	124 (81)	0.84
Long-term virological response	169	41 (76)	128 (83)	0.24
Long-term CD4 cell count increase (median, range)		156 (−67 to + 610)	165 (−164 to +667)	0.58
Outcomes				
Changes in the first PI	83	23 (43)	60 (39)	0.63
Clinical events	29	11 (20)	18 (12)	0.11
Admissions	31	11 (20)	20 (13)	0.18
Lost to regular follow-up	20	8 (15)	12 (8)	0.13
Adherence				
> 90%	132	28 (52)	104 (68)	0.058
> 1 month of interruption	50	22 (41)	28 (18)	0.001

MMP, Methadone maintenance programmes; PI, protease inhibitor.
[a]No active injection drug users (IDU).
[b] and [c]Excluding patients without baseline viral load (n = 13) or CD4 cell counts (n = 6).
[d]Patients with a viral load decline greater than 1 log_{10} or reaching undetectable levels (< 400 or < 50 copies/ml) within the first 3 months of therapy.

ART [14]. In our cohort, most patients maintained an adequate adherence level regardless of methadone use, but a significantly greater proportion of methadone users reported drug interruptions of at least 30 consecutive days. Other drug abuse maintenance therapies, such as buprenorphine, have been able to increase the adherence to HAART among this population [15]. The association between poor adherence, virological failure and resistance has been clearly established [16], and therefore it was difficult to explain why it did not translate into a worse long-term prognosis in our methadone patients. Previous reports [17] suggested a lack of deleterious effect on reinitiated therapy in patients who temporarily discountinued ART. If a successful therapy is completely and abruptly interrupted, the absence of drug selection applied on the virus could avoid the emergence of resistant strains. However, an extended treatment interruption might erase the benefits of a long period under HAART and bring the patient back to the pre-treatment starting point. Active efforts should thus be made to improve adherence further in these patients, through a trusting and accessible relationship with experienced physicians in charge of HIV-infected IDU [18] along with adequate support services [19].

In summary, our results showed that in the population of IDU, current stable enrolment in a MMP could allow the adequate management of HIV infection, and that methadone patients are able to reach similar outcomes to the overall ART-naive population after the administration of potent PI-based therapy. However, their adherence to ART should be supported by strict monitoring and timely collaboration with primary care services.

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Ana Moreno, María J. Perez-Elías, Jose L. Casado, Vicente Muñoz, Antonio Antela, Fernando Dronda, Enrique Navas and Santiago Moreno, *Infectious Diseases Service, Ramón y Cajal Hospital, Madrid, Spain.*

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Persistence of zidovudine-resistance mutations in HIV-1 isolates from patients removed from zidovudine therapy for at least 3 years and switched to a stavudine-containing regimen

Because of the widespread use of zidovudine during the 1990s, the treatment history of many HIV-1-infected individuals relied on the first line of zidovudine-containing regimens followed by second lines of stavudine-containing regimens when virological failure occurred.

After the removal of zidovudine pressure, some studies reported a slow rate of reversion of zidovudine resistance mutations, suggesting that these mutations have only a modest impact on viral replication in the absence of the drug [1–3], whereas others reported a fast reappearance of wild-type virus strains [4,5]. On the other hand, several recent studies have demonstrated that HIV-1 isolated from individuals failing stavudine-containing regimens often contain thymidine analogue mutations [6–8], which include M41L, D67N, K70R, L210W, T215Y/F and K219Q/E, previously associated only with zidovudine resistance.

We have investigated whether the potential ongoing selective pressure exerted by a second line of stavudine-containing regimen might prevent the reversion of zidovudine-associated genotypic mutations in patients who have not been exposed to zidovudine for at least 3 years.

Fourteen patients who had been treated with a first line of zidovudine-containing regimen for a median duration of 36 months (range 5–72 months) and had switched to a stavudine-containing regimen because of virological failure, were retrospectively included in the study. The median follow-up period after zidovudine discontinuation was 48 months (range 36–84 months). Patients were tested for HIV-1 drug resistance just before stopping their more recent zidovudine-containing regimen (baseline) and every year after zidovudine discontinuation (mean 3.5 follow-up samples per patient). Only patients with zidovudine-related genotypic resistance before the switch from zidovudine- to stavudine-containing regimen were selected. Reverse transcriptase (RT) genotype analysis was performed using an in-house procedure on the new eight capillary array sequencer CEQ 2000 System (Beckman Coulter Inc., Fullerton, CA, USA).

Table 1 summarizes the treatment history, viral load and sequencing results for successive samples of these patients. The plasma viral load remained detectable during the whole treatment period: median (range) plasma viral loads at baseline and at the end of follow-up were 30 600 (2900–1 174 000) and 7782 (2253–1 021 260) HIV-1-RNA copies/ml, respectively.

At baseline, the mean of mutations in the RT gene correlated with zidovudine-resistance was 3.4/RT gene. More than 3 years after the cessation of zidovudine therapy, reversal to wild-type amino acid for mutant codons in the RT gene did not occur under stavudine therapy (mean 3.4 zidovudine-mutations/RT gene at the end of follow-up). The main zidovudine resistance mutations: M41L, D67N, L210W, and T215Y/F were observed in eight, seven, eight, and 13 out of 14 baseline isolates, respectively. They persisted at the end of follow-up in all isolates but one (no. 9) with a disappearance of L210W and T215Y mutations within the second year after the discontinuation of zidovudine.

These results are in discordance with reports of the fast reappearance of wild-type virus after stopping therapy [4,5] or switching nucleoside reverse transcriptase inhibitors to protease inhibitors [4]. On the other hand, previous studies have shown the persistence in the plasma of several mutations associated with zidovudine, 9 months after the discontinuation of zidovudine and switch to a triple combination regimen including stavudine, lamivudine and indinavir [3], or 14–15 months after the switch from zidovudine monotherapy to didanosine monotherapy in two patients [2]. Our results suggest that the switch from zidovudine to stavudine maintains a selective pressure that prevents the reversal of zidovudine-related mutations or the reappearence of virus with wild-type genotype. This switch does not allow the resensitizing of HIV to zidovudine, and leads to a genotypic resistance to stavudine and a decrease in susceptibility to abacavir [9]. This finding may have clinical implications when deciding on the optimal combination of nucleoside or non-nucleoside RT inhibitors as a component of multidrug regimens after first lines of therapy including zidovudine or stavudine, even more than 3 years after the discontinuation of zidovudine. Finally, these results offer further support to the concept that genotyping is necessary to select the components of antiretroviral regimens for patients experiencing treatment failure.

Isabelle Pellegrin, Isabelle Garrigue, Anne Caumont, Jean-Luc Pellegrin, Patrick Merel, Marie Hélène Schrive, Pascal Bonot and Hervé Fleury, *Departments of Virology and Infectious Diseases, Bordeaux University Hospital, Bordeaux, France.*

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Table 1. Treatment history, viral load and sequencing results for successive samples of patients who switched from zidovudine- to stavudine-containing regimens.

	Treatment history				Reverse transcriptase codon substitutions									
Patient	Previous ZDV therapies – duration (months)	Switch to d4T regimens	Sampling months	HIV RNA	41 M	67 D	69 T	70 K	74 L	151 Q	184 M	210 L	215 T	219 K
1	ZDV then ZDV+ddC (36)	1,d4T+3TC+SQV	0	8500	–	–	–	–	–	–	–	W	Y	–
			12	21 200	–	N	–	–	–	–	V	W	Y	–
			24	1 1800	L	–	–	–	–	–	V	W	Y	–
			36	15 463	–	N	–	–	–	–	V	W	Y	–
		2,d4T+ddI+EFV	48	3339	L	–	–	–	–	–	–	W	Y	–
			60	2955	L	–	–	–	–	–	–	W	Y	–
2	ZDV then ZDV+ddC (16)	1,d4T+3TC	0	2900	L	–	–	–	–	–	–	–	Y	–
			12	2300	L	–	–	–	–	–	–	–	Y	–
		2,d4T+ddI+NFV	36	5955	L	–	–	–	–	–	–	–	Y	–
			48	12 868	L	–	–	–	–	–	–	–	Y	–
			60	6156	L	–	–	–	–	–	–	–	Y	–
3	ZDV then ZDV+ddI (36)	1,d4T+3TC+RTV	0	37 100	–	N	–	R	–	–	–	–	Y	–
			12	153 060	–	N	–	R	–	–	V	–	Y	Q
		2,d4T+3TC+NFV	24	142 044	–	N	–	R	–	–	V	–	Y	Q
			36	9815	–	N	–	R	–	–	V	–	Y	Q
			48	5511	–	N	–	R	–	–	V	–	Y	Q
4	ZDV then ZDV+ddI then AZT+37C+SQV (33)	1,d4T+ddI+SQV	0	21 000	L	N	–	–	–	–	V	W	F	Q
			12	8800	L	N	–	–	–	–	–	W	F	–
		2,d4T+ddI+NFV	24	14 349	L	N	–	–	–	–	–	W	F	–
			36	6500	L	N	–	–	–	–	–	W	F	–
5	ZDV then ZDV+ddC then AZT+3TC+SQV (40)	1,d4T+3TC+IDV	0	44 000	L	–	–	–	–	–	V	W	Y	–
		2,d4T+3TC+NFV	12	12 500	L	–	–	–	–	–	V	W	Y	–
			24	4418	L	–	–	–	–	–	V	W	Y	–
			36	23 338	L	–	–	–	–	–	V	W	Y	–
6	ZDV+ddI then ZDV+3TC+SQV (56)	1,d4T+ddI+IDV	0	7100	L	–	–	–	–	–	V	W	Y	–
			12	4300	L	N	–	–	–	–	–	W	Y	–
			24	23 411	L	N	–	–	–	M	–	W	Y	–
			36	17 654	L	N	–	–	–	M	–	W	Y	Q
7	ZDV then ZDV+ddI (13)	1,d4T+3TC	0	27 200	L	–	–	–	I	–	–	–	–	–
			12	4500	L	–	–	–	I	–	–	W	Y	–
		2,d4T+3TC+NVP	24	900	L	–	–	–	–	–	–	W	Y	–
			36	6751	L	–	–	–	–	–	V	W	Y	–
			48		L	–	–	–	–	–	V	W	Y	–
			60	11 860	L	–	–	–	–	–	V	W	Y	–
8	ZDV then ZDV+ddC (12)	1,d4T+3TC+IDV	0	1 174 000	L	–	–	–	–	–	–	W	Y	–
			12	21 600	L	–	–	–	–	–	–	W	Y	–
			24	1700	L	–	–	–	–	–	V	W	Y	–
			36	3184	L	–	–	–	–	–	V	W	Y	–
			48	3380	L	–	–	–	–	–	V	W	Y	–
			60	2253	L	–	–	–	–	–	V	W	Y	–
9	ZDV+ddC (9)	1,d4T+ddI+NFV	0	59 400	–	N	–	R	–	–	V	W	Y	Q
			12	1400	–	–	–	R	–	–	V	–	Y	–
			24	2000	–	N	–	R	–	–	–	–	–	Q
			36	5465	–	N	–	R	–	–	–	–	–	Q
			48	29 532	–	–	–	–	–	–	–	–	–	–
10	ZDV then ZDV+ddC then ZDV+3TC (41)	1,d4T+3TC+NFV	0	34 000	L	N	–	–	–	–	V	W	F	Q

	12	33 100	L	N	–	–	V	W	F	Q		
	24	10 000	L	–	–	–	V	W	F	–		
	36	16 564	L	–	–	–	V	W	F	–		
2,d4T+ddI+SQV	48	32 651	L	–	–	–	V	W	F	–		
	60	4401	L	–	–	–	V	W	F	–		
11 ZDV then ZDV+ddC (8)	0	54 600	–	N	–	R	–	–	Y	–		
	12	1500	L	–	–	–	V	–	Y	–		
	48	2651	L	–	–	–	V	–	Y	–		
12 ZDV+ddC then ZDV+3TC (10) 1,d4T+3TC+NFV	0	4800		–	–	–	V	–	Y	–		
	12	5700	M/L	–	–	–	V	–	Y	–		
	24	43 859	L	–	–	–	V	–	Y	–		
	48	22 209	L	–	–	–	V	–	Y	–		
13 ZDV then ZDV+3TC then ZDV+3TC+IND (72) 1,d4T+3TC+IDV	0	37 600	L	N	D	–	V	–	Y	–		
	12	305 400	L	N	D	–	V	–	Y	–		
2,d4T+3TC+ABC	24	156 600	L	N	D	–	V	–	Y	–		
	36	500 000	L	N	D	–	V	–	Y	–		
	48	1 021 260	L	N	D	–	V	–	Y	–		
14 ZDV then ZDV+ddI (40) 1,d4T+3TC+IDV	0	13 400	L	N	–	–	–	W	Y	–		
	12	4700	L	N	–	–	V	W	Y	–		
	24	2900	L	N	–	–	V	W	Y	–		
	36	38 035	L	N	–	–	V	W	Y	–		
	48	9064	L	N	–	–	V	W	Y	–		

1, First line of treatment with d4T; 2, second line of treatment with d4T. HIV-1 RNA, copies/ml.
ABC, Abacavir; ddC, zalcitabine; ddI, didanosine; d4T, stavudine; EFV, efavirenz; IDV, indinavir; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir; SQV, saquinavir; 3TC, lamivudine; ZDV, zidovudine.

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Lactic acid levels in children perinatally treated with antiretroviral agents to prevent HIV transmission

Nucleoside-analogue reverse-transcriptase inhibitors (NRTI) reduce mother-to-child transmission (MTCT) of HIV by approximately 70% [1]. NRTI are also substrates for DNA polymerase, the enzyme required for the replication of mitochondrial DNA. Decreased concentrations of mtDNA have been observed in cultured cells exposed to NRTI, and in muscle cells from patients with zidovudine-induced myopathy [2].

In a series of 1754 infants treated perinatally with NRTI, Blanche *et al*. described eight children with possible mitochondrial dysfunction [3]. Although this observation was not confirmed by preliminary analyses of other large cohort studies [4], the frequency of mitochondrial dysfunction reported by Blanche *et al*. [3] is much higher than would be expected in the general population.

Lactic acid (LA) in plasma is a sensitive but not specific marker of mitochondrial dysfunction [5]. Five of the children described by Blanche *et al*. [3] had persistent lactic acidosis, with plasma levels above 2.5 mmol/l. Our objective was to evaluate whether mild mitochondrial damage, as shown by elevated plasma LA levels, is associated with prenatal or perinatal exposure or therapy with antiretroviral agents (ARV) in HIV-infected children.

Plasma LA levels were determined in two groups of children; group A included 20 infants who had experienced varying prenatal or perinatal exposure to ARV; group B consisted of 36 HIV-infected children who had either received ARV therapy or were not treated.

In group A clinical, virological and immunological parameters were monitored monthly in the first 3 months of life and thereafter at 3 monthly intervals. A fasting blood sample was taken from an antecubital vein at every visit and plasma LA levels were measured within 1 h from sampling using a standardized quantitative enzymatic method. Values were considered normal if they were below 2.5 mmol/l [5].

Seventeen of these infants had been exposed to ARV that contained NRTI (two zidovudine; one stavudine/lamivudine; seven zidovudine/lamivudine; seven protease inhibitor-containing regimens) *in utero*. All their mothers, plus an additional one also received intravenous zidovudine during delivery. All infants were treated with oral zidovudine during the first 6 weeks of life. Three mothers were not treated during pregnancy, and one child was diagnosed after birth as HIV infected.

Seventeen out of these 20 children (85%) at least once showed an LA level exceeding 2.5 mmol/l. All elevated LA levels returned to normal during follow-up and the mean decrease between the first and the second test was -0.52 mmol/l ($P = 0.12$) (Table 1). In three patients with high LA levels the lactate : pyruvate ratio was normal. During follow-up (mean length 7.4 months, range 0.5–11) none of the children developed any clinical signs or symptoms consistent with mitochondrial damage.

In group B, we conducted a cross-sectional study to assess the fasting plasma LA concentrations. The mean

Table 1. Lactic acid levels in children exposed to antiretroviral agents to prevent mother-to-child transmission (group A).

Variable	Mean (median)	SD	Range	*P*-value
Duration of pregnancy (weeks)	37.2 (38)	2.5	30–40 weeks	
Lactic acid first sample (days)	33.5 (32.6)	19.8	10–92	
Level of first lactic acid	2.78	1.01	1.7–5.3	
< 1 month	2.35(00.0)	0.48	1.7–3.1	
> 1 month	3.1(0.0)	1.19	1.8–5.3	0.18 (Mann–Whitney test)
Intravenous treatment during labour				
Nothing (first LA level)	2.37(00.0)	0.35	2–2.7	
Zidovudine (first LA level)	2.9(0.0)	1.11	1.7–5.3	0.54 (Mann–Whitney test)
Treatment during pregnancy				
Nothing (first LA level)	2.45(00.0)	0.33	2.0–2.7	
Mono therapy zidovudine (first LA level)	2.75(00.0)	0.50	2.4–3.1	
Zidovudine/lamivudine (first LA level)	3.7(0.0)	1.7	1.7–5.3	
Triple therapy (first LA level)	2.3(0.0)	0.4	1.8–2.7	0.18 (ANOVA)
Change in lactic acid level				
Between first and second (n = 9)	−0.52 (−0.5)0	0.81	−1.6–0.6−	0.12 (Wilcoxon paired test)

ANOVA, Analysis of variance; LA, lactic acid.
All values are expressed in mmol/l.

age was 9 years (range 5 months to 17 years); 29 children (81%) were treated with at least one NRTI and 24 were on triple therapy with protease inhibitor-containing regimens.

Only three out of 29 treated children (8%, 95% confidence interval 0–17%) had LA levels that were slightly above the normal range (mean 2.9 mmol/l). LA levels were not related to age.

In this small study, we observed that infants who were exposed to NRTI during gestation had an increased LA level during the first weeks of life. Our study did not have the power to demonstrate a significant association between an eventual small increase in LA levels and age, exposure to ARV during gestation or the type of perinatal treatment. The slight increase of LA in the NRTI exposed infants could reflect the possibility of transient mitochondrial toxicity; however, no clinical implications were observed. Although more data are needed to be conclusive, our findings do not support a change in the current recommendations to use ARV in pregnant women to prevent MTCT of HIV.

Carlo Giaquinto[a], Antonia De Romeo[a], Vania Giacomet[a], Osvalda Rampon[a], Ezia Ruga[a], Alberto Burlina[a], Anita De Rossi[b], Miriam Sturkenboom[c] and Ruggiero D'Elia[a], *[a]Department of Paediatrics, and [b]Institute of Oncology, University of Padova, Padua, Italy; and [c]Department of Epidemiology and Biostatistics, Erasmus University Medical School, Rotterdam, the Netherlands.*

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'Do HIV-infected injecting drug users over-report adherence to highly active antiretroviral therapy?' A comparison between patients' self-reports and serum protease inhibitor concentrations in the French Manif 2000 cohort study

Patients' self-reports about adherence to highly active antiretroviral therapy (HAART) were compared with serum concentrations of indinavir in a sample of 57 HIV-infected injecting drug users on an indinavir-containing HAART regimen at their last follow-up visit in the French MANIF 2000 cohort study. Among the 39 patients who reported adherence (≥ 80%), only three had indinavir serum concentrations less than 50 ng/ml, whereas half (nine out of 18) of those who reported adherence less than 80% had serum concentrations of 50 ng/ml or greater. Cross-validation between patients' self-reports and the determination of protease inhibitor (PI) serum concentration can improve the validity of measurement of adherence to HAART.

The assessment of adherence to HAART remains a matter of debate. Patients' self-reports are often suspected to be invalid measures [1,2]. Among patients HIV-infected through injecting drug use, enrolled and followed between 1 October 1995 and 31 December 1999 in the French Manif 2000 cohort study [3], we retrospectively selected the subgroup of 57 patients who were on an indinavir-containing HAART regimen (indinavir 800 mg three times a day in combination with nucleoside reverse transcriptase inhibitors), for at least one month, at their last follow-up visit in the cohort. In this sample, we had the opportunity to evaluate the relationships between self-reports about adherence to HAART, serum concentrations of indinavir and virological success of therapy.

Patients were classified into three groups according to their self-reports on adherence. A total of 26 patients (45.6%) were considered 'strictly adherent' because they declared consistently, in both a face-to-face interview administered by a nurse and a self-administered questionnaire, that they had taken 100% of their prescribed doses of indinavir during the week before the visit. An additional 13 patients (22.8%), who declared having missed no more than 20% of their prescribed doses of indinavir, were classified as 'adherent'. The remaining 18 individuals (31.6%) were 'non-adherent' patients who had taken less than 80% of their scheduled indinavir doses. No difference was found

between these three groups with regard to clinical data (viral load, CD4 cell count, Centers for Disease Control and Prevention AIDS stage, antiretroviral pretreatment) and sociodemographic characteristics at enrolment in the cohort. The median time since the initiation of HAART at the last follow-up visit was 11.3 months [interquartile range (IQR) 6.0–20.6] in the whole sample and was not significantly different between the three groups. At the last visit, 'non-adherent' patients had significantly higher viral load titres [median (IQR) log copies/ml 3.80 (2.60–5.04)] than 'adherent' [2.30 (2.30–3.64)] and 'strictly adherent' [2.30 (2.30–3.45)] patients (Mann–Whitney test, $P = 0.001$). They also had a significantly lower proportion of patients with undetectable viral load (22.2%) than 'adherent' (69.2%) and 'strictly adherent' (73.1%) patients (chi-square test, $P = 0.002$). They tended to have lower CD4 cell counts [median (IQR) cells/mm^3 403 (218–534) versus 446 (355–517) and 518 (389–655), respectively] (Mann–Whitney test, $P = 0.06$).

Table 1. Relationships between self-reported adherence, serum concentrations of indinavir and viral load titres at last follow-up visit in the Manif 2000 cohort study of HIV-infected injecting drug users (n = 57).

	Serum concentration of indinavir		*P*
	< 50 ng/ml N (%)	⩾ 50 ng/ml N(%)	value*
Self-reported adherence to prescribed doses of indinavir			
< 80%	9 (50.0)	9 (50.0)	0.001
80–99%	0 (0.0)	13 (100.0)	
100%	3 (11.5)	23 (88.5)	
Undetectable viral load[a]			
Yes	2 (6.2)	30 (93.8)	0.002
No	10 (40.0)	15 (60.0)	

*Fisher's exact test.
[a]⩽ 200 copies/ml.

At each visit, patients' blood samples were drawn, centrifuged within 4–6 h and stored at −70°C. For the determination of indinavir concentrations in frozen serum, we used a previously described high performance liquid chromatography method [4]. The intra- and inter-assay coefficient of variation ranged from 0.97 to 3.58% and from 11.1 to 12.3%, respectively, for quality control concentrations (50, 500, 2500 and 8000 ng/ml). Because the exact schedule of the last dose taken by the patient was not available, and the range of residual concentrations of indinavir (8 h after the last dose) has been estimated between 50 and 300 ng/ml [5,6], we used a 50 ng/ml threshold for considering that a patient had a sub-optimal indinavir serum concentration. Such a low threshold allowed us to take into account inter-individual variability in PI concentrations as a result of patient differences in drug absorption and clearance [7].

Table 1 shows that 12 patients (21.1%) had indinavir serum concentrations lower than 50 ng/ml (including 10 patients below the limit of detection of 20 ng/ml), and they were less likely to have undetectable viral loads. None of these 12 patients had been prescribed metabolic inducers of liver cytochrome P450 3A4, which may have decreased serum indinavir exposure.

Among the 39 patients who declared adherence of 80% or greater, only three had indinavir serum concentrations lower than 50 ng/ml, and two out of these three had a detectable viral load; nine patients had a detectable viral load but indinavir serum concentrations of 50 ng/ml or greater; finally, 27 patients had both undetectable viral loads and indinavir concentrations of 50 ng/ml or greater.

Among the 45 patients with indinavir serum concentrations of 50 ng/ml or greater, nine (20.0%) declared having been 'non-adherent'; six out of these nine patients (67%) had a detectable viral load, whereas only nine of the 36 (25%) 'adherent' patients with indinavir serum concentrations of 50 ng/ml or greater had detectable viral loads (Fisher test, $P = 0.04$).

Various studies among HAART-treated patients have shown that self-reports tend to overestimate adherence slightly more than alternative methods of measurements (such as unannounced pill counts and electronic medication monitors) [2,8]. However, many studies, including ours, have found reasonably good correlation between self-reports of adherence to HAART and the virological success of HAART [8,9].

A previous study by Murri and colleagues [10] had already shown that self-report of missing a dose of antiretroviral medication the previous day was related to an unmeasurable plasma PI level. Our study in a sample of injecting drug users, who are often suspected to be more likely than other HIV-infected patients to make biased declarations, brings the additional evidence that most patients (92.3%) who self-reported adherence behaviour in the previous week also presented a quantifiable serum level (⩾ 50 ng/ml) of indinavir.

On the contrary, half of the patients who explicitly recognized problems of non-adherence when answering questionnaires in our sample would not have been identified as such on the only basis of indinavir serum concentration. It suggests that the determination of antiretroviral serum concentrations cannot provide a unique and direct method for measuring adherence in routine clinical practice, because they predominantly reflect the most recently ingested dose, and do not necessarily provide a measure of long-term exposure to

HAART. What is known as the 'dentist effect' (people brush their teeth more regularly right before and after scheduled dental visits) may also influence patients' behaviour towards prescribed medicines in the very short period before an hospital follow-up visit [11].

Because there is still no gold standard to assess medication adherence [12], cross-validation between patients' self-reports and an assessment of drug serum concentration can provide a useful tool for the clinical follow-up of HAART-treated HIV-infected patients.

Ségolène Duran[a], Caroline Solas[b], Bruno Spire[a], Maria Patrizia Carrieri[a], Jean-Gabriel Fuzibet[c], Dominique Costagliola[d], Bruno Lacarelle[b], Jean-Paul Moatti[a], and the cohort Manif 2000 Study Group, *[a]INSERM Research Unit 379 'Epidemiology and Social Sciences applied to medical innovation', Marseille, France; [b]Pharmacokinetic and Toxicokinetic Department, Timone Hospital, Marseille; France; [c]Department of Internal Medicine and Hematology, Archet Hospital, Nice, France; and [d]INSERM Research Unit SC4 'Centre Coopérateur de Données Epidémiologique sur l'Immunodéficience Humaine', Paris, France.*

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Low incidence of genotypic and phenotypic resistance in paediatric HIV-infected patients on long-term first-line antiretroviral triple therapy

Starting antiretroviral treatment with a combination of three drugs in both HIV-infected adults and children is widely accepted and recommended according to various guidelines [1– 3]. Intention-to-treat analysis demonstrated plasma HIV-1-RNA levels of less than 500 copies/ml in 43–70% of treatment-naive children and less than 50 copies/ml in 26–50% of patients after 48 weeks of combination therapy [4]. In 30–60% of these children increased viral replication may contribute to the emergence of resistant HIV-1 strains. Discussion on the clinical relevance of resistant strains and the need to change antiretroviral regimens is controversial. Until now, clinical data on drug susceptibility assessed by genotypic and phenotypic assays have been reported in HIV-infected adults, but data on children on initial triple therapy are rare [5,6].

In an intention-to-treat study we investigated 16 perinatally infected patients (eight females and eight males), aged 1.5–13 years (median 7.5). Antiretroviral treatment was initiated and dosages were chosen according to American and German paediatric guidelines [2,3]. None of the children had had previous antiretroviral treatment. All children received antiretroviral triple therapy, including the protease inhibitor nelfinavir in combination with two nucleoside reverse transcriptase inhibitors (NRTI). Informed consent was obtained from the parents or legal guardians. Baseline characteristics, the course of CD4 cell counts and reduction of viral load after 12 months of treatment have been reported previously [7].

The observation period was 24 months. All 16 patients received nelfinavir, eight patients in combination with zidovudine and lamivudine, and eight patients in combination with stavudine and didanosine. Adherence to therapy was questioned at every visit. Compliance was considered poor when more than 20% of the dose was missed or inadequate nelfinavir levels were repeatedly found [7–9].

The viral load (Amplicor-test, version 2; Hoffmann

LaRoche, Grenzach Whylen, Germany) expressed in HIV-1-RNA copies per ml (limit of detection 50 copies/ml) was measured every 4–8 weeks. Phenotypic and genotypic resistance assays were performed simultaneously (VIRCO, Belgium/Ireland) when the viral load increased above 500 copies/ml.

Phenotypic susceptibility was assessed using the MTT-MT4 assay (Antivirogram, VIRCO, Belgium) and results are expressed as fold resistance (fold-increase in IC_{50}) relative to the wild type [10].

Genotypic resistance was measured by the determination of drug-related point mutations in the *pol* gene. HIV-1 complementary DNA was obtained directly from amplified protease and reverse transcriptase coding regions including mutations at positions 10–90 (HIV-protease) and at positions 41– 400 (HIV-reverse transcriptase) [11].

After 24 months of treatment a viral load of less than 500 copies/ml was found in 11 out of 16 patients (69%), and a viral load of less than 50 copies/ml was found in eight out 16 patients (50%). Two patients (12%) had a viral load of between 500 and 5000 copies/ml and three patients (19%) had a viral load greater than 5000 copies/ml. In three of these five children, non-compliance was proved by questioning and the finding of inadequate nelfinavir levels. Phenotypic and genotypic resistance assays were performed in all five patients, but genetic material could be amplified only from the plasma of four patients (one of two patients with HIV-1 copies between 500 and 5000/ml and in all three patients with greater than 5000 copies/ml).

Data on phenotypic and genotypic resistance assays are given in Table 1.

In the phenotypic assay, three patients had a maximum resistance to lamivudine and a more than 10-fold resistance to nelfinavir after 12 months or more of treatment. No cross-resistance to other NRTI was found. Limited resistance (fivefold or less) to ritonavir and indinavir but not to saquinavir could be demonstrated in two out of four patients. Genotypic assays confirmed phenotypic lamivudine resistance as a result of the M184V mutation. In three out of four patients the D30N mutation was seen, corresponding with phenotypic resistance to nelfinavir. All patients had more than one point mutation (2– 3) in the protease gene, leading to decreased susceptibility.

In summary, during a long-term follow-up of 24 months, 11 out of 16 paediatric patients had a reduction in viral load to less than 500 copies/ml, and only three had a viral load greater than 5000 copies/ml. In four patients resistant virus strains were detected. According to phenotypic assays, relevant

Table 1. Therapy regimen, duration of treatment, drug susceptibility and viral load in four perinatally HIV-infected patients on initial antiretroviral triple therapy.

Patient	Therapy regimen	Interval	Genotypic resistance (points of mutation)[b]								Phenotypic resistance (fold increase in IC_{50})[c]				HIV-1 RNA[d]
no.	**NRTI**	(months)[a]	41	62	65	**70**	74	77	151	**184**	215	219	ZDV	**3TC**	ddI	d4T	(copies/ml)
1	d4T/ddI	8				**X**							1	1	1	1	< 5000
2	ZDV/3TC	12				**X**				**X**			1	**58**	1	1	> 5000
3	ZDV/3TC	15								**X**			1	**78**	1	2	> 5000
4	ZDV/3TC	20								**X**			1	**96**	3	1	> 5000
	PI		24	**30**	36	46	48	71	77	82	88	90	**NFV**	RTV	SQV	IDV	(copies/ml)
1	NFV	8			X							X	4	4	1	5	< 5000
2	NFV	12		**X**	X							X	**11**	**5**	1	4	> 5000
3	NFV	15		**X**				X					**19**	1	1	1	> 5000
4	NFV	20		**X**					X		X		**42**	1	1	1	> 5000

[a]Interval between onset of treatment and resistance assay.
[b]Point mutation with evidence of resistance: to zidovudine/stavudine (ZDV/d4T): **M41L**, **K70R** and **T215Y**; to lamivudine (3TC): **M184V**; to didanosine (ddI): **L74V**; multi-drug resistance to nucleoside reverse transcriptase inhibitors (NRTI): 62, 77, 151; to nelfinavir (NFV): **D30N**, L90M; to ritonavir (RTV): M36I, A71V/I, **V82F**; to saquinavir (SQV): **G48V**, L90M; to indinavir (IDV): L24I, **M46L/I, V82F**.
[c]Susceptibility of patient sample is classified as sensitive: ⩽ fourfold; as intermediate: 5–10-fold; and as resistant: > 10-fold increase relative to wild type.
[d]Viral load measured when resistance assays were performed.

resistance (> 10-fold) to lamivudine and nelfinavir appeared in three patients. No cross-resistance to other NRTI, but limited resistance to ritonavir and indinavir was demonstrated, confirming former studies [6,12]. Non-compliance was the major cause of virological failure, as reported by others [8,9]. In our opinion, patients with a viral load greater than 5000 copies/ml need to change their therapeutic regimen, but not patients with a viral load of less than 5000 copies/ml. A change of treatment should be discussed according to resistance data and the possibility of improving compliance [9].

Richard Linde[a], Markus B. Funk[a], Tobias Schuster[a], Uwe Wintergerst[b], Gundula Notheis[b], Thomas Klingebiel[a] and Wolfhart Kreuz[a], *[a]Children's Hospital, Johann Wolfgang Goethe-University Frankfurt, 60590 Frankfurt, Germany; and [b]Children's Hospital, Ludwig Maximilian-University Munich, 80337 Munich, Germany.*

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Is indinavir crystalluria an indicator for indinavir stone formation?

The HIV-protease inhibitor, indinavir sulphate, used for the treatment of HIV-1-infected patients is prone to precipitate [1–3] form deposits inside cortical and medullary collecting ducts [4,5] and form kidney stones in 4–12% [1,6–8] of patients. The associated symptoms [9,10] may necessitate lowering the drug dosage or drug withdrawal [1,2]. Indinavir stone formation probably follows the sequence supersaturation → crystal formation → stone formation. Indeed, a relationship between the indinavir plasma level and urological complaints has been shown [11,12]. Crystal formation is considered to start intra-renally [13], and indinavir crystals are found inside cortical and medullary collecting ducts in renal biopsies of indinavir-treated patients [1–3]. Indinavir crystalluria seems a likely candidate for monitoring the risk of urolithiasis [1,3,14]. It is found in 20–67% of indinavir-treated patients. Of these, 8–36% develop urological symptoms. However, false-positive findings as a result of crystallization occurring in the time between urine production in the kidney and urinalysis may disturb the applicability.

We analysed the conditions under which indinavir crystals form spontaneously during a time period of 4 h, applying a range of pH values, 5.8–6.5, and indinavir concentrations, 250–500 mg/l, which cover those found in HIV-treated patients [9,14]. This concentration range was based on known plasma indinavir concentrations, up to 10 mg/l, 61% protein binding by indinavir and a concentration factor of urine versus plasma of 100–180 times. Twenty-four hour urine samples were collected and pooled at room temperature from two healthy young men without a history of urolithiasis, renal diseases, metabolic disorders, or immunodeficiencies. The pooled urine had a pH of 6.09, showed normal on urine dipstick analysis (Multistix 8S6, Bayer, Leverkusen, Germany) and had a Coulter Counter particle count (Multisizer II with 100 μm orifice from Beckman Coulter, Mijdrecht, the Netherlands) of over 200 000 particles/ml, which is normal for unfiltered human urine. The urine was acidified to pH 3 with 3 M hydrochloric acid and passed through a filter (CH2 system with S1Y30 ultrafilter and

30 000 M_r cut-off, Millipore, Bedford, MA, USA), to remove insoluble particles. This lowered the particle count to less than 1600 particles/ml. The urine was divided into 250 ml samples to which pure indinavir sulphate (Merck, Sharp and Dohme, the Netherlands) was added to achieve final concentrations of 250, 300, 350, 400, 450, and 500 mg/l. The background particle count remained less than 1600 particles/ml for prolonged times. Four 50 ml urine portions of each indinavir concentration were rapidly titrated with sodium hydroxide to pH values of 5.8, 6.0, 6.2, and 6.5. Above pH 6.5 immediate indinavir precipitation occurred at all concentrations. Immediately after the target pH was reached, a 0 min particle count was performed. The absence or presence of particles was reconfirmed by light microscopy and scanning electron microscopy. Particles were identified as indinavir crystals on the basis of their starburst appearance and infrared spectrophotometric analysis. This was repeated every 30 min. At each time-point three particle size measurements were performed. When the average exceeded two times the initial particle number, we considered nucleation to have started.

As shown in Fig. 1, the lag time for indinavir crystallization is inversely related to its concentration and to urine pH. At pH values, 5.5–6.1, and indinavir concentrations, 250–500 mg/l, likely to be present in the urine of indinavir-treated patients, indinavir precipitates within 1–2.5 h. This time is reduced as the pH approaches 6.5 or the indinavir concentration approaches 400 mg/l. False-positive findings of crystalluria may occur after prolonged urine storage time (known to affect urine pH [15]), in samples after medication and in samples collected during postprandial peaks in urine pH [16]. To exclude false-positive findings, analysis should be performed without delay on freshly voided urine, collected after the bladder has been emptied, to avoid mixing with standing bladder urine, and after a fluid load has been ingested to wash out crystals from the renal distal tubuli.

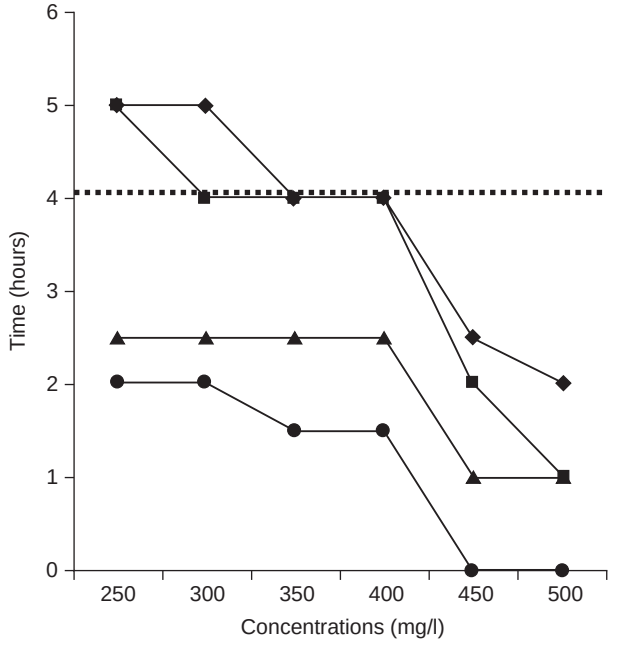

Fig. 1. Indinavir precipitation in urine.
Values below the dotted line (the maximum observation time) represent conditions in which precipitation was observed within 4 h at the given concentration and pH.
pH values: ——◆—— 5.8; ——■—— 6.0; ——▲—— 6.2; ——●—— 6.5.

Saima Salahuddin, Yeffrey S. Hsu, Niels P. Buchholz, Jeanne P. Dieleman, Inge C. Gyssens and Dik J. Kok,
Departments of Urology and Internal Medicine, EMCR, Rotterdam, the Netherlands.

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Homocysteinaemia in HIV-infected patients treated with highly active antiretroviral therapy

Metabolic abnormalities, i.e. hypercholesterolaemia, hypertriglyceridaemia and hyperglycaemia, are increasingly reported in HIV-infected patients treated with a protease inhibitor (PI)-containing regimen [1]. Therefore, there is a need to recognize additional cardiovascular risk factors that could predispose this population to accelerated atherosclerosis.

A substantial body of epidemiological evidence suggests an association between cardiovascular risk and moderately increased plasma homocysteine levels [2]. Like smoking or hypercholesterolaemia, elevated plasma homocysteine levels confer an independent risk of vascular disease. Moreover, it shows a multiplicative effect on risk among cigarette smokers and patients with hypertension.

We investigated the prevalence of hyperhomocysteinaemia, assessing total plasma homocysteine (tHcy), in patients on PI-containing highly active antiretroviral therapy (HAART) and compared the plasma levels of tHcy in HIV-infected patients with those in healthy controls. One study performed on HIV-infected patients in the pre-HAART era [3] found normal total homocysteine concentrations.

The 82 adult subjects (male : female ratio 53 : 29; median age: men 39 years, women 36 years, range 26–59) were participants in the Swiss HIV Cohort Study. At the time of blood collection they were all on PI-containing antiretroviral therapy. Blood was sampled after overnight fasting and ethylene diamine tetraacetic acid plasma was stored at −80°C for a maximum of 530 days. The tHcy concentration was measured in the plasma by high performance liquid chromatography (and fluorometric detection; column Suplelcosil LC 18–25 cm × 4.6 mmID) under isocratic conditions at room temperature using an acetate buffer (flow rate 1.2 ml/min). Hyperhomocysteinaemia was defined as plasma tHcy levels exceeding the 95th percentile of distribution among healthy controls (N = 80; male : female ratio 51 : 29; median age: men 33 years, women 26 years, range 20–49): for women over 11.3 μmol/l, for men over 12.6 μmol/l.

Differences between medians were analysed using the Wilcoxon–Mann–Whitney test. Differences between frequencies of hyperhomocysteinaemia in the study groups were analysed by the Fisher exact test for independent samples. The STATA for Windows package (version 6.0; Stata Corporation, College Station, TX, USA) was used for statistical analysis.

We found significantly higher fasting plasma homocysteine values in HIV-infected patients on HAART than in healthy controls (Table 1). According to our data analysis by sex, the difference between both groups, i.e. HIV-infected patients and healthy controls, was only statistically significant for men, but not for women. Although men on HAART were on average 6 years older, and women 10 years older than healthy controls, this difference was too small to affect the total homocysteine values consistently. Furthermore, the prevalence of hyperhomocysteinaemia was higher in patients on HAART than in healthy controls for both sexes (Table 2).

Genetic defects in the enzymes involved in homocysteine metabolism, nutritional deficiencies in the vitamin co-factors (folate, vitamin B_{12}, and vitamin B_6), and less frequently, the use of drugs interfering in homocysteine metabolism, can lead to elevated plasma tHcy [4]. As we were unable to assess accurately the vitamin concentrations in the stored plasma, we can not speculate on the possible cause of elevated plasma tHcy in HIV-infected patients on HAART. Whereas the use of trimethoprim has been reported to increase the total homocysteine concentration, no difference was found in the total homocysteine concentration between patients receiving and those not receiving trimethoprim as a component of prophylaxis against *Pneumocystis carinii* pneumonia (data not shown).

Hyperhomocysteinaemia was mild in almost all cases, but one man showed a moderate to high plasma tHcy of 59 μmol/l, related to a homozygous C677T mutation of the methylene-tetrahydrofolate reductase gene as assessed by polymerase chain reaction.

Hyperhomocysteinaemia may have a multiplicative effect on vascular risk in HIV-infected patients with PI-induced hyperlipidaemia. In this regard, our anecdotal observation of a man with hyperhomocysteinaemia and concomitant hyperlipidaemia who developed

Table 1. Median (range) total plasma homocysteine concentrations in healthy controls and in patients on highly active antiretroviral therapy.

		Healthy controls		HAART patients	*P* value
Male tHcy (μmol/l)	n = 51	7.8 (5.0–14.0)	n = 47	9.1 (5.1–15.9)	0.021
Female tHcy (μmol/l)	n = 29	7.4 (4.3–12.1)	n = 26	8.25 (4.3–13.4)	0.19
All	n = 80	7.6 (4.3–14.0)	n = 73	8.7 (4.3–15.9)	0.0094

HAART, Highly active antiretroviral therapy; tHcy, total plasma homocysteine.

Table 2. Prevalence of hyperhomocysteinaemia in healthy controls and in patients on highly active antiretroviral therapy.

Subjects	Hyperhomocysteinaemia	*P* value
Male healthy controls (n = 51)	3 (5.9%)	
Male HAART patients (n = 47)	6 (12.8%)	0.31
Female healthy controls (n = 29)	1 (3.4%)	
Female HAART patients (n = 26)	3 (11.5%)	0.34

HAART, Highly active antiretroviral therapy.

an incidental stroke is of particular concern [5]. HIV-infected patients with multiple risk factors for vascular disease could thus benefit from a determination of the total homocysteine concentration. This recommendation is justified considering the fact that a simple intervention, i.e. folate and eventually vitamin B_{12} and vitamin B_6 supplementation, successfully and rapidly decreases plasma homocysteine concentrations within a few weeks after the initiation of vitamin therapy.

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Appendix

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Enos Bernasconi[a], Mario Uhr[b], Lorenzo Magenta[a], Aldo Ranno[b], Amalio Telenti[c] and the Swiss HIV Cohort Study, *[a]Infectious Disease Unit, Department of Medicine, Regional Hospital, Lugano, Switzerland; [b]Laboratory for Special Biochemical Analysis, Ligornetto, Switzerland; and [c]Division of Infectious Diseases, CHUV University Hospital, Lausanne, Switzerland.*

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Prevalence of antiretroviral resistance in a South London cohort of treatment-naive HIV-1-infected patients

Data from North America, Europe and Australia suggest that the transmission of drug-resistant HIV is an emerging public health problem [1]. Conservative estimates indicate that the prevalence of resistance to at least one antiretroviral drug may be as high as 14% in treatment-naive patients with early infection (< 12 months' duration) and 12% in those with established infection [1]. The transmission of multidrug-resistant HIV has also been reported at a prevalence of 1–4% [1]. As baseline resistance profiles predict responses to antiretroviral therapy [2], the consensus is emerging that resistance testing should be considered for all patients before the initiation of therapy [3,4]. However, variations in prevalence rates between different geographical areas and risk groups are to be expected, and epidemiological surveys are needed to inform local and national guidelines for testing. Data from the UK remain limited [5]. The benefit of testing patients with established infection also needs to be defined, as resistant mutants may revert to wild type soon after transmission in the absence of drug pressure.

Our aim was to determine the prevalence of antiretroviral resistance in treatment-naive patients with defined seroconversion dates between January 1996 and October 2000 attending two HIV clinics in south London. These centres care for a heterogeneous population, including a large proportion of patients from sub-saharan Africa and the Caribbean basin. Of the 60 eligible patients, 14 had acute seroconversion illness, 25 had early infection (< 12 months interval between first positive and last negative

Table 1. Antiretroviral resistance mutations in 60 treatment–naive HIV–infected patients.

Patient[a]	Ethnicity[b]	Subtype	Year of HIV diagnosis	Length of infection	Risk group[c]	Mutations	
						RT	Protease
M75	C	B	1997	Acute	MSM	–	L63P V77I
M20	C	B	1997	Acute	MSM	–	L63P A71T/A
F92	AC	C	1998	Acute	Hetero	–	M36I
M52	C	B	1998	Acute	MSM	–	V77I
M87	C	B	1998	Acute	MSM	–	L10V
M23	C	B	1998	Acute	MSM	–	–
M09	C	B	1999	Acute	MSM	–	–
M81	C	B	1999	Acute	MSM	–	L63P
M73	C	B	1999	Acute	MSM	–	V77I
M90	C	C	1999	Acute	Hetero	–	M36I
F98	AC	B	2000	Acute	Hetero	–	L63P V77I
F99	AC	B	2000	Acute	Hetero	–	L63P
F60	SSA	G	2000	Acute	Hetero	K103T	K20I M36I M46I V77I
M19	C	B	2000	Acute	MSM	–	L63T/P
M96	C	B	1996	Early	MSM	–	L63P, A71V, V77I
M10	C	B	1996	Early	MSM	–	–
M67	C	B	1996	Early	MSM	–	L63P
F04	AC	D	1997	Early	Hetero	K103K/R/I	M36I L63P V77I
M93	C	B	1997	Early	MSM	–	V77I/V
M98	C	B	1997	Early	MSM	M184V	–
M57	C	B	1998	Early	MSM	–	M36I L63P
M22	C	B	1998	Early	MSM	–	L63P V77I
M84	C	B	1999	Early	MSM	–	L63P V77I
M16	C	B	1999	Early	MSM	K103R	L63P
M58	C	B	1999	Early	MSM	–	V77I
M94	C	B	1999	Early	Hetero	–	V77I
M89	AC	B	1999	Early	MSM	–	L10I
M74	C	B	1999	Early	MSM	–	L63P V77I
M55	SSA	B	1999	Early	Hetero	–	K20I M36I
M01	AC	B	1999	Early	MSM	–	–
F69	AC	B	2000	Early	Hetero	–	K20I M36I
M18	C	B	2000	Early	MSM	–	V77I/V
M56	AC	B	2000	Early	MSM	–	K20I M36I L63P
M70	C	B	2000	Early	MSM	–	V77I
M42	C	B	2000	Early	MSM	–	L63P
M02	C	B	2000	Early	MSM	–	L10V M36I/M
M99	C	B	2000	Early	MSM	M41L T215D	–
M36	C	B	2000	Early	MSM	–	L63P
M14	C	B	2000	Early	MSM	–	M36I
F17	SSA	B	1996	Established	Hetero	–	K20I M36I
M21	C	B	1996	Established	MSM	–	M36I
M50	C	B	1996	Established	MSM	–	–
M38	C	B	1996	Established	MSM	–	V77I
M33	C	B	1997	Established	MSM	M41L T215C	L63P/L
M32	C	B	1997	Established	MSM	–	–
F18	SSA	A	1998	Established	Hetero	–	M36I L63P/L
M08	SSA	D	1998	Established	Hetero	–	K20R M36I
F49	AC	B	1999	Established	Hetero	–	–
M15	C	B	1999	Established	MSM	–	–
M77	C	B	1999	Established	MSM	–	M36I L63P
M26	C	B	1999	Established	MSM	–	–
F09	C	G	2000	Established	IVDU	–	K20I M36I
M39	C	B	2000	Established	MSM	–	–
M43	C	B	2000	Established	MSM	–	L63P V71V
M27	C	B	2000	Established	MSM	–	L63P
M29	C	A	2000	Established	MSM	–	M36I
M25	SSA	C	2000	Established	Hetero	A98A/S	M36I, L63P/S
M12	C	B	2000	Established	MSM	–	L63P V77I
M11	AC	B	2000	Established	MSM	–	M36I L63P
M51	C	B	2000	Established	MSM	–	V77I

[a]M and F numbers indicate male and female patients, respectively.
[b]AC, Afro-Caribbean; C, Caucasian; SSA, sub-saharan African.
[c]Hetero, Heterosexual; IVDU, intravenous drug user; MSM, men who have sex with men.

antibody test), and 21 had established infection (> 12 months interval between first positive and last negative antibody test). Forty-four (73%) were Caucasian, 10 (17%) afro-Caribbean, and six (10%) sub-saharan Africans. Risk factors for infection were male homosexual contact (45, 75%), heterosexual contact (14, 23%), and intravenous drug use (one). Genotypic resistance was determined by direct sequencing of RNA from stored plasma samples (TruGene assay, Visible Genetics, Evry, France). Viral subtypes were determined from consensus reverse transcriptase (RT) and protease sequences (NCBI HIV-1 Genotyping Tool).

Fifty-one (85%) patients were infected with B and nine (15%) with non-B subtype virus (Table 1). Three patients (subtype B) had key mutations conferring resistance to nucleoside RT inhibitors. Two patients had M41L with either T215D or T215C. M41L arises during treatment with zidovudine and to a lesser extent stavudine, and confers high-level resistance to zidovudine when present with other mutations, including T215F/Y. T215D/C are unusual mutations, which may represent incomplete reversion from zidovudine-resistant to wild-type virus [6]. T215C has also been associated with resistance to zalcitabine. The two patients had early and established infection, respectively, suggesting persistence and good replication competence of the transmitted mutants. The third patient had the M184V mutation, which confers high-level resistance to lamivudine, and may also affect susceptibility to didanosine, zalcitabine and abacavir.

Four patients had mutations at RT codons involved in resistance to the non-nucleoside RT inhibitors. However, only one (subtype G) had a key mutation, K103T, which confers resistance to delavirdine [7], and may also have resistance effects for nevirapine and efavirenz. Other mutations were K103R (subtype B), K103K/R/I (subtype D), and A98S (subtype C), which are likely to represent natural polymorphisms.

The key protease mutation M46I was detected in one patient (subtype G). In combination with other mutations, M46I reduces susceptibility to indinavir and other protease inhibitors (PI). Forty-eight (80%) patients had accessory protease mutations (codons 10, 20, 36, 63, 71 and 77), which are likely to reflect natural polymorphisms. Although individually these mutations have limited effects on drug susceptibility, they may influence the rate at which PI-resistant viruses are selected during therapy [8]. Whereas most patients had only one or two mutations, four patients had three, with potential cumulative effects on PI resistance. The predominant mutations in B subtypes were those at codons 63 (22, 43%) and 77 (16, 31%), whereas M36I was found in all non-B subtypes.

In conclusion, in south London there remains a relatively low prevalence (4/60, 7%) of key resistance mutations in treatment-naive patients with defined seroconversion dates between 1996 and 2000. The prevalence is lower than that reported in other studies of patients with similar risk factors [1,9], suggesting that as yet undefined demographic factors influence the likelihood of acquiring drug-resistant HIV. As patients with defined seroconversion dates are a highly selected subgroup of treatment-naive patients, a study of a south London cohort that is representative of all risk groups is ongoing. Consistent with previous findings [10], a high degree of polymorphism was detected in the protease gene of both B and non-B subtypes. The clinical significance of this observation and the implications on the choice of antiretroviral therapy remain to be established.

Anna Maria Geretti[ab], Melvyn Smith[b], Natasha Osner[b], Siobhan O'Shea[c], Ian Chrystie[c], Philippa Easterbrook[a] and Mark Zuckerman[b], *[a]Academic Department of HIV Medicine, and [b]Department of Virology, King's College Hospital, London, UK; and [c]Department of Infection, Virology Section, St Thomas' Hospital; King's, Guy's and St Thomas' School of Medicine, London, UK.*

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