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Increased haemoglobin A_2 percentage in HIV infection: disease or treatment?

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An elevated haemoglobin A_2 percentage has been reported in HIV-infected patients, possibly attributable to therapy. In cross-sectional and cohort studies we have established that A_2 is often elevated in untreated patients; a further rise during treatment is attributable specifically to zidovudine. The haemoglobin A_2 may be high enough to lead to a misdiagnosis of beta thalassemia trait if there is a lack of awareness of this unexpected effect of HIV infection and its treatment.

An increased haemoglobin A_2 percentage is an important diagnostic criterion for the diagnosis of beta thalassemia trait. Other causes of an increased percentage have, until recently, been uncommon, but in 1993 Routy and colleagues [1] described an increased A_2 percentage in HIV-infected patients, some of whom were being treated with zidovudine. They found that 24 out of 78 patients on zidovudine had an A_2 above 3.5%, whereas this was seen in none of the 68 patients not on zidovudine [1]. No comparison of HIV-infected and normal individuals was performed. The increased A_2 percentage in HIV-infected patients on treatment was confirmed by Howard and colleagues [2] in 2005. They reported two patients, both on zidovudine, with A_2 percentages of 4.3 and 3.9, respectively (normal range 1.5–3.5%), in one of whom beta thalassemia trait was excluded by DNA analysis. In both these reports the increased A_2 percentage was attributed to the therapy. Our own observations during routine thalassemia screening suggested that HIV-positive patients who were not on antiretroviral therapy also sometimes had an increased A_2 percentage. We therefore carried out a systematic study to determine the frequency of an increase in haemoglobin A_2 and to establish to what extent this was related to the HIV infection itself and to what extent to the treatment.

We performed both cross-sectional and cohort studies. In the cross-sectional study we performed a full blood count and measured the haemoglobin A_2 percentage in 60 HIV-positive patients, 30 on HAART and 30 not on therapy. Three other HIV-positive patients were excluded because they were found to have sickle cell trait, which increases the measured haemoglobin A_2 percentage by the technique we used (two patients) or thyrotoxicosis, which also raises the haemoglobin A_2 percentage (one patient). The A_2 percentages were compared with those of healthy haematologically normal volunteers using an unpaired *t*-test, and the proportions of elevated levels were compared using a χ^2 test. The A_2 percentage was related to haematological variables. A χ^2 test was used to study the relationship between elevated haemoglobin A_2 and individual drugs.

In the cohort study the same tests were performed on 23 HIV-positive patients immediately before starting on HAART and 4 months or more after starting treatment. The haemoglobin A_2 percentages were compared using a paired *t*-test.

Full blood counts were performed on a Coulter LH700 automated blood cell analyser (Beckman–Coulter, Florida, USA). Haemoglobin A_2 was determined by high performance liquid chromatography on a Bio-Rad variant II instrument (Bio-Rad, Hercules, California, USA) using the Beta Thal Short Programme. A reference range for haemoglobin A_2 was derived from 40 healthy northern European volunteers with normal red cell indices.

The study was approved by our institutional research ethics committee. All patients were given detailed information as to the purpose of the study and gave written informed consent. Healthy volunteers were fully informed and gave verbal consent.

The normal volunteers were found to have a mean haemoglobin A_2 of 2.89% (SD 0.179). HIV-positive patients not on treatment had a mean haemoglobin A_2 of 3.09% (SD 0.255). This was significantly different from the values in the normal volunteers on an unpaired *t*-test ($t = -3.711$, $P < 0.0001$). HIV-positive patients on HAART had a mean haemoglobin A_2 of 3.36% (SD 0.50; Fig. 1). The haemoglobin A_2 percentage was significantly higher than values in normal volunteers ($t = -5.449$, $P < 0.0001$) and also values in untreated HIV-positive patients ($t = -2.664$, $P < 0.01$). Half of the treated patients were taking zidovudine and half were not. A χ^2 test showed a significant association between this drug and an elevated haemoglobin A_2 (elevated in 13/15 on zidovudine versus 3/15 not on zidovudine, $P < 0.0001$). No significant relationship to any other individual antiretroviral agent was found.

In the prospective cohort study, haemoglobin A_2 percentages were significantly different from those of normal controls both before and after starting HAART ($P < 0.001$ pretreatment and $P < 0.02$ post-treatment).

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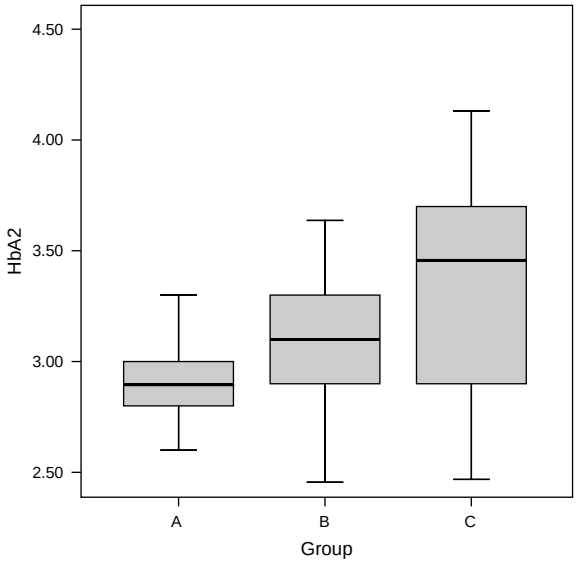

Fig. 1. Mean haemoglobin A_2 percentage in normal subjects (A), untreated HIV-positive patients (B) and HIV-positive patients taking antiretroviral therapy (C). The bold black horizontal lines in the centre of each of the boxes represent the median haemoglobin A_2 percentage (HbA_2%) for each of the groups. The boxes represent the upper and lower centiles. The whisker lines projected above and below the boxes represent the 2.5 and 97.5% values of the haemoglobin A_2 percentage found in each group.

Two patients (two of the three who were taking zidovudine) had an appreciable rise in haemoglobin A_2 after commencing HAART, both rising from high in the normal range to clearly above the normal range (3.9 and 4.4%, respectively). This occurred simultaneously with a marked increase in the mean cell volume (MCV), from 83 to 116 fl and from 89 to 120 fl; in the third patient with only a minor change in the haemoglobin A_2 percentage (3 to 3.2), the rise in the MCV was less (91 to 101 fl). Two patients had an appreciable fall in haemoglobin A_2; otherwise changes were minor. Overall, there was no significant change in the haemoglobin A_2 percentage with therapy of 4 months or more duration (mean haemoglobin A_2 pretherapy 3.0957, mean haemoglobin A_2 post-therapy 3.0870, $t = 0.117$, $P > 0.05$).

We have confirmed that HIV infection is associated with a significant increase in the haemoglobin A_2 percentage. This difference is seen in both treated and untreated patients. In a cross-sectional study, patients on HAART had a significantly higher haemoglobin A_2 percentage than those not on treatment. The higher percentage of haemoglobin A_2 in treated patients correlates with zidovudine therapy.

The rise in the haemoglobin A_2 percentage in HIV-infected patients is clinically significant. If haematologists are not aware of this effect of HIV infection and its treatment, a misdiagnosis of beta thalassemia trait may occur, the absence of typical red cell indices being attributed to antiviral agents that raise the MCV. Diagnostic confusion is even more likely if the haemoglobinopathy laboratory is not aware of the patient's HIV status. Increasing numbers of HIV-infected women are becoming pregnant, and zidovudine remains an integral part of regimens to prevent mother-to-child transmission. A lack of awareness of the effect of HIV *per se* and of therapy with zidovudine on haemoglobin A_2 may lead to unnecessary investigation and anxiety.

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References

1. Routy JP, Monte M, Beaulieu R, Toma E, St Pierre L, Dumont M. **Increase of hemoglobin A_2 in human immunodeficiency virus-1-infected patients treated with zidovudine.** *Am J Hematol* 1993; **43**:86–90.
2. Howard J, Henthorn JS, Murphy S, Davies SC. **Implications of increased haemoglobin A_2 levels in HIV-positive women in the antenatal clinic.** *J Clin Pathol* 2005; **58**:556–558.

WHO HIV clinical staging or CD4 cell counts for antiretroviral therapy eligibility assessment? An evaluation in rural Rakai district, Uganda

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The ability of WHO clinical staging to predict CD4 cell counts of 200 cells/μl or less was evaluated among 1221 patients screened for antiretroviral therapy (ART). Sensitivity was 51% and specificity was 88%. The positive predictive value was 64% and the negative predictive value was 81%. Clinical criteria missed half the patients with CD4 cell counts of 200 cells/μl or less, highlighting the importance of CD4 cell measurements for the scale-up of ART provision in resource-limited settings.

The World Health Organization (WHO) guidelines recommend that antiretroviral therapy (ART) be started for all patients with CD4 cell counts of 200 cells/μl or less irrespective of clinical stage or total lymphocyte counts less than 1200 cells/μl in symptomatic patients. When CD4 cell counts or total lymphocyte counts cannot be performed, patients with WHO clinical stage III or IV disease may be offered ART [1]. In sub-Saharan Africa, when access to CD4 cell counts or total lymphocyte

counts is limited, clinical criteria are frequently used to initiate therapy. It is not known, however, how well the clinical criteria predict a CD4 cell count of 200 cells/μl or less. Studies have documented a higher mortality risk or development of AIDS among patients who started ART with very low CD4 cell counts [2,3].

If WHO clinical criteria lag behind immunological criteria, this could potentially result in treatment delays and poorer outcomes among patients started on ART based on clinical criteria alone.

There are limited data to evaluate the utility of the WHO clinical criteria to predict CD4 cell counts of 200 cells/μl or less. One hospital-based study in Cambodia compared the use of clinical criteria alone versus a combination of the 2003 WHO clinical and immunological criteria, and showed high sensitivity (96%) and accuracy (89%) [4]. Findings from that study only apply to very sick patient populations, because approximately 86% of the study population had clinical stage III or IV disease. Another study conducted in primary healthcare facilities in south Africa showed that 24% of patients classified as WHO stage 1 and 46% classified as stage II had CD4 cell counts of 200 cells/μl or less [5]. A substantial proportion of stage I and II cases would thus not have initiated ART if therapy was based on clinical criteria alone. Clinical assessment in that study was primarily done by nurses and there was a time lag of up to 90 days between clinical assessment and CD4 cell counts, potentially leading to misclassification.

We analysed data on patients seen in the Rakai Health Sciences Programme (RHSP) community-based ART programme to assess whether WHO clinical stages III or IV could be used to identify individuals with CD4 cell counts of 200 cells/μl or less. The majority of patients were participants in the population-based Rakai Community Cohort Study (RCCS) and thus were likely to be representative of the HIV-infected population in Rakai.

In 2004, RHSP started the provision of ART in rural Rakai district in southwestern Uganda, with funding from the Presidential Emergency Plan for AIDS Relief through the Centers for Disease Control and Prevention, Uganda. Patients for this evaluation participated in RCCS and the ART-related clinical study evaluating the epidemiological, demographic and behavioural effects of antiretroviral drugs in Rakai. Through the RCCS, serosurveys are conducted in 48 communities every 12–16 months among 13 000 participants aged 15–49 years. Approximately 94% of participants provided blood for HIV testing and approximately 80% agreed to receive their HIV results and post-test counseling. HIV-positive participants were referred to 16 community-based clinics, to screen for ART eligibility. Patients enrolled in the clinics were invited to participate in the ART-related clinical study.

A fluorescence-activated cell sorter count (Becton Dickson, Franklin Lakes, New Jersey, USA) located at the RHSP facility in Rakai was used for CD4 cell counts. Clinical staging was conducted by clinicians who were trained in HIV care at the Infectious Diseases Institute in Kampala, Uganda; additional weekly technical support was provided on-site in Rakai by an expert infectious disease physician from the Infectious Diseases Institute. Clinical assessment was carried out 2 weeks after the CD4 cell counts.

The RCCS and ART-related clinical studies were approved by the Ugandan Science and Ethics committee and the US Western internal review board. Patients provided consent to have their clinical data used for research.

Sensitivity and specificity were calculated by computing 2×2 tables, comparing clinical stage III or IV with CD4 cell counts of 200 cells/μl or less (as the reference). The Wilson score method without continuity correction was used to calculate 95% confidence intervals (CI) around the estimates. The analysis used STATA software (release 8.0; Stata Corp., College Station, Texas, USA).

As shown in Table 1, a total of 1221 patients were evaluated. The median age was 34 years (interquartile

Table 1. Perfomance of World Health Organization clinical criteria against CD4 cell counts for antiretroviral therapy eligibility assessment in Rakai, Uganda.

	CD4 cell counts (cells/μl)				
	≤100	101–200	Sub-total (≤200)	>200	Total
WHO stage					
Total	195	169	364	857	1221
III or IV	124	62	186	106	292
I or II	71	107	178	751	929
Sensitivity (%)	64	37	51		
Specificity (%)	88	88	88		
Positive predictive value (%)	54	37	64		
Negative predictive value (%)	91	88	81		

WHO, World Health Organization. The comparison group with CD4 cell counts above 200 cells/μl was kept constant for all the subgroup analyses.

range 29–40), and 65% of patients were women. Overall, 24% of patients had clinical stage III or IV, 16% had CD4 cell counts of 100 cells/μl or less, 14% had CD4 cell counts between 100 and 201 cells/μl, and 70% had CD4 cell counts above 200 cells/μl.

The sensitivity of clinical stage III or IV to detect individuals with CD4 cell counts of 200 cells/μl or less was 51% (95% CI 46–56%), specificity was 88% (95% CI 85–90%), the positive predictive value was 64% (186/292, 95% CI 58–69%), and the negative predictive value was 81% (751/929, 95% CI 78–83%). Sensitivity was higher (64%, 95% CI 57–70%) for the subgroup of patients with CD4 cell counts of 100 cells/μl or less compared with patients with CD4 cell counts of 100–200 cells/μl (37%, 95% CI 30–44%; $P < 0.001$).

The WHO clinical criteria missed approximately half the patients with CD4 cell counts of 200 cells/μl or less. The proportion of patients missed was highest (63%) for patients with CD4 cell counts between 100 and 201 cells/μl and lowest (36%) for patients with CD4 cell counts of 100 cells/μl or less.

Our findings support previous evidence that WHO clinical criteria provide an unsatisfactory screening tool for ART eligibility in settings in which CD4 cell technologies are unavailable. The exclusive use of clinical criteria would better identify patients with CD4 cell counts of 100 cells/μl or less, but this would translate into the initiation of ART in patients with advanced disease and potentially led to poor treatment outcomes.

As the patient population with advanced HIV declines, as a result of mortality and the availability of antiretroviral drugs, the clinical criteria will become less sensitive and thus less useful as a screening tool.

Our findings have important implications for the scale-up of ART in resource-limited settings in which the exclusive use of the clinical criteria could deny or delay ART to approximately half the patients with CD4 cell counts of 200 cells/μl or less, and patients who eventually start ART would potentially be at an increased risk of poor treatment outcomes.

In conclusion, CD4 cell screening facilities are urgently needed as an adjunct to clinical criteria, to facilitate screening for ART in resource-limited settings.

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References

1. World Health Organization. *Antiretroviral therapy for HIV infection in adults and adolescents in resource-limited settings: towards universal access: recommendations for a public health approach* (2006 revision). Geneva, Switzerland: WHO; 2006.
2. Egger M, May M, Chene G, Phillips AN, Ledergerber B, Dabis F, *et al*. **Prognosis of HIV-1-infected patients starting HAART: a collaborative analysis of prospective studies.** *Lancet* 2002; **360**:119–129.
3. Kaplan JE, Hanson DL, Cohn DL, Karon J, Buskin S, Thompson M, *et al*. **When to begin highly active antiretroviral therapy? Evidence supporting initiation of therapy at CD4+ lymphocyte counts < 350 cells/microl.** *Clin Infect Dis* 2003; **37**:951–958.
4. Lynen L, Thai S, De Munter P, Leang B, Sokkab A, Schrooten W, *et al*. **The added value of a CD4 count to identify patients eligible for highly active antiretroviral therapy among HIV-positive adults in Cambodia.** *J Acquir Immune Defic Syndr* 2006; **42**: 322–324.
5. Martinson N, Heyer A, Steyn J, Struthers H, McIntyre J, Gray G, Pronyk P. **Does WHO clinical stage reliably predict who should receive ARV treatment?** In: *3rd IAS Conference on HIV Pathogenesis and Treatment*. Rio de Janeiro, Brazil, 24–27 July 2005 [Abstract WeFo0304].

Key amprenavir resistance mutations counteract dramatic efficacy of darunavir in highly experienced patients

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In highly experienced HIV-1-infected patients, a ritonavir-boosted darunavir-containing regimen was associated with dramatic immunological and virological efficacy. Patients harbouring viruses with amprenavir-specific resistance profiles, such as I50V or V32I + I47V, failed on a darunavir/ritonavir-containing regimen. These key amprenavir mutations were also selected at the time of failure, suggesting their impact on darunavir efficacy.

The extensive cross-resistance that exists between currently approved protease inhibitors (PI) concerns a large number of HIV-1-infected patients. Despite the pharmaco-enhancement of PI plasma exposure with ritonavir and consequently the increase in the resistance

level, the efficacy of boosted PI combinations remains greatly influenced by the extent of baseline PI resistance mutations [1]. The accumulation of resistance mutations on the protease gene limits the sequential use of these agents [2].

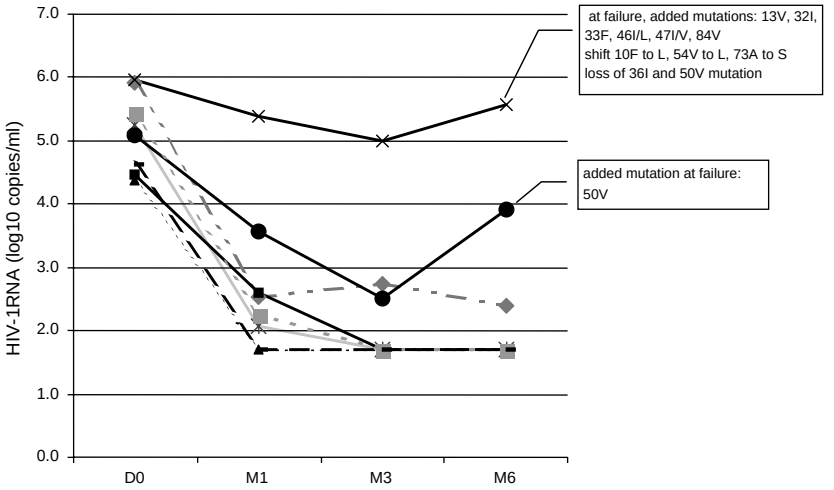
Darunavir (TMC 114) is an investigational PI now available for heavily treatment-experienced patients through an expanded access programme. In-vitro data suggest that darunavir has extremely potent antiretroviral activity (EC_{50} 1–5 nmol) and is able to maintain this activity against HIV variants that are highly cross-resistant to current PI. The in-vitro selection experiments starting from wild-type HIV-1 also showed that darunavir has a very high genetic barrier to the development of resistance [3]. This potent activity against PI-resistant HIV indicates that this new antiretroviral agent could be particularly useful in treating patients failing a PI-containing regimen [4]. In Power randomized clinical studies [5,6], many PI-experienced subjects receiving darunavir/ritonavir (600/100 mg twice a day) achieved significantly greater viral load declines at 24 weeks than comparative PI. In a recently reported resistance substudy [7], the specific baseline PI mutations associated with reduced response to darunavir/ritonavir 600/100 mg were V11I, V32I, L33F, I47V, I50V, I54L/M, G73S, L76V, I84V, and L89V. That study also described the emergence of mutations V32I, L33F, I47V, I54L, and L89V in more than 10% of non-responder patients. It is therefore important to collect, in clinical practice, complementary resistance data that impact darunavir/ritonavir virological response. Factors associated with virological outcome to darunavir/ritonavir, particularly resistance mutations profile, in eight heavily experienced patients harbouring multidrug resistance viruses, were studied.

Since February 2006, the French Drug Agency has authorized the prescription of darunavir/ritonavir to HIV-1-infected patients with no available treatment option, through a temporary authorization for use. Patients who received a darunavir/ritonavir (600/100 mg twice a day)-containing regimen as salvage therapy for at least 3 months during precommercialization use were studied. Inclusion criteria were: (i) multiple failures to all current classes of antiretroviral agents, including ritonavir-boosted PI; (ii) plasma HIV-1 RNA greater than 10 000 copies/ml; and (iii) the presence of protease mutations associated with resistance to available approved PI. The baseline characteristics including CD4 cell count, HIV-1 viral load and genotypic resistance test, therapeutic history, and genotypic sensitive score (GSS) of associated antiretroviral agents were collected. Plasma darunavir trough concentrations were determined using a high-performance liquid chromatography-coupled fluorimetric detector (limit of quantitation 5 ng/ml).

Eight patients with severe immunosuppression [median (range) nadir of CD4 cell count 45 cells/μl (1–190)] began rescue therapy with darunavir/ritonavir associated with at least two reverse transcriptase inhibitors and enfuvirtide (Fig. 1). They had received a median of eight antiretroviral regimens (four to 12), with a mean of 6.2 nucleoside reverse transcriptase inhibitors, 1.4 non-nucleoside reverse transcriptase inhibitors and 4.7 PI (including amprenavir or fosamprenavir in all cases). Four patients were pretreated with tipranavir, four with enfuvirtide and three with both drugs. The median baseline CD4 cell count and plasma HIV-1 RNA were 19 cells/μl (4–550) and 5.2 $\log_{10}$ copies/ml (4.4–6), respectively. A baseline genotypic resistance test reported the median number of six nucleoside reverse transcriptase inhibitors (3–7), one non-nucleoside reverse transcriptase inhibitor (0–2) and 13 PI (10–15)-associated resistance mutations (Fig. 1). Among the darunavir-associated mutations, three patients harboured one, four patients harboured two and one patient harboured three mutations. According to the ANRS algorithm, all but one patient harboured darunavir/ritonavir-susceptible viruses. In contrast, darunavir resistance was intermediate for all viruses with regard to the Stanford algorithm. GSS was calculated with the baseline genotype and enfuvirtide was considered active only in non-pre-exposed patients. The median GSS was 1.25, ranging from zero to three active drugs associated with darunavir/ritonavir in the salvage regimen. The median darunavir trough concentration was 2612 ng/ml (1173–6932).

The median decrease in HIV-1 viral load from baseline was -2.8 $\log_{10}$ copies/ml at month 1, at month 3, and at month 6, respectively (Fig. 1). A plasma HIV-1 RNA of less than 400 and less than 50 copies/ml were observed at month 1 for six out of eight and two out of eight patients, at month 3 for six out of eight and five out of eight patients, and at month 6 for six out of eight and five out of eight patients, respectively. The median increase in CD4 cell counts from baseline was +57, +61, and +113 cells/μl at months 1, 3 and 6, respectively. Virological failure was reported in two patients (Fig. 1). In patient 4, the viral load was never suppressed and resistance analysis at month 6 showed new mutations 13V, 32I, 33F, 46I/L, 47I/V and 84V, a shift at mutated codons 10F to I, 54V to L, 73A to S and a loss of 36I and 50V. In patient 6, viral rebound was observed at month 6 with the selection of I50V to the same backbone protease resistance profile.

Risk factors leading to virological failure, such as multiple therapeutic regimens, tipranavir/enfuvirtide pre-exposure, a high degree of baseline drug resistance, low number of active drugs (low GSS), high baseline viral load and low baseline CD4 cell count were similar between the two darunavir regimen-failing patients and the six others. In spite of the high number of PI mutations and extensive amprenavir resistance according to ANRS and Stanford (HIV database) algorithms for all patients (Fig. 1), it seems important to emphasize that specific amprenavir resistance profiles, such as I50V and



	Drug associated with DRV	GSS	Baseline PI mutations (IAS & ANRS list)*	interpretation §			
				DRV/r		FPV/r	
				A	DB	A	DB
pt1	EFV, FTC, ABC, ENF	0.5	10I, 13V, 20R, **33F**, 43T, 54V, 60E, 62V, 63P, 71V, 82A, 83D, **84V**, 90M	S	I	R	R
pt2	TDF, FTC, ENF	1.5	10I, 13I/V, **33F/L**, 36L/M, 46L, **54L/V**, 58Q/E, 60E/D, 62V, 63I, 71V, 82A, 90M	S	I	R	I
pt3	TDF, FTC, ABC, ENF	3	10I, 35D, 36I, 41K, 43T, 46I, 54V, 63P, 71V, **73S**, 77I, 82F, 90M	S	I	R	I
pt4	TDF, FTC, ZDV, ENF	0	10F, **11V/I**, 20V, 36I, **50I/V**, 54V, 63P, 71V, 73A, 82T, **89L/V**, 90M	I	I	R	R
pt5	TDF, FTC, ENF	1	10I, 20R, 33I, 35D, 36I, 41K, 46I, 54V, 62V, 69K, 71V, 82T, **84V**, 89I	S	I	R	R
pt6	TDF, FTC, ENF	1	10I, 20R, **32I/V**, 35D, 36I, 46I, **47V**, 53L, 54V, 62V, 69K, 74P, 77I, 82T, 89M, 90M	S	I	R	R
pt7	ABC, 3TC, ENF	2	10I, 13I/V, **33F**, 41K, 46I, 54T/A, 63P, 71V, 82A, **84I/V**, 90M	S	I	R	R
pt8	TDF, FTC, ENF	2	10I, 20R, 35D, 36I, 46L, 54V, 62V, 63P, 71V, **73S**, 82A, 90M	S	I	R	R

Fig. 1. Virological response to darunavir/ritonavir-including regimen in eight heavily experienced patients. *Darunavir-associated resistance mutation among 11I, 32I, 33F, 47V, 50V, 54L/M, 73S, 76V, 84V, 89V is indicated in **bold**. §A, ANRS algorithm; DB, HIV database Stanford algorithm (S, sensitive; I, possible resistance or intermediate; R, probable resistance or high level of resistance). ABC, Abacavir; ANRS, Agence Nationale de Recherches sur le Sida; DRV, darunavir; DRV/r, darunavir/ritonavir; EFV, efavirenz; ENF, enfuvirtide; FTC, emtricitabine; GSS, genotypic sensitive score; IAS, International AIDS Society; PI, protease inhibitor; TDF, tenofovir; 3TC, lamivudine; ZDV, zidovudine.

V32I + I47V, were detected at baseline only in the two darunavir-failing patients. Moreover, the emergence of these two key resistance profiles occurred at virological failure in these two patients (Fig. 1). Interestingly, darunavir trough plasma concentrations were below the target value for resistant viruses of 2000 ng/ml [8] for the two failing patients (1173 and 1453 ng/ml) compared with the mean (3776 ng/ml) for the six others.

As darunavir was chemically similar to amprenavir [9], this result could be expected. In vitro data, however, showed that most clinical isolates resistant to amprenavir exhibited susceptibility to darunavir [3,10]. No impact of the previous use of fosamprenavir, tipranavir and lopinavir was observed on the darunavir/ritonavir virological response in Power studies [11]. These findings did, however, not take into account key amprenavir-selected mutations (I50V and V32I + I47V) [12], but only overall amprenavir susceptibility. As shown with other PI, the relative weight of resistance mutations may be determined to predict the efficacy of darunavir/ritonavir in experienced patients. This result suggests that antiretroviral treatment with darunavir in patients with key amprenavir resistance should be considered with caution. A further analysis of large clinical databases could assess the impact of the specific amprenavir resistance profile on the response to darunavir.

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References

1. Barreiro P, Camino N, de Mendoza C, Valer L, Nunez M, Martin-Carbonero L, *et al*. **Comparison of the efficacy, safety and predictive value of HIV genotyping using distinct ritonavir-boosted protease inhibitors.** *Int J Antimicrob Agents* 2002; **20**:438–443.
2. Johnson VA, Brun-Vezinet F, Clotet B, Kuritzkes DR, Pillay D, Schapiro JM, *et al*. **Update of the drug resistance mutations in HIV-1: fall 2006.** *Top HIV Med* 2006; **14**:125–130.
3. De Meyer S, Azijn H, Surleraux D, Jochmans D, Tahri A, Pauwels R, *et al*. **TMC114, a novel human immunodeficiency virus type 1 protease inhibitor active against protease inhibitor-resistant viruses, including a broad range of clinical isolates.** *Antimicrob Agents Chemother* 2005; **49**:2314–2321.

4. Poveda E, Blanco F, Garcia-Gasco P, Alcolea A, Briz V, Soriano V. **Successful rescue therapy with darunabir (TMC114) in HIV-infected patients who have failed several ritonavir-boosted protease inhibitors.** *AIDS* 2006; **20**:1558–1560.
5. Katlama C, Carvalho MT, Cooper D, De Backer K, Lefebvre E, Pedro R, *et al*. **TMC114/r outperforms investigator-selected PI(s) in 3-class-experienced patients: week 24 primary efficacy analysis of Power 1 (TMC114-C213).** In: *3rd IAS Conference on HIV Pathogenesis and Treatment*. Rio de Janeiro, Brazil, 24–27 July 2005 [Abstract WeOaLB0102].
6. Wilkin T, Haubrich R, Steinhart C, Becker S, Conant M, Vangeneugden T, *et al*. **TMC114/r superior of care in 3-class-experienced patients: 24-wks primary analysis of the Power 2 study (C202).** In: *45th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, USA, 16–19 December 2005 [Abstract H-413].
7. De Meyer S, Hill A, De Baere I, Rimsky L, Azijn H, VanBaelen B, *et al*. **Effect of baseline susceptibility and on-treatment mutations TMC114 and control PI efficacy.** In: *13th Conference on Retroviruses and Opportunistic Infections*. Denver, CO, USA, 5–8 February 2006 [Abstract 157].
8. Sekar V, Spinosa-Guzman S, Lefebvre E, Hoetelmans R. **Clinical pharmacology of TMC114 – a new HIV protease inhibitor.** In: *XVIth International AIDS Conference*. Toronto, Canada, 13–18 August 2006 [Abstract TUPE0083].
9. King NM, Prabu-Jeyabalan M, Nalivaika EA, Wigerinck P, de Bethune MP, Schiffer CA. **Structural and thermodynamic basis for the binding of TMC114, a next-generation human immunodeficiency virus type 1 protease inhibitor.** *J Virol* 2004; **78**: 12012–12021.
10. Picchio G, Staes M, Van Craenenbroeck E, Vermeiren H, Bacheler L, De Bethune MP, *et al*. **HIV-1 susceptibility to TMC114 among routine clinical samples with different levels of protease inhibitor susceptibility using linear regression model-based fold change predictions.** In: *46th Interscience Conference on Antimicrobial Agents and Chemotherapy*. San Francisco, USA, 27–30 September 2006 [Abstract 402].
11. Lefebvre E, de Bethune MP, De Meyer S, Vangeneugden T, De Pauw M, Cefalone M, *et al*. **Impact of use of TPV, LPV, and(f)APV at screening on TMC114/r virologic response in treatment-experienced patients in POWER 1, 2 and 3.** In: *46th Interscience Conference on Antimicrobial Agents and Chemotherapy*. San Francisco, USA, 27–30 September 2006 [Abstract H-1387].
12. Maguire M, Shortino D, Klein A, Harris W, Manohitharajah V, Tisdale M, *et al*. **Emergence of resistance to protease inhibitor amprenavir in human patients: selection of four alternative viral protease genotypes and influence of viral susceptibility to coadministered reverse transcriptase nucleoside inhibitors.** *Antimicrob Agents Chemother* 2002; **46**:731–738.

An outbreak of HIV-1 subtype G among Italian injecting drug users

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We describe an outbreak of subtype G among injecting drug users (IDU) in northern Italy newly infected with HIV. We analysed *pol* gene sequences from samples of 139 individuals from different risk groups. Non-B subtypes were more frequently detected among IDU than in homosexual or heterosexual contacts. All G subtypes but one were found among IDU. The phylogenetic analysis indicated that the outbreak was of monophyletic origin and was caused by HIV-1 strains similar to those from western Africa.

Although subtype B is the dominant HIV-1 subtype in western countries, there has been increasing circulation of non-B subtype strains in Europe [1]. In Italy, the proportion of subtypes that were non-B was reported to be as low as 1.9% before 1997, but since that time this proportion has tended to increase, ranging from 4.7 to 12.6% [2–5]. Most non-B subtypes and circulating recombinant forms (CRF) have been found in hetero- sexual contacts, whereas among injecting drug users (IDU) the proportion of non-B subtypes and CRF remains very low [2].

Herein we describe an outbreak of subtype G among IDU in northern Italy newly infected with HIV. The samples were collected for the Italian Seroconversion Study (ISS), a prospective cohort of HIV seroconverters; the inclusion criteria for the ISS are the availability of a documented seronegative HIV test result, followed by a seropositive result within 12 months [6].

We analysed sequences from a subgroup of ISS participants recruited from five clinical centres, located in Milan, Turin and Rome. The subgroup consisted of 139 individuals (6.7% of the entire ISS cohort). Of them, 25 (18.0%) were IDU, 63 (45.3%) homosexual men, 47 (33.8%) heterosexual contacts, and four (2.9%) belonged to other/unknown exposure categories.

Protease and reverse transcriptase *pol* sequences were generated after RNA extraction, reverse transcriptase–polymerase chain reaction amplification, and automatic sequencing. A commercially available assay (ViroSeq v2 kit; Abbott Laboratories, Abbott Park, Illinois, USA) was used. The nucleotide sequences used in this study have been submitted to Gene-Bank (National Center for Biotechnology Information) under the following accession numbers EF219434 to EF219447.

All of the HIV-1 *pol* sequences were analysed using the REGA HIV-1 subtyping tool [7].

All G subtype sequences of the HIV-1 *pol* gene present in the HIV database (http://hiv-web.lanl.gov/content/index) were aligned with our 11 sequences (691 base pairs long), partial protease and partial RT region), using a subtype B reference sequence (B.US.98.1058) as the outgroup; the alignment was then manually edited using the Bioedit program [8]. The maximum likelihood phylogenetic tree was generated with the Hasegawua, Kishino and Yano (HKY-85) nucleotide substitution model under gamma distribution, using a neighbour-joining tree (Jukes–Cantor distance) as a starting tree [9]. The evolutionary model was chosen as the best-fitting nucleotide substitution model, according to the hierarchical likelihood ratio test implemented in the Model Test V3.0 software [10]. The statistical robustness and reliability of the branching order in the maximum likelihood phylogenetic tree was confirmed with a bootstrap analysis using 1000 replicates and with the

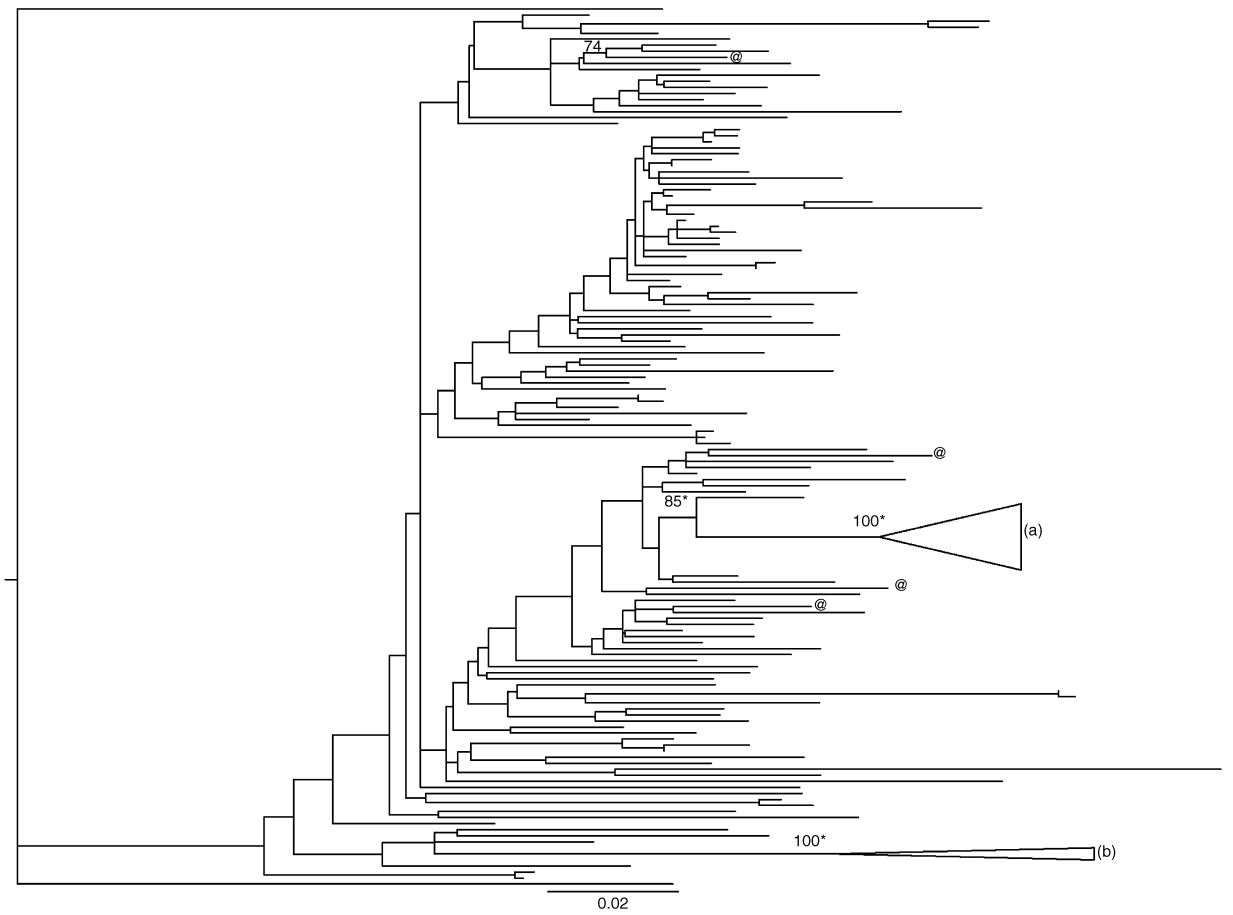

Fig. 1. Phylogenetic maximum likelihood unrooted genealogies of HIV-1 subtype G. Italian subtype G clades are represented by a triangle (a), previous Italian subtype G sequences are indicated by @, subtype G clades for Nigerians in Italy are represented by a triangle (b). Bootstrap scores greater than 700 (70% of 1000 replicates) are shown; $*P < 0.001$ (zero length branch test). Branch lengths are drawn to scale, with the bar at the bottom indicating 0.2 nucleotide substitutions per site.

zero branch length test. All calculations were performed with PAUP*4.0 software written by Swofford [9].

As expected, most participants were infected with subtype B (112 individuals, 80.6%). Non-B subtypes were more frequently detected among IDU (48.1%) than among homosexual men (25.9%) or heterosexual contacts (22.2%). The majority of non-B subtypes were subtype G (12 individuals, 9%), or undetermined (10 individuals, 7%).

Surprisingly, all G subtypes but one (11 of the 12 isolates) were found among IDU (the remaining individual was a woman belonging to the category of heterosexual contacts), and all of them were from Turin (north-western Italy); only one of them was not Italian (i.e. a man from Tunisia). The proportion of HIV-1 G subtype strains isolated after 1998 in Turin was 34.3%, of which 50% were isolated from individuals who seroconverted only in 2004, suggesting an increase over time of such a subtype in the northen Italian city. Overall, this subtype represented 64.7% of all strains identified among IDU between 1999 and 2004 (data not shown).

The phylogenetic analysis (Fig. 1) indicated that the outbreak was of monophyletic origin and was caused by HIV-1 strains that were similar to those from western Africa [11]. The phylogenetic relationships were supported by the bootstrap analysis, which produced a value of 100% between the Italian G subtypes, 85% between the Italian G subytpes and one of thewestern African strains (accession no. AY181075); the relationships were also confirmed by zero branch length test ($P < 0.001$).

This is the first report of an outbreak of HIV-1 subtype G among IDU in Italy. This was largely unexpected, because most infections reported among Italian IDU are caused by subtype B. In a previous study, all sequences from 145 European IDU, including 20 Italian IDU from Turin, belonged to subtype B [12], whereas few HIV-1 non-B clades were detected among IDU in Finland, Spain, and Switzerland [13–16]. Large epidemics of subtype A have occurred among IDU in several states of the former Soviet Union, and a new epidemic caused by a CRF03 A/B was reported among IDU in northern Russia [15].

With specific regard to subtype G, this has been detected only rarely in Italy, and only among heterosexual contacts [2,5]. Similarly, in other countries in the same geographical area (e.g. France), none of the identified G subtypes were detected among IDU [17].

The monophyletic origin of this outbreak probably indicates a single point of introduction. Although the likely source was not identified, our strain was similar to those from west Africa (i.e. Nigeria). The hypothesis that the introduction of the subtype is related to drug trafficking conducted by west African immigrants remains to be tested.

In conclusion, this outbreak of HIV-1 subtype G among Italian IDU suggests that viral diversity is also increasing in population groups that had been exclusively characterized by subtype B. The monophyletic origin and the explosive dynamic of this outbreak can be attributed to the efficiency of blood transmission among IDU. This report suggests that the introduction of different subtypes in a specific risk population may give rise to future epidemic waves of unpredictable outcome, highlighting the importance of continuing molecular surveillance of the HIV-1 non-B strains in Italy, as well as in other western European countries.

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References

1. Perrin L, Kaiser L, Yerly S. **Travel and the spread of HIV-1 genetic variants.** *Lancet* 2003; **3**:22–27.
2. Balotta C, Facchi G, Violin M, Van Dooren S, Cozzi-Lepri A, Forbici F, *et al*. **Increasing prevalence of non-clade B HIV-1 strains in heterosexual men and women, as monitored by analysis of riverse transcriptase and protease sequences.** *J Acquir Immune Defic Syndr* 2001; **27**:499–505.
3. Tramuto F, Vitale F, Bonura F, Romano N. **Detection of HIV type 1 non-B subtype in Sicily, Italy.** *AIDS Res Hum Retroviruses* 2004; **20**:251–254.
4. Buonaguro L, Tagliamonte M, Tornesello ML, Pilotti E, Casoli C, Lazzarin A, *et al*. **Screening of HIV-1 isolates by riverse heteroduplex mobility assay and identification of non-B subtypes in Italy.** *J Acquir Immune Defic Syndr* 2004; **37**:1295–1306.
5. Monno L, Brindicci G, Lo Caputo S, Punzi G, Scarabaggio T, Riva C, *et al*. **HIV-1 subtypes and circulating recombinant forms (CRFs) from HIV-infceted patients residing in two regions of Central and Southern Italy.** *J Med Virol* 2005; **75**:483–490.
6. Rezza G. **Determinants of progression to AIDS in HIV-infected individuals: an update from the Italian Seroconversion Study.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; **17 (Suppl. 1)**:S13–S16.
7. De Oliveira T, Deforche K, Cassol S, Salminen M, Paraskevis D, Seebregts C, *et al*. **An automated genotyping system for analysis of HIV-1 and other microbial sequences.** *Bioinformatics* 2005; **21**:3797–3800.
8. Hall TA. **Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98 NT.** *Nucl Acids Symp Ser* 1999; **41**:95–98.
9. Swafford DL. *PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b4. 4.0 edn*. Sunderland, MA, USA: Sinauer Associates Inc.; 1999.
10. Posada D, Crandall KA. **Model test: testing the model of DNA substitution.** *Bioinformatics* 1998; **14**:817–818.
11. Agwale SM, Zeh C, Paxinos E, Odama L, Pienazek D, Wambebe C, *et al*. **Genotypic and phenotypic analyses of human immunodeficiency virus type 1 in antiretroviral drug-naive nigerian patients.** *AIDS Res Hum Retroviruses* 2006; **22**:22–26.
12. Op de Coul ELM, Prins M, Cornelissen M, Van der Schoot A, Boufassa F, Brettle PR, *et al*. **Using phylogenetic analysis tot race HIV-1 migration among western European injecting drug users seroconverting from 1984 to 1997.** *AIDS* 2001; **15**:257–266.
13. Delgado E, Thomson MM, Villahermosa ML, Ocampo A, Miralles C, Rodriquez-Perez R, *et al*. **Identification of a newly characterized HIV-1 BG intersubtype circulating recombinant form in Galicia, Spain, which exhibits a pseudotype-like virion structure.** *J Acquir Immune Defic Syndr* 2002; **5**:536–543.
14. Thomson MM, Delgado E, Manjon N, Ocampo A, Villahermosa ML, Marino A, *et al*. **HIV-1 genetic diversity in Galicia Spain: BG intersubtype recombinant viruses circulating among injecting drug users.** *AIDS* 2001; **15**:509–516.
15. Liitsola K, Tashkinova I, Laukkanen T, Korovina G, Smolskaja T, Momot O, *et al*. **HIV-1 genetic subtype A/B recombinant strain causing an explosive epidemic in injecting drug users in Kaliningrad.** *AIDS* 1998; **12**:1907–1919.
16. Liitsola K, Ristola M, Holmstrom P, Salminen M, Brummer-Korvenkontio H, Simola S, *et al*. **An outbreak of the circulating recombinant form AECM240 HIV-1 in the Finnish injection drug user population.** *AIDS* 2000; **14**:2613–2615.
17. Granjean M, Bourlet T, Berthelot P, Fresard A, Fascia P, Cazorla C, *et al*. **Human immunodeficiency virus type 1 subtypes in cohort of infected patients, Saint-Etienne, France, from 1984 to 2003.** *Med Mal Infect* 2005; **35**:82–87.

Atazanavir-associated nephrolithiasis: cases from the US Food and Drug Administration's Adverse Event Reporting System

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The risk of nephrolithiasis associated with atazanavir is not well characterized. The US Food and Drug Administration's Adverse Event Reporting

System was searched for reports of nephrolithiasis in HIV-infected patients taking an atazanavir-based regimen. Thirty cases were identified. Many patients required hospitalization for management, including lithotripsy, ureteral stent insertion, or endoscopic stone removal. Some cases of nephrolithiasis resulted in atazanavir discontinuation. Healthcare professionals and patients should be informed that nephrolithiasis is a possible adverse event with atazanavir.

Two cases of nephrolithiasis due to atazanavir (ATV) were recently described in the literature [1,2]. The risk of nephrolithiasis associated with the use of ATV is not well characterized.

The Adverse Event Reporting System (AERS) of the US Food and Drug Administration (FDA) is a voluntary reporting system. This database contains spontaneous reports generated by health professionals, consumers, and manufacturers from the United States and other countries [3]. The AERS was searched for reports of nephrolithiasis in HIV-infected patients taking an atazanavir-based regimen. The search was conducted using the Medical Dictionary for Regulatory Activities high level term 'renal lithiasis'. This term includes three preferred terms: 'nephrolithiasis', 'nephrocalcinosis', and 'stag horn calculus'. Data on demographics, co-morbidities, concurrent medications, atazanavir exposure (defined as the time between atazanavir initiation and the onset of signs or symptoms of nephrolithiasis), diagnostic evaluation (radiology, laboratory results, stone analysis), patient management, and clinical outcomes were reviewed for each case [4,5].

From December 2002 to January 2007, 30 cases of nephrolithiasis in HIV-infected patients taking an atazanavir-based regimen were reported in the AERS database, including the published cases [1,2]. The clinical characteristics of these patients are shown in Table 1. Twenty-one patients were men, five patients were women, and four patients had no sex reported. Five patients (17%) had underlying liver disease: four patients had hepatitis C (one with cirrhosis), and one patient had hepatitis B with cirrhosis. Three patients had pre-existing renal disease and five patients (17%) had a history of nephrolithiasis.

Of the 20 cases reporting complete antiretroviral information (medications and doses), 13 patients received concomitant tenofovir and 17 patients received 100 mg ritonavir. Among 14 cases reporting stone analysis, 12 had atazanavir confirmed by infrared spectrophotometry or other analysis. In six cases with available data, atazanavir concentrations in the stone ranged from 40 to 100%. In 17 cases with complete atazanavir treatment history, the median time between atazanavir initiation and the onset of nephrolithiasis was 1.7 years (range 5 weeks to 6 years). Among the 30 patients, 18 required hospitalization, seven received outpatient care, and five lacked data. Interventions for stone removal were performed in eight patients (27%). Two patients required lithotripsy only, one patient underwent both lithotripsy and ureteral stent insertion, one patient needed nephrostomy tube placement for the relief of severe hydronephrosis because of ureteral obstruction by the calculus, two patients had ureteral stent insertion and endoscopic surgical extraction of the stone, and two patients needed endoscopic surgical extraction of the stone. In addition to clinical symptoms associated with nephrolithiasis, some patients also reported concurrent laboratory and radiological abnormalities [4,5]. Five patients developed renal insufficiency (four with acute renal insufficiency and one with a worsening of baseline chronic renal insufficiency) at the time of nephrolithiasis. In all four cases of acute renal insufficiency, renal function returned to baseline after stone removal and atazanavir discontinuation. In the patient who developed a worsening of baseline chronic renal insufficiency, renal function improved but had not returned to its previous baseline after stone removal. Three patients had radiological documentation of hydronephrosis. Of the 30 cases, atazanavir was reported as discontinued in nine cases (30%) after nephrolithiasis was diagnosed.

Data submitted to a voluntary reporting system such as the AERS are often limited by the absence of detailed and pertinent information, such as demographics, weight (only available for seven patients in the study), medical co-morbidities (particularly conditions that predispose to nephrolithiasis [4,5]), duration of atazanavir exposure, continuation of atazanavir after nephrolithiasis was diagnosed (only available for 15 patients), atazanavir drug levels (only available for one patient [1]), doses of any concurrent medications, diagnostic evaluation, patient management, and clinical outcome. As these adverse drug events are obtained voluntarily from a population of unknown size, the ability to make reliable estimates in frequency or establish a causal relationship with drug exposure is not always possible. Despite these limitations, our study findings from the AERS database suggest that atazanavir can be associated with nephrolithiasis. In the AERS database, 30 cases were identified, of which 12 cases had confirmed atazanavir by infrared spectrophotometry or other analysis. Only two of the confirmed cases (patients 18 and 23) have been reported in the literature [1,2]. In addition to the 12 confirmed atazanavir-associated cases, five cases were excluded that could have been confirmed as atazanavir-associated cases with better follow-up information. Three patients had reports that stated 'kidney stones observed through lab work', but provided no details regarding the composition of the stones (patients 8, 9, and 10). Furthermore, two patients had stone analysis but incomplete characterization of the stone metabolite (patient 25) or possible inaccurate reporting of the data (patient 30). It is conceivable that, with better follow-up information, some or

Table 1. Characteristics of patients with nephrolithiasis associated with the use of atazanavir.

Patient	Sex	Age (years)	Co–morbidities	Previous history of nephrolithiasis	Atazanavir dose (mg/day)	Other antiretroviral agents	Other medications	Atazanavir exposure[a] (weeks)	Documentation of stone	Was atazanavir discontinued?
1	M	55	HCV, cirrhosis	–	400	TDF, d4T	TMP–SMX, fluconazole, ciprofloxacin	5	A/P CT (bilateral calculi, severe left hydronephrosis), left nephrostomy tube placement	Yes
2	–	–	–	–	–	–	–	–	Clinical narrative	–
3	–	–	–	–	–	–	–	–	Clinical narrative	–
4	M	50	–	–	300	RTV, TDF, 3TC	–	32	Clinical narrative	Yes
5	M	49	–	No	300	RTV, TDF, 3TC	–	28	U/S (bilateral calculi), lithotripsy	Yes
6	F	41	GERD, peripheral neuropathy	No	300	RTV, TDF, 3TC	Ranitidine, gabapentin	33	A/P CT (two calculi, moderate right hydronephrosis)	–
7	M	–	–	–	–	–	–	–	Biochemical analysis: ATV crystals in renal calculus	No
8	–	–		–	–	–	–	–	Laboratory analysis[b]	–
9	–	–	–	–	300	RTV, TDF, FTC	Valacyclovir	–	Laboratory analysis[b]	–
10	F	38	Diabetes, hypertriglyceridemia	–	300	RTV, TDF, ddI	Glipizide, gemfibrozil	–	Laboratory analysis[b]	–
11	F	–	–	Yes, with IDV	–	–	–	104	Clinical narrative	–
12	M	34	–	No	300	RTV, TDF, 3TC	–	82	Clinical narrative	–
13	M	29	–	–	300	RTV, TDF, ddI	–	–	Infrared spectrometry: ATV in renal calculus	–
14	M	37	–	–	300[c]	ZDV, 3TC	–	44	Infrared spectroscopy: ATV in renal calculus	–
15	M	43	HCV, hyperlipidemia	No	300	RTV, ddI, 3TC	Calcium folinate	–	U/S (bilateral calculi), infrared spectrometry: 60% ATV in renal calculus	No
16	F	54	Hypertension, hyperthyroid	No	300	RTV, TDF, ABC	Levothyroxine	–	U/S, infrared spectrometry: ATV in renal calculus	ATV stopped with complete symptom resolution. ATV later restarted at 400 mg/day
17	M	–	–	No	–	–	–	–	U/S, A/P CT	No
18[d]	M	44	HCV, renal insufficiency, CMP, COPD, adrenal insufficiency	No	300	RTV, TDF, FTC	TMP–SMX, carvedilol, testosterone, hydrocortisone	126	A/P CT (right calculi with hydronephrosis), endoscopic surgical extraction and stent insertion; renal calculus was mixture of 60% metabolite of ATV and 40% calcium phosphate	Yes
19	M	44	–	–	2 doses daily	ZDV, 3TC	–	26	Clinical narrative	–
20	M	–	–	Yes	300	RTV, ABC, 3TC	–	121	Infrared spectrometry: 100% ATV in renal calculus	Yes
21	M	54	Congenital cystic kidney disease	Yes	–	–	–	–	Lithotripsy; lithiasis specimen contained ATV	No
22	M	50	–	Yes	400	d4T, ddI	–	320	Lithotripsy, stent insertion	No
23[e]	M	38	HBV, cirrhosis	Yes	300	RTV, TDF, FTC	–	56	U/S (right calculi), endoscopic surgical extraction and stent insertion; infrared spectrometry: 100% ATV in renal calculus	Yes
24	M	53	HCV, condyloma acuminata	No	300	RTV, ABC, 3TC	Phloroglucinol, ribavirin, peginterferon	112	U/S, A/P CT, renal echogram; stone analysis: crystallized ATV	Yes

(*continued overleaf*)

Table 1. (*continued*)

Patient	Sex	Age (years)	Co–morbidities	Previous history of nephrolithiasis	Atazanavir dose (mg/day)	Other antiretroviral agents	Other medications	Atazanavir exposure[a] (weeks)	Documentation of stone	Was atazanavir discontinued?
25	M	–	–	–	300	RTV (other HAART not listed)	–	–	Stone analysis: unspecified metabolite	–
26	M	64	–	–	300	RTV, EFV, 3TC	–	117	Clinical narrative	–
27	M	42	Osteoporosis, hypertension, diabetes, psoriasis, migraine	No	300	RTV, TDF, FTC	Calcium, vitamin D, alendronate, irbesartan, fenofibrate, zolpidem	–	Stone analysis: ATV 40% in renal calculus	–
28	M	65	Renal insufficiency, BPH	No	300	RTV, TDF, 3TC	–	134	Endoscopic surgical extraction	No
29	M	68	–	No	250 mg/day (per report)	–	–	93	Stone analysis: crystallized ATV	Yes
30	F	52	Diabetes, hyperlipidemia, hypertension	No	300	RTV, ZDV, 3TC	Furosemide, gliclazide, metformin, pravastatin, senna	90	Endoscopic surgical extraction; stone analysis: IDV stone despite NOT taking this medication	Yes

ABC, abacavir; A/P, abdominal/pelvic; ATV, atazanavir; BPH, benign prostatic hyperplasia; CMP, cardiomyopathy; COPD, chronic obstructive pulmonary disease; CT, computerized tomography; ddI, didanosine; d4T, stavudine; EFV, efavirenz; FTC, emtricitabine; GERD, gastroesophageal reflux disease; HBV, hepatitis B virus; HCV, hepatitis C virus; IDV, indinavir; 3TC, lamivudine; RTV, ritonavir; TDF, tenofovir; TMP–SMX, trimethoprim–sulfamethoxazole; U/S, ultrasound (renal); ZDV, zidovudine.

[a]Atazanavir exposure is the time between atazanavir initiation and the development of signs or symptoms of nephrolithiasis.

[b]No additional information provided.

[c]It is likely that patient 14 was also receiving ritonavir 100 mg/day along with atazanavir 300 mg/day; however, information on ritonavir was not provided in the report.

[d]Patient 18 was reported by Chang and Pella [2].

[e]Patient 23 was reported by Pacanowski *et al.* [1].

all of these five cases could also be confirmed as being associated with the use of atazanavir.

The mechanism for the development of atazanavir-associated nephrolithiasis is unknown. Further information is needed to determine whether patients with pre-existing hepatic or renal impairment or a history of nephrolithiasis are at an increased risk of this event. Data are not currently available to determine whether increased atazanavir exposures or prolonged atazanavir use is associated with these events.

Nephrolithiasis associated with atazanavir can cause significant morbidity, including renal dysfunction and hydronephrosis. Many cases required hospitalization for management and symptom relief. Lithotripsy, ureteral stent insertion, nephrostomy tube placement, or endoscopic stone removal was needed in a subset of cases. Some cases of nephrolithiasis resulted in the discontinuation of atazanavir. Healthcare professionals and patients should be informed that nephrolithiasis is a possible adverse event with the use of atazanavir. If signs or symptoms of nephrolithiasis occur, healthcare providers should consider temporary interruption or discontinuation of atazanavir therapy.

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Conflicts of interest: None.

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References

1. Pacanowski J, Poirier JM, Petit I, Meynard JL, Girard PM. **Atazanavir urinary stones in an HIV-infected patient.** *AIDS* 2006; **20**:2131.
2. Chang HR, Pella PM. **Atazanavir urolithiasis.** *N Engl J Med* 2006; **355**:2158–2159.
3. Kessler DA. **Introducing MEDWatch. A new approach to reporting medication and device adverse effects and product problems.** *JAMA* 1993; **269**:2765–2768.
4. Pietrow PK, Karellas ME. **Medical management of common urinary calculi.** *Am Fam Physician* 2006; **74**:86–94.
5. Asplin JR, Coe FL, Favus MJ. **Nephrolithiasis.** In: *Harrison's internal medicine*. Edited by Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, Isselbacher KJ. New York: The McGraw-Hill Companies, Inc; 2005. pp. 1710–1714.

Serosorting can potentially increase HIV transmissions

David M. Butler and Davey M. Smith

The effectiveness of a serosorting strategy for HIV prevention depends on the accuracy of individuals'

serostatus disclosures. We modeled the risks of sexual transmission of HIV under various circumstances differing by the type of disclosures made. Accounting for rates of unrecognized HIV infection, treatment status and differences in infectivity by stage of infection, we found that serosorting can increase the transmission risk for some groups.

Recommendations for preventing sexual transmissions of HIV emphasize measures that might reduce infection risk, such as consistently using latex condoms or serosorting [1,2]. In serosorting, partners make decisions about how they have sex on the basis of their assumed or reported HIV serostatus. If other prevention methods, such as latex condoms, are not employed with every sex partner but only with those having a discordant serostatus, then knowing the true HIV status is imperative. Without lying, some HIV-infected individuals may mistakenly believe they are not infected and disclose as 'HIV negative' [3]. Because the effectiveness of serosorting depends upon accurate disclosure, we decided to model the risk of transmission from 'HIV-positive' versus 'HIV-negative' disclosers to someone who is not infected and not using condoms. Our model accounts for the prevalence of unrecognized infections, treatment status and differences in infectivity by stage of infection.

Individuals who incorrectly disclose as 'HIV negative' are either chronically or recently infected. Although standard antibody tests diagnose chronic infection with a very low false-negative rate, acute infections cannot be diagnosed by standard HIV testing until after detectable antibodies have been produced [4]. Individuals may erroneously believe themselves to be uninfected after a negative antibody test result, and disclose themselves as 'HIV negative' to sexual partners when, in fact, they are highly contagious [5]. Many factors affect the incidence of HIV within groups [6–9]; and, for some groups, the density rate of new infections (and therefore the number of individuals with recent infections) can be quite high [10].

During the period of recent HIV infection, individuals typically have a much higher viral burden than they do for most of the time that they are infected, and the viral load has been shown to correlate with infectiousness [9,11,12]. During acute infection, which lasts approximately 6–8 weeks [13], infectiousness is probably greater than at any other time. Infectiousness, however, remains elevated even after the acute period for up to 25 months [11]. As the proportion of recently infected potential sex partners in a population increases, the effectiveness of disclosure for preventing HIV transmissions by serosorting decreases. Therefore, the same serosorting strategy will not have the same effect on the transmission risk for individuals from low-risk populations as individuals from high-risk populations. As a result of differences in the proportion of potential partners who are recently infected, the risk of acquiring HIV from one sexual exposure with one randomly selected 'HIV-negative' discloser from a high-risk population may actually be greater than the risk associated with a randomly selected 'HIV-positive' discloser (Table 1).

These provocative results are based upon the simple modeling of empirical data when they exist [11], epidemiological patterns found in the United States for disease and treatment rates [14], and conservatively biased estimates of transmission rates, which do not factor the relative frequency of anal versus vaginal sex, the rates of

Table 1. The transmission rate per exposure by the recently infected increases the risk of acquiring HIV from a member of the high-risk population disclosing as HIV negative; thus, when the prevalence of recently HIV-infected individuals in this population is 4%, there is less risk of acquiring HIV from a member of the group disclosing as HIV positive.

Group	Actual HIV status	Transmission rate per act A	Prevalence B	Individual risk of transmission per partner C = A × B	Cumulative risk of transmission per group D = C1 + C2 + C3...
'HIV-negative' low-risk population	HIV-negative	0 in 10 000	99%	0 in a million	
	Chronic, stable off treatment	7 in 10 000	0.75%	5.3 in a million	
	Chronic, advanced disease	36 in 10 000	0.05%	1.8 in a million	
	Recently infected	82 in 10 000	0.20%	16.4 in a million	
Combined risk of transmission per act with one randomly chosen member of this lower risk HIV-negative group					23.5 in a million
'HIV-negative' high-risk population	HIV-negative	0 in 10 000	86%	0 in a million	
	Chronic, stable off treatment	7 in 10 000	9.5%	66.5 in a million	
	Chronic, advanced disease	36 in 10 000	0.5%	18 in a million	
	Recently infected	82 in 10 000	4%	328 in a million	
Combined risk of transmission per act with one randomly chosen member of this higher risk HIV-negative group					412.5 in a million
'HIV-positive'	Chronic, stable off treatment	7 in 10 000	20%	140 in a million	
	Chronic, stable on treatment	1 in 10 000	75%	75 in a million	
	Chronic, advanced disease	36 in 10 000	5%	180 in a million	
Combined risk of transmission per act with one randomly chosen member of this HIV-positive group					395 in a million

sexually transmitted infections, or recreational drug use, any of which would probably increase the rate of transmission above the estimates we do use [6,7,12]. The limitations of our model include that, whereas clade B virus predominates in the United States where men who have sex with men comprise the majority of infected individuals, the only source of empirical data for calculating rates of sexual transmission is a heterosexual cohort in Africa with non-clade B virus [11]. Few in this cohort were on effective therapy, so the rate for transmission from a source with chronic, treated HIV was very conservatively estimated to be one-seventh the rate from an untreated source on the basis of an expected reduction in viral load and its correlation with infectiousness [9]. In addition, we assumed that newly diagnosed individuals with recent infection will not participate in high-risk activities before antiretroviral therapy effectively controls their viral load, and we assigned them to the 'on treatment' group of 'HIV-positive' disclosers, but this may not always be so.

Our conservative calculations show that serosorting based on disclosure is not likely to be an effective prevention strategy when the prevalence of recently infected 'HIV-negative' disclosers comprises approximately 4% of the potential sex partner population. This is a realistic estimate based on current data [15]. MacKellar *et al.* [3] found that among young men who have sex with men who had disclosed as 'HIV negative', 6% had unknowingly seroconverted in the previous 6 months. Using 6% as our estimated rate, the total cumulative risk of transmission per sexual exposure for someone who serosorts with the group of 'HIV-negative' disclosers would be 576.5 in a million compared with a risk of 395 in a million by sorting with the 'HIV-positive' disclosers group. In populations such as these, HIV-uninfected individuals who try to serosort may be more likely to become HIV infected than if they had not tried to serosort in the first place.

In conclusion, the effectiveness of serosorting on the basis of mutual disclosure of perceived HIV status is a flawed strategy for reducing sexual transmissions of HIV when it does not consider the prevalence of recent HIV infections in specific populations. Importantly, the individuals at greatest risk of HIV infection predictably belong to these very groups having the greatest proportions of recently infected people. By ignoring the increased potential for HIV transmission by recently infected individuals, serosorting may paradoxically increase the number of new HIV infections in certain populations.

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References

1. Varghese B, Maher JE, Peterman TA, Branson BM, Skeketee RW. **Reducing the risk of sexual HIV transmission: quantifying the per-act risk for HIV on the basis of choice of partner, sex act, and condom use.** *Sex Transm Dis* 2002; **29**:38–43.
2. Parsons JT, Scrimshaw EW, Wolitski RJ, Halkitis PN, Purcell DW, Hoff CC, Gomez CA. **Sexual harm reduction practices of HIV-seropositive gay and bisexual men: serosorting, strategic positioning, and withdrawal before ejaculation.** *AIDS* 2005; **19 (Suppl. 1)**:S13–S25.
3. MacKellar DA, Valleroy LA, Behel S, Secura GM, Bingham T, Celentano DD, *et al*. **Unintentional HIV exposures from young men who have sex with men who disclose being HIV-negative.** *AIDS* 2006; **20**:1637–1644.
4. Constantine N. University of Maryland. HIV InSite Knowledge Base Chapter. Antibody Assays. May 2006. Available from: http://hivinsite.ucsf.edu/InSite?page=kb-02-02-01. Accessed: March 2007.
5. Pilcher CD, Tien HC, Eron JJ, Vernazza PL, Leu SY, Stewart PW, *et al*. **Brief but efficient: acute HIV infection and the sexual transmission of HIV.** *J Infect Dis* 2004; **189**:1785–1792.
6. Centers for Disease Control and Prevention. **Methamphetamine use and HIV risk behaviors among heterosexual men–preliminary results from five northern California counties, December 2001–November 2003**. *MMWR Morb Mortal Wkly Rep* 2006; **55**:273–277.
7. Cohen MS. **Sexually transmitted diseases enhance HIV transmission: no longer a hypothesis.** *Lancet* 1998; **351 (Suppl. 3)**:5–7.
8. Katz MH, Schwarcz SK, Kellogg TA, Klausner JD, Dilley JW, Gibson S, McFarland W. **Impact of highly active antiretroviral treatment on HIV seroincidence among men who have sex with men: San Francisco.** *Am J Public Health* 2002; **92**:388–394.
9. Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, Wabwire-Mangen F, *et al*. **Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group.** *N Engl J Med* 2000; **342**:921–929.
10. Weinstock H, Sweeney S, Satten GA, Gwinn M. **HIV seroincidence and risk factors among patients repeatedly tested for HIV attending sexually transmitted disease clinics in the United States, 1991 to 1996. STD Clinic HIV Seroincidence Study Group.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; **19**:506–512.
11. Wawer MJ, Gray RJ, Sewankambo NK, Serwadda D, Li X, Laeyendecker O, *et al*. **Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda.** *J Infect Dis* 2005; **191**:1403–1409.
12. Royce RA, Sena A, Cates W Jr, Cohen M. **Sexual transmission of HIV.** *N Engl J Med* 1997; **336**:1072–1078.
13. Little SJ, McLean AR, Spina CA, Richman DD, Havlir DV. **Viral dynamics of acute HIV-1 infection.** *J Exp Med* 1999; **190**:841–850.
14. Centers for Disease Control and Prevention. *AIDS cases, by geographic area of residence and metropolitan statistical area of residence, 2004*. Atlanta, GA: Centers for Disease Control and Prevention; 2006.
15. Doll LS, Holtgrave DR. **The HIV/AIDS Prevention Research Synthesis Project: implications for federal HIV prevention policy.** *J Acquir Immune Defic Syndr* 2002; **30 (Suppl. 1)**: S130–S133.