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AIDS 2007, **21**:2355–2367

Primary penile angiosarcoma in a patient with AIDS: a case report

Primary penile malignancies represent approximately 0.2% of cancers in men in the United States, with approximately 1530 new cases diagnosed in 2006, according to the American Cancer Society. Penile carcinoma is most commonly seen in uncircumcised men and is associated with poor hygiene. There are few reports of angiosarcoma of the genitalia, and local control with surgery, followed by chemotherapy or radiotherapy for widespread disease, is the suggested mode of treatment. Differential diagnosis of angiosarcoma includes epithelioid hemangioendothelioma, Kaposi's sarcoma, and alveolar rhadomyosarcoma. We report, to our knowledge, the first known case of penile angiosarcoma in an AIDS patient, and present a brief review of the pertinent literature.

Case report

A 44-year-old man with AIDS, Kaposi's sarcoma, and *Pneumocystis carinii* pneumonia, presented with a complaint of bleeding penile lesions, which had been enlarging and increasing in number over several months. On physical examination, there was a friable mass encompassing the left half of the penis extending to the scrotum (Fig. 1a). The foreskin was not retractable, and minimal handling of the penis caused bleeding. Inguinal lymph nodes were non-palpable. Initial laboratory values demonstrated a hematocrit of 27%, serum creatinine level of 1.4 mg/dl, and CD4 cell count of 22 cells/μl. Computed tomography of the abdomen and pelvis showed numerous low-density lesions in the liver, bilateral enlarged inguinal lymph nodes without pelvic lymphadenopathy, and multiple bony lytic lesions. Biopsy of the tumor revealed a malignant vascular tumor consistent with angiosarcoma. The patient underwent radical penectomy and partial scrotectomy, with formation of thigh pouches and perineal urethrostomy.

Pathological examination of the specimen showed multiple areas of ulceration on the surface of the penis, including a focus of condyloma acuminatum just proximal to the glans. On microscopy, irregular vascular channels infiltrating the dermis were identified among areas of spindle cells, along with red blood cell containing vacuoles (Fig. 1b). The surgical margins were negative, and the final pathology correlated with the initial biopsy results. Extensive review of the specimen confirmed that this was indeed angiosarcoma and not Kaposi's sarcoma. During his hospitalization, the patient was evaluated by the Pulmonology and Gastroenterology services, which performed bronchoscopy and colonoscopy, revealing no obvious lesions. He was discharged on postoperative day 14, but was lost to follow-up with the medical oncology service, which recommended palliative chemotherapy. He was re-admitted 3 months postoperatively with worsening cough secondary to parenchymal Kaposi's sarcoma, failure to thrive and malnutrition, with a serum albumin level of 1.0 g/dl. Examination of the perineum demonstrated gross recurrence at the penectomy site. He died 3 weeks later as a result of metastatic angiosarcoma and disseminated Kaposi's sarcoma; no postmortem examination was performed.

Non-squamous malignancy of the penis is rare, with sarcomatous lesions representing the smallest percentage of these malignancies [1,2]. Previous reports of penile angiosarcoma include lesions presenting as a fungating

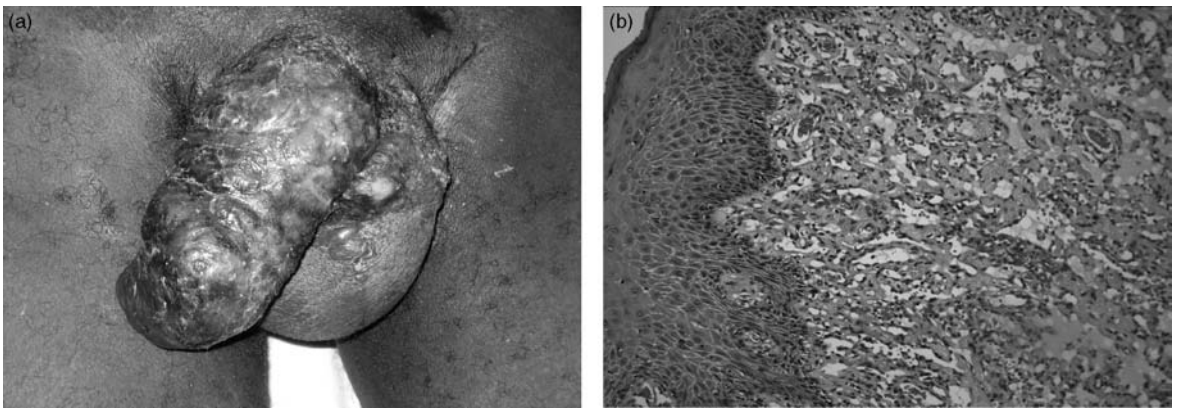

Fig. 1. Photograph of penis and microscopy. (a) Gross photograph of penis demonstrating extensive lesions extending to the scrotum. The tissue was friable and bled with minimal manipulation. (b) Histological analysis of the pathological specimen revealed spindle cell areas with vacuoles filled with red blood cells. The irregular vascular channels infiltrated the dermis.

mass [3], a Peyronie's plaque [4], a mass extending into the bladder [5], in a patient with von Recklinghausen's disease [6], and in a patient who received radiation for penile squamous carcinoma [7]. Another patient with low exposure to polyvinyl chloride presented with hepatic lesions, similar to this patient, which were consistent with hepatic angiomas [8]. The natural course of sarcomata of the penis is of local recurrence, therefore total penectomy with or without radiotherapy is suggested even for superficial disease, and unless there is palpable inguinal lymphadenopathy, inguinal node dissection is not necessary [1,3]. Metastatic disease is rare with aggressive local control, and is treated with either palliative chemotherapy or radiotherapy [1,3,9]. Our report of penile angiosarcoma represents, to our knowledge, the first known case in a patient with AIDS, and underscores the unique lesions that can be seen in immunodeficiency states. Importantly, histological diagnosis of angiosarcoma, and avoiding the assumption that the patient's lesion were Kaposi's sarcoma, led to the appropriate treatment. Genitourinary malignancy is common is HIV/AIDS, and is frequently more aggressive. Renal cell carcinoma has been shown to occur 8.5 times more commonly in HIV patients, and occurs in younger patients [10]. Similarly, prostate cancer seems to occur in younger patients and acts more aggressively [11]. Testicular tumors are the third most common malignancy in HIV-associated cancers, frequently presenting as non-Hodgkin's lymphoma and Burkitt's lymphoma, as well as traditional germ-cell tumors [11]. This case highlights the fact that an atypical presentation of genitourinary pathology requires the consideration of less common malignancies in the differential, particularly in a severely immunocompromised patient.

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Response to Brown *et al.*, 'Incident and prevalent herpes simplex virus type 2 infection increases risk of HIV acquisition among women in Uganda and Zimbabwe'

The recent paper by Brown and colleagues [1] concerning the impact of incident and prevalent herpes simplex virus type 2 (HSV-2) on the acquisition of HIV among women in Uganda and Zimbabwe provides further evidence for the role of the former in the spread of the latter and confirms the results of an earlier longitudinal study among female sex workers [2] and a recent meta-analysis [3]. Of particular importance is the relative contribution of incident and prevalent herpes simplex virus infection. Brown and colleagues [1] show that incident HSV-2 infection has a greater impact on the risk of HIV acquisition than does prevalent HSV-2 infection. The hazard ratio for the risk of acquiring HIV among individuals with incident and prevalent infection does not, however, quite reach significance at the 5% level in either of their two data sets. In Figure 1 we compare the data given by Brown and colleagues [1] with the corresponding data from South Africa [2].

The three estimates do not differ significantly and the weighted average hazard ratio suggests that women with incident HSV-2 infections are 2.46 (1.53–3.95) times more likely to be infected with HIV-1 than women with prevalent HSV-2 infections. These data suggest that the risk of acquiring HIV-1 infections wanes over time after infection with HSV-2, but none of the studies provide sufficient data to measure the rate at which this happens directly. The South African study may tend to give a higher estimate than the studies carried out in Uganda and Zimbabwe because of the much shorter time between visits (one month versus 3 months), which would mean that women were identified as being HSV-2 positive sooner after being infected in South Africa than

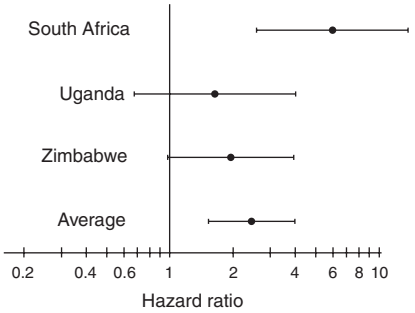

Fig. 1. The hazard ratio for the risk of HIV acquisition among women with incident and prevalent herpes simplex virus type 2 infection comparing women in the period after herpes simplex virus type 2 seroconversion to women who were herpes simplex virus type 2 positive at the start of the study. The hazard ratios are South Africa 6.0 (2.6–14.0) [2], Uganda women 1.64 (0.67–4.01) [1], Zimbabwe women 1.95 (0.98–3.91) [1], weighted average 2.46 (1.53–3.95).

in Uganda. Furthermore, the South African women were sex workers and at a higher overall risk with an HSV-2 incidence over the course of the study of 35% per year compared with 10 and 9% in Uganda and Zimbabwe, respectively, [1].

In a study in Carletonville, South Africa, among young adults from the general population [4] HIV infection was very strongly associated with HSV-2 infection; the odds ratios for HIV infection among those with and without HSV-2 infection was 5.3 (2.7–10.3) for men and 8.4 (4.9–14.2) for women. Furthermore, by the time they reached 24 years of age, 89% of women and 51% of men were infected with HSV-2. The high population prevalence and the very high incidence of HSV-2 among women between the ages of 15 and 25 years adds to the urgency of finding ways to prevent and manage HSV-2 infection.

We fully endorse the conclusions drawn by Brown and colleagues [1] concerning the need to find ways to control HSV-2 as part of the overall strategy for controlling HIV. If, as seems to be the case, the effect of HSV-2 on HIV acquisition wanes over time then prevention of HSV-2 is likely to be even more effective for HIV prevention than cure or symptomatic treatment of HSV-2. The study in South Africa adds important evidence in support of their arguments, especially in protecting young women from being infected with HIV.

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Atazanavir crystal nephropathy

Atazanavir (Reyataz, Bristol-Myers Squibb Laboratories, New York, New York, USA), an azapeptide inhibitor of HIV protease, is not considered directly nephrotoxic despite recent reports of urinary stones [1–3]. We describe a patient in whom direct nephrotoxic effects of atazanavir appeared to develop.

The patient was a 61-year-old white man known to be HIV/hepatitis C coinfected for 20 and 10 years, respectively. He also had a history of renal insufficiency of unknown cause, with a baseline creatinine level of 1.4 mg per decilitre since 2003. At this time, atazanavir 150 mg twice daily was added to his prior treatment, including lamivudine (300 mg/day), didanosine (100 mg/day), and ritonavir (100 mg/day). During the same period, serum creatinine had gradually risen from 1.5 mg/dl to 3.63 mg/dl and the patient had no urinary symptoms. Blood pressure was 120/70 mmHg. Urinalysis revealed 300 mg of protein per deciliter in a 24-h collection, urine pH 7.0, and the urine sediment showed granular casts, epithelial cells and rodlike-shaped crystals (Fig. 1a). There was no hematuria, leukocyturia and culture remained

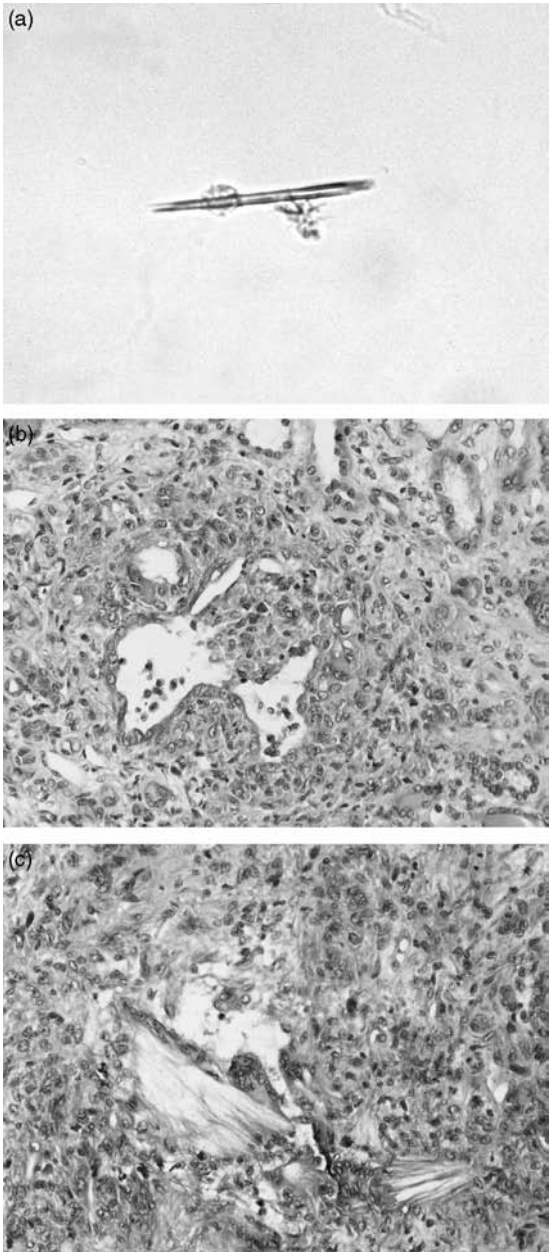

Fig. 1. (a) Rodlike-shaped mildly birefringent urine crystal, measuring 8–20 nm and thrusting the white cell. (b) Crystalline precipitation within tubular lumens, and (c) interstitium, with damaged tubules and aggregates of multinucleated giant cells related to the crystal material.

negative. Renal ultrasound revealed normal-sized kidneys without pyelocalyceal distension. A renal biopsy showed unremarkable glomeruli, crystalline precipitation within tubular lumens (Fig. 1b), and interstitium (Fig. 1c) associated with granulomatous reaction. Polarized light microscopy analysis of urine crystals revealed that it was composed of a mixture of 60% atazanavir metabolite and 40% calcium phosphate. Plasma atazanavir concentrations measured 12 h after the last drug intake were 4737 ng/ml (normal 149–219 ng/ml), four-fold higher than the normal range of concentrations reported after boosted atazanavir administration combined with ritonavir (1023 ng/ml) [4].

Atazanavir was withdrawn and replaced by saquinavir with no renal function improvement. One month later, the patient was treated with oral prednisone 1 mg/kg daily for 4 weeks followed by rapid tapering, resulting in partial recovery of renal function (serum creatinine level improved to 0.90 mg/dl).

Only 7% of an atazanavir dose is excreted as unchanged drug in the urine. Furthermore, atazanavir sulphate is slightly soluble in water and its solubility increase with acidity of the urine (pH < 3) [4]. Thus, it is not surprising that intratubular crystal formation might occur and cause renal parenchymal injury. An unusually high atazanavir plasma level, alkaline urine, with pre-existing chronic kidney and liver diseases predisposed our patient to develop this complication. Indeed, the mean AUC $(0-\infty)$ was 42% greater in patients with impaired hepatic function than healthy volunteers [4].

One report suggested that atazanavir caused acute interstitial nephritis related to a hypersensitivity-like reaction [5]. However, there have been no previous reports indicating that atazanavir can induce renal failure due to tubular and/or interstitial crystal deposition associated with granulomatous reaction. This case suggests that atazanavir needs to be considered among the possible causes of crystal nephropathy in highly-active antiretroviral therapy-treated HIV-infected patients. It seems reasonable to recommend a short duration steroid therapy if no improvement of the renal function is noted 2 weeks after stopping Atazanavir. This is in order to limit the risk of progression to chronic renal insufficiency.

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Geographical biases and convenience sampling in HIV molecular epidemiology estimates

Molecular epidemiology studies of HIV diversity around the world are of pivotal importance to the containment of an HIV/AIDS pandemic. An increasing body of evidence suggests that differences of HIV types, groups and subtypes influence on their biological characteristics and on the impact of clinical intervention strategies [1]. This is particularly true for the effective design of HIV vaccine candidates, where one should know the genetic variants of the virus that circulate in specific targeted regions [2]. HIV-1 group M, the pandemic group of the virus, is divided into nine distinct subtypes (A–D, F–H, J and K) and over 30 intersubtypic circulating recombinant forms [3,4]. In a report recently published in *AIDS*, Hemelaar and colleagues have analyzed the most updated global and regional distribution of HIV-1 genetic strains, comprising over 20 000 strains isolated from 2000–2004 in 70 countries representing all continents [5]. Data were compiled according to the relative contributions of investigators from those countries, and our group was one of the Brazilian contributors.

Our group is mostly interested in the spread of HIV-1 subtype C, which is still a minor variant in Brazil, but highly concentrated to the southernmost part of the country [6–9]. Even although a large number of the contributed HIV sequences by our group derived from a nationwide HIV diversity survey [7], that survey was highly represented by samples from the southernmost states of Brazil, notably from Rio Grande do Sul and Paraná. Additional sequences were also obtained from our HIV diversity studies conducted in southern Brazil [6,8,9]. Rio Grande do Sul concentrates most of subtype C infections in Brazil, but it harbored only 10% of all Brazilian HIV infections in 2004 [10]. In addition, subtype C infections outside southern and southeastern Brazil and in other Latin American countries are only anecdotal.

Table 1. Estimated relative contributions of HIV-1 subtype C to total infections in Brazil according to state, 2004.

State of Brazil	% of total AIDS cases[a]	% Subtype C infections[b]	% Total contribution[d]
Rio Grande do Sul	10.1	45	4.5
Santa Catarina	5.1	37[c]	1.9
Paraná	4.7	30	1.4
São Paulo	27.4	3	0.8
Rio de Janeiro	16.2	6	1.0
Total[e]			8.7

[a]Percentage of total AIDS cases in Brazil in 2004, as estimated previously [10].
[b]As estimated previously [7].
[c]Estimates for this state were not performed, but were inferred as the average from the two neighboring states, Rio Grande do Sul and Paraná.
[d]Relative contributions of subtype C to total infections in Brazil.
[e]Total contribution of subtype C infections in Brazil in 2004.

In order to evaluate potential geographical and convenience sampling biases in the estimates of Latin America proposed in [5], we have estimated subtype C infections in Brazil based on the relative contributions of cases from each state of the country where subtype C prevalence is significant (over 1%) in 2004 [10]. We have also used data from the most recent HIV molecular epidemiology nationwide [7] and local surveys [8,9]. The five Brazilian states where subtype C circulate in proportions above 1% harbors over 63% of HIV/AIDS cases in the country (Table 1). However, by using the estimates of subtype C infections in those states, we find it to represent less than 9% of the total HIV infections in the country. As subtype C infections have so far been only sporadically reported in other Latin American countries, and considering that Brazil harbors 35% of the HIV infections in Latin America [5], the total contribution of subtype C to the epidemic in that continent can be as low as 3.1%. This is less than one quarter of the 12.6% reported by Hemelaar *et al.* [5].

In this report, we were able to verify the effect of potential biases in the estimate of subtype C prevalence in Latin America. We anticipate that similar geographical concentrations and convenient samplings, mostly related to the use of HIV *pol* genomic regions sequenced for drug resistance surveys, may have taken place in other parts of the world, where restricted investigators conduct HIV molecular surveys. Although the monitoring of HIV epidemiology is of extreme importance to rational policy contention strategies, any results relating to HIV subtype prevalence should be taken with caution and more prospective, unbiased designed surveys should be carried out.

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Resistance mutation patterns in plasma and breast milk of HIV-infected women receiving highly-active antiretroviral therapy for mother-to-child transmission prevention

Mother-to-child transmission via breastfeeding accounts for a relevant proportion of pediatric HIV infections in resource-limited countries [1–2]. Highly-active antiretroviral therapy (HAART) prophylaxis administered to breastfeeding mothers has been proposed as a possible strategy to prevent postnatal transmission [3] and, indeed, the recently reported results of two studies assessing this approach are encouraging [4,5]. One of the possible obstacles to the use of this preventive strategy is that the different penetration of antiretroviral drugs into the breast milk could favour the emergence of resistance and be associated to the transmission of resistant strains.

In this study, we analyzed the resistance mutation patterns of plasma and breast milk viral populations in women receiving HAART for mother-to-child transmission prevention in order to assess the frequency of mutations in these women and to evaluate if different patterns arise between plasma and breast milk.

We studied 26 pregnant women attending the AnteNatal Clinic in Matola, Maputo, Mozambique [part of the Drug Resource Enhancement against AIDS and Malnutrition (DREAM) program, designed and managed by the Community of S. Egidio] who had received zidovudine (or stavudine if Hb < 8 g/dl), lamivudine and nevirapine from 28 weeks of gestation until 1 month postpartum [6]. Within 1 week after delivery, 10 ml of blood and 10 ml of breast milk were collected from all women. Plasma and 1 ml of unprocessed breast milk were stored at -80°C. The remaining 9 ml of breast milk were centrifuged at $1000\,\boldsymbol{g} \times 10$ min. Breast milk cells were washed once in phosphate-buffered saline and stored at -80°C as dry pellets. HIV-RNA in plasma and whole breast milk and proviral DNA in breast milk cells were quantified as previously described [6]. Concentrations of nevirapine, lamivudine and zidovudine were determined by the high-performance liquid chromatography method with ultraviolet detection [6].

Sequence analysis of viruses in plasma, breast milk and in breast milk cells was performed with the TruGene HIV-1 Assay (Bayer Diagnostics, Milan, Italy). Only resistance mutations included in the IAS-USA 2006 classification were considered as significant. The hivdb6 internet-accessible database (http://hivdb6.stanford.edu) was used for subtype assignment.

Women had received a median of 77 days of prophylaxis (range 27–137 days), the median CD4+ cell count at delivery was 620/mm^3 (range 183–1275/mm^3), the median HIV-RNA level was 2.6 $\log_{10}$ copies/ml (range 1.7–4.7 $\log_{10}$ copies/ml) in plasma, and 2.7 $\log_{10}$ copies/ml (range 1.7–4.7 $\log_{10}$ copies/ml) in breast milk. Median HIV-DNA content was 10 copies/10^6 breast milk cells (range 10–667 copies/10^6 breast milk cells).

Sequences were obtained from plasma and from breast milk in 23 cases and from breast milk cells in 18 women (Table 1). All strains belonged to subtype C with the exception of one subtype A. Major resistance mutations (K103N + M184V, K103N, V108I, M184I) were detected in the plasma viruses of four women; two of them had the same pattern in breast milk (although in one woman only in the cell-free virus) whereas the other two had no mutation in breast milk. In two cases, viral strains present in breast milk harboured major resistance mutations (M184I + M46I in the cell-associated virus in one patient and V106A in the cell-free virus in the second patient) not present in the plasma virus. Although the patterns of resistance were different, the prevalence of nonnucleoside reverse transcriptase inhibitor (NNRTI)-associated mutations was 13% (3/23) both in plasma and in breast milk and that of lamivudine-associated mutations was 8.7% (2/23) in both plasma and breast milk (considering either cell-free or cell-associated viruses). Several patients had differences in the number and type of minor protease mutations in the different viral populations.

Patients with or without mutations did not have significantly different plasma or breast milk drug concentrations that could possibly explain the differences in resistance mutations, although it must be emphasized that the number of our patients was small and the statistical power to detect these differences was limited.

The frequency of major resistance mutations in plasma in our women (4/23, 17.4%) is comparable to that reported in previous studies performed in women receiving

Table 1. Drug-resistance mutations in plasma, breast milk and breast milk cells.

	Plasma		Breast milk		Breast milk Cells	
Pt	RT	PR	RT	PR	RT	PR
1		M36I		M36I		M36I
2		M36I		M36I	M184I	M36I M46I G73S
3		M36I L63P		M36I L63P	ND	ND
4		M36I L63P		M36I L63P	ND	ND
5		M36I		M36I		M36I
6	ND	ND		L10I M36I		L10I M36I
7		K20R M36I	ND	ND	ND	ND
8		K20R M36I		K20R M36I		K20R M36I
9	ND		ND	M36I		M36I
10		M36I	ND	ND		ND
11		K20R M36I L63P		M36I L63P		M36I L63P
12	ND	ND		M36I L63P	ND	ND
13		M36I L63P		M36I L63P		M36I L63P
14	V179D	K20R M36I L63P	K103T	M36I		M36I L63P
15		K20R M36I		K20R M36I		K20R M36I
16	K103N M184V	M36I	K103N M184V	M36I	K103N M184V	M36I L63P
17	K103N	M36L L63P	K103N	M36L L63P		M36L L63P
18		K20R M36I L63P		K20R M36I L63P		K20R M36I L63P G73S
19	M184I	M36I L63P		M36I L63P	ND	ND
20		M36I		M36I A71V		M36I
21	–	–	–	–	ND	ND
22	K101E V108I	M36I		M36I		M36I
23		K20R M36I L63P	V106A	K20R M36I L63P G73S		K20R L63P
24	–	–	–	–	–	–
25		M36I	ND	ND	ND	ND
26		K20R L63P		K20R L63P M36L		M36I

RT, Reverse transcriptase; PR, protease; ND, not determined.

HAART [7,8] whereas the only available study assessing the rate of mutations in breast milk reported a considerably higher frequency with the use of single-dose nevirapine (NNRTI resistance was present in breast milk in 65% of the women tested) [9]. In conclusion, in our study, the same proportion of women receving HAART prophylaxis had resistance-associated mutations in plasma and in breast milk. However, since there were differences in the mutational patterns, our data indicate that postnatal transmission may occur with viral variants that cannot predicted by those present in plasma.

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Hepatic involvement with tuberculosis-associated immune reconstitution disease

We read with interest the article by Hoffman and colleagues concerning hepatotoxicity in patients receiving antiretroviral therapy (ART) in a South African cohort [1]. Hepatotoxicity is a common problem in resource-limited settings where less costly but more hepatotoxic agents are often used and where patients frequently have comorbidity. Of particular note, concurrent treatment for tuberculosis is common; in a cohort in Cape Town, South Africa, we reported that approximately 30% of patients during the first year of ART receive overlapping tuberculosis treatment [2]. Hoffman and colleagues found that such overlap of tuberculosis treatment with ART was associated with an 8.5-fold increased risk of grade 3 or 4 hepatotoxicity (as defined by the AIDS clinical trials network) [1].

Development of hepatic dysfunction during the early weeks of concurrent tuberculosis treatment and ART presents a common clinical challenge, which is complicated greatly by the fact that potential causes are multiple. Each of the major classes of antiretroviral agents is hepatotoxic, with hypersensitivity reactions to nonnucleoside reverse transcriptase inhibitors being the most commonly implicated cause during early ART [3]. Rifampicin, isoniazid and pyrazinamide and co-trimoxazole are all well recognised causes of hepatitis. Moreover, patients who also have coinfection with chronic hepatitis infections B or C may develop a transient rise or 'flare' in serum concentrations of hepatic transaminases during early immune recovery with ART [4]. In addition, however, we suggest that immune reconstitution disease associated with tuberculosis (TB-IRD) is a further underrecognised cause of hepatic disease in this period.

TB-IRD develops in approximately one third of patients who commence ART within the first 2 months of tuberculosis treatment and results from rapid restoration of host inflammatory responses to residual mycobacterial antigen [5]. A wide spectrum of manifestations may occur but these most commonly include the development or recurrence of fever, respiratory manifestations or lymphadenopathy. A range of intra-abdominal manifestations has also been reported, including development of lymphadenopathy, intestinal lesions, ascites, splenomegaly and psoas abscesses [5]. However, hepatic involvement was described in only two case reports among a total of 86 cases of TB-IRD reported in the literature up to 2005 [5,6].

Our clinical experience in South Africa is that hepatic involvement is not infrequent in patients with disseminated tuberculosis who develop TB-IRD. In a series of 19 previously reported cases, seven (37%) had intra-abdominal manifestations with hepatic involvement in four (21%) [7]. All four cases developed hepatomegaly associated with elevated serum concentrations of predominantly the bile cannalicular hepatic enzymes but without ultrasound evidence of biliary duct obstruction. Median levels (with normal ranges) of hepatic enzymes were: alkaline phosphatase 493 U/l (40–120 U/l), γ-glutamyl transferase 338 U/l (0–35 U/l), alanine transaminase 66 U/l (5–40 U/l) and aspartate transaminase 68 U/l (5–40 U/l); mild hyperbilirubinaemia developed in one patient. In all four cases, hepatic involvement was associated with manifestations of TB-IRD at another anatomical site, such as intra-abdominal lymphadenopathy or an exacerbation of respiratory disease. Disease was mild and self-limiting in these cases, but a degree of cholestasis sufficient to cause clinical jaundice may develop in some patients.

Hepatic manifestations of TB-IRD are likely to result from rapid restoration of host granulomatous inflammation in response to mycobacteria trapped within the hepatic portal tracts during lympho-haematogenous dissemination. Histopathology of liver biopsy specimens typically shows large epithelioid granulomas, the formation of which would explain the rapid development of hepatomegaly. Hepatic discomfort appears to be a frequent accompanying symptom, presumably due to stretching of the capsule during rapid liver expansion.

In conclusion, we believe that TB-IRD should not be overlooked within the complex differential diagnosis of hepatic disease during the initial weeks of ART in patients receiving concurrent tuberculosis treatment. Careful evaluation of the patient for other manifestations consistent with TB-IRD and the observation of elevated serum concentrations of bile cannalicular hepatic enzymes may provide important clues to the diagnosis. Recognition of this condition may help to avoid unnecessary drug interruptions that could potentially result from misdiagnosis of drug-induced hepatotoxicity.

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Dysfunctional natural killer cells, *in vivo,* are governed by HIV viremia regardless of whether the infected individual is on antiretroviral therapy

Progression to AIDS during HIV-infection is associated with a decrease in the number and function of natural killer (NK) cells [1–4]. The reduction in NK cells expressing CD56 and CD16 correlates with low CD4+ T-cell count [5] and increasing viral load [6,7]. Moreover, higher viral loads are associated with an increased presence of a highly dysfunctional NK cell subpopulation lacking CD56 but retaining CD16 [7,8], whereas suppression of viral replication with highly-active antiretroviral therapy (HAART) results in improvements in both NK cell function and NK cell phenotype [7]. These observations have been used to propose that high level viral replication is a consequence rather than a cause of alterations in the NK cell phenotypic patterns [7], although this remains controversial [9]. Of note, most of these studies involved individuals who were either not controlling HIV replication or whose virus was fully controlled with HAART. No study has systematically included individuals whose virus was low in the absence of therapy, and presumably being controlled immunologically.

The present study determines the characteristics of NK cell subsets across a broad range of chronically-infected patients. A total of 76 individuals from four well characterized groups of individuals were enrolled: (i) 'elite' controllers: plasma HIV RNA levels < 75 copies/ml in the absence of any therapy ($n = 19$) (Fig. 1a); (ii) 'partial' controllers on antiretroviral therapy (PCAT): plasma HIV RNA levels between 75 and 10^4 copies/ml ($n = 19$) (Fig. 1a); (iii) 'HAART suppressed': plasma HIV RNA levels < 75 copies on long-term HAART ($n = 19$) (Fig. 1a); and (iv) 'noncontrollers': plasma HIV RNA levels $> 10^4$ copies RNA/ml ($n = 19$) (Fig. 1a). All subjects were recruited from the UCSF Study of the Consequences of the Protease Inhibitor Era (SCOPE) cohort and all provided their written informed consent. The first two groups were selected because previous work from these individuals have the strongest evidence for immune-mediated virus control [10].

Peripheral blood lymphocytes from the study subjects were stained with flurochrome-conjugated CD56, CD16, CD3 and CD69 antibodies (BDIS, San Jose, California, USA). After staining, cells were washed twice with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin and 0.1% sodium azide. Cells were fixed with 2% paraformaldehyde in PBS, pH 7.2, acquired on a FACsCalibur (BDIS) flow cytometer and analyzed using Cell Quest Software, version 3.2.1 (BDIS). Plasma HIV RNA levels were determined by the bDNA amplification technique (Quantiplex HIV RNA; Chiron, Emeryville, California, USA), as described previously [11].

The virologic noncontrollers had the lowest total percentage of classically defined NK cells (CD56+/CD3−) (mean $\pm$ SD $= 3.4 \pm 2.3\%$). This percentage was significantly lower than that observed in the HAART-suppressed group (mean $\pm$ SD $= 5.7 \pm 3.9\%$; $P = 0.03$) and is consistent with prior observations [1–4]. The percentage of CD56+ NK cells was intermediate in the elite controllers and PCAT subjects, and did not differ significantly from the noncontroller group (Fig. 1b).

Of the three different NK cell subsets, based on CD56 and CD16 expression, the $CD56^{dim}$/CD16+ NK cell subset possesses the greatest capacity to mediate a cytotoxic response [6,7]. The two viremic groups (noncontrollers and PCAT) had the lowest percentage of these cells (Fig. 1c) and were not statistically different from one another. The differences in the percentage of CD56dim/CD16+ NK cells between the noncontrollers and either aviremic group were statistically significant ($P = 0.004$ in noncontrollers versus elite controllers, and $P = 0.007$ in noncontrollers versus HAART-suppressed).

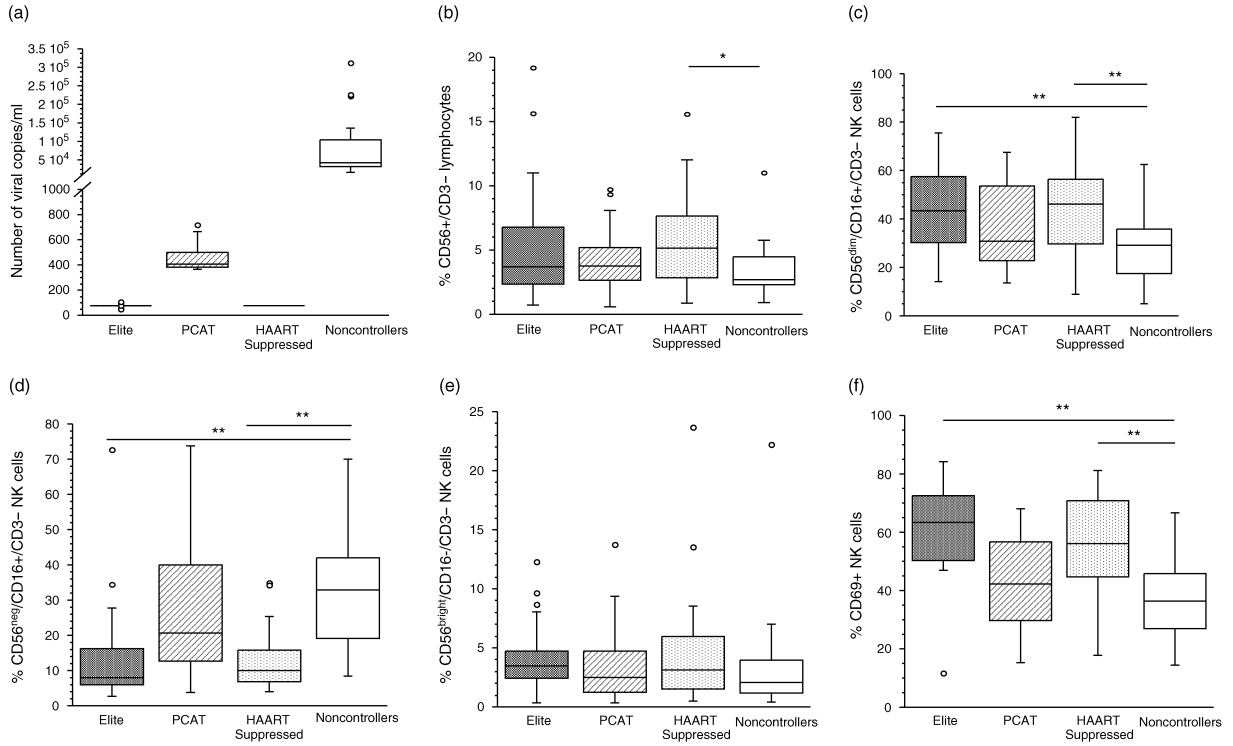

Fig. 1. Viral load and percentage of natural killer (NK) cells and their subsets in viremic and aviremic HIV-infected individuals. (a) HIV viral load and percentage (b) natural killer cells, (c) $CD56^{dim}$, (d) $CD56^{neg}$ and (e) $CD56^{bright}$ expressing natural killer cells (CD16+/CD3−) of aviremic and viremic HIV-infected individuals. CD69 expression was evaluated with all NK cell subpopulations (f). Error bars indicate SD. Dots represent outliers. $^{*}P < 0.05$, $^{**}P < 0.01$ based on Wilcoxon test. PCAT, 'Partial' controllers on antiretroviral therapy; HAART, highly-active antiretroviral therapy.

$CD56^{neg}$/CD16+ subset of NK cells are thought to be largely dysfunctional [8]. In contrast to the trends observed above, we saw a striking association between high viral load and a high percentage of these cells. Subjects whose virus was controlled therapeutically (HAART) or presumably immunologically (elite controllers) had the lowest percentage of these cells overall compared with the viremic individuals. Since little if any of these cells exist in uninfected individuals [12], our findings suggest that the presence of this subpopulation of cells occurred as a consequence of virus infection and that virus control appears to maintain this population of NK cells to significantly lower levels than when virus is not controlled (Fig. 1d; $P < 0.0001$ for either elite or HAART suppressed compared to either viremic population). The percentage of $CD56^{bright}$/$CD16^{neg}$ NK cells, which tend to be effector NK cells with the greatest capacity to produce cytokines [13], was similar across all groups tested (Fig. 1e).

We next evaluated the activation status of NK cells in the different infected individuals studied, focusing on the expression of CD69 on CD56dim/CD3−/CD16+ and CD56neg/CD3−/CD16+ cells (Fig. 1f). In contrast to what has been observed from this cohort with T cell activation [11,14], patients who were viremic either in the presence or absence of therapy had lower levels of NK cell activation ($P < 0.0001$ for comparison between either aviremic group with either viremic group).

In summary, we did not find any consistent NK cell phenotypic pattern in our subsets of individuals who are likely to be controlling their virus immunologically (i.e. elite controllers, PCAT subjects) as compared to either HAART-suppressed or the noncontroller populations. These data suggest that NK cells, at least as measured in the peripheral blood using classical cell surface markers, are not causally associated with control of HIV replication. We did, however, observe consistent trends that tracked more closely with the level of viremia. For example, undetectable viremia in either elite controllers or HAART-suppressed subjects was associated with a higher percentage of activated effector NK cells ($CD56^{dim}$/CD16+) and lower numbers of presumably dysfunctional NK cells ($CD56^{neg}$/CD16+). As these observations were independent of whether the virus was controlled immunologically or therapeutically, they suggest that the level of viral replication was a cause rather than a consequence of the observed NK cell phenotypic patterns. This conclusion is supported by our previous study performed *in vitro* indicating that NK cells do not kill HIV infected cells effectively [15]. Further work is needed to correlate these phenotypic changes with NK cell function in both peripheral blood and in tissues of infected individuals. Given that NK cells are important in

control of tumors and virus infected cells, it would be important to understand how HIV replication leads to an alteration in NK cells.

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Entecavir can select for M184V of HIV-1: a case of an HIV/hepatitis B (HBV) naïve patient treated for chronic HBV

Entecavir, a nucleoside analogue approved for the treatment of chronic hepatitis B (HBV), has been recommended as a treatment in co-infected HIV-1/HBV patients who do not require antiretroviral therapy (ART) [1], in part because it was thought to have no activity against HIV-1. However, a recent report [2] suggests that entecavir has activity against HIV-1 replication leading to an approximately 1 log decline in HIV-1 viral load. Entecavir can also select for the M184V mutation in HIV-1. Emergence of M184V resistance during entecavir treatment has been demonstrated only in patients who had prior lamivudine exposure, a drug which also selects for M184V. Here, we report an HIV-1/HBV co-infected patient never previously treated with ART, who received entecavir only for his HBV, suppressed HIV-1 viral load (<400 copies/ml) on two occasions and then developed the M184V mutation.

A 51-year-old African–American bisexual male co-infected with HIV-1 and HBV was diagnosed with HIV-1 in September 2004 when his CD4 count was 877 cells/m^3 with a percentage of 46.2% and an HIV-1 viral load of 2600 copies/ml (3.41 log). Routine HIV resistance testing (Trugene; Siemens Diagnostics, Tarrytown, New York, USA) revealed wild-type virus. His HBV surface antigen was positive. He drank alcohol daily but had reduced his intake to 40-50 ounces/day since 2004. He reported intermittent crack cocaine use. He was not initiated on ART during his initial evaluation because of his laboratory profile.

In July 2005, his HBV load was >17 857 143 IU/ml and his HBV e-antigen was positive. Entecavir 0.5 mg daily was initiated for HBV treatment [CD4 count was 1445 cells/m^3 and his HIV-1 viral load was 10 000 copies/ml (4 log)]. Eight weeks later, his HIV-1 viral load was undetectable [< 400 copies/ml (< 2.6 log)] (Fig. 1). This finding persisted for 6 months. From January to April 2006, the patient began actively using both alcohol and cocaine and became homeless. He did

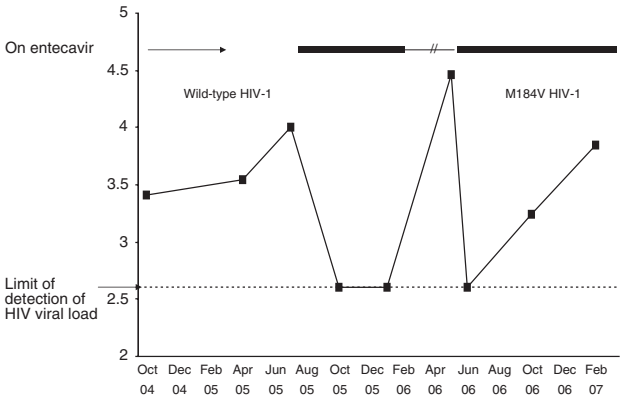

Fig. 1. The patient was initiated on entecavir only for anti-hepatitis B treatment because antiretroviral therapy was not indicated. HIV-1 viral load became undetectable with entecavir, became detectable with break in therapy, and then suppressed again with re-initiation of entecavir. Subsequently, the patient developed HIV-1 resistance and could not be suppressed with entecavir. ■, Log HIV-1 viral load.

not return for follow-up and failed to refill entecavir during this time. He was hospitalized in April 2006 with bacteremia secondary to a septic joint and re-entered medical care in May 2006. Laboratory values indicated his highest HIV-1 viral load of 28 700 copies/ml (log 4.46). Entecavir was reinitiated and remarkably his HIV-1 viral load suppressed to undetectable levels again within 6 weeks from initiation. In October 2006, his HIV-1 viral load became detectable despite continued use of entecavir. Pharmacy prescription records indicate the patient was adherent to entecavir. Routine HIV resistance testing (Trugene) in May 2007 revealed the development of an M184V mutation. HBV DNA, measured only once during the follow-up period, had decreased to 642 IU/ml by May 2007.

This HIV-1/HBV co-infected patient, naïve to ART, received entecavir monotherapy for the treatment of HBV and completely suppressed his HIV-1 RNA (approximately 2 log drop) on two occasions after initiating entecavir, and subsequently developed the M184V mutation. To our knowledge, this is the first case report of an HIV-1 patient receiving entecavir to fully suppress his HIV-1 viral load on two occasions without concomitant use of antiretroviral medications. It is important to note that all of our laboratory samples were evaluated using testing methods in routine clinical practice, differing from other reports [2] that utilized assays available only in the research setting. Entecavir 0.5mg daily was initiated for treatment of HBV in this patient to avoid use of medications with known activity against both HIV-1 and HBV, such as tenofovir, lamivudine, or emtricitabine, so that future HIV treatment options could be preserved. Our case confirms that entecavir can select for HIV-1 resistance and should not be used as monotherapy in HIV-1/HBV naïve patients [3,4]. In addition, HIV providers treating HBV should monitor both HIV and HBV viral loads to assess for efficacy [5].

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Effects of atazanavir/ritonavir and lopinavir/ritonavir on glucose uptake and insulin sensitivity

We read with interest the article by Noor *et al.* [1] examining the differential effects on glucose metabolism of the combinations of atazanavir/ritonavir versus lopinavir/ritonavir. Their paper deals with an important issue, having both theoretical interest and clinical impact. We think that some comments concerning aspects of the experimental procedure, data analysis and interpretation of the results could be helpful to other readers.

The euglycemic hyperinsulinemic clamp is the golden standard for the assessment of insulin sensitivity. This experimental approach requires that the glucose

concentration is maintained at a constant level throughout the study [2]. However, in the study by Noor *et al.* [1], the glucose concentration increased remarkably (up to 100 mg/dl) during the initial portion of the clamp (their fig. 3a), a defect which may influence the clamp-derived estimate of insulin sensitivity. During the clamp, it is assumed that endogenously secreted insulin is inhibited and only peripherally administered insulin contributes to insulin elevation. If the glycemic increase triggered an endogenous insulin response, this likely influenced the degree of inhibition of endogenous glucose production because the acute elevation of the portal insulin level has been shown to have a long-lasting effect on the liver [3]. As a result, the clamp-derived estimate of insulin sensitivity would also be affected. Additional data on the C-peptide would be welcome to verify the time course of endogenous insulin secretion, as a bias cannot otherwise be ruled out.

In analyzing the oral glucose tolerance test (OGTT), Noor *et al.* [1] used plasma glucose and insulin levels to calculate insulin resistance for each time point of the OGTT by means of the homeostasis model assessment (HOMA) (their fig. 3c). That, however, is a misuse of the HOMA method, which has been developed to provide an index of insulin resistance from the concentrations of glucose and insulin measured at the basal steady state [4]. This method cannot be applied to nonsteady-state conditions when insulin and glucose concentrations continuously change. Thus, the authors had no methodological support for calculating the HOMA index at each time point during the OGTT and performing statistical comparisons among groups.

In discussing their data obtained *in vivo*, Noor *et al.* [1] faced the problem of reconciling a discrepancy between the euglycemic, hyperinsulinemic clamp and OGTT results. Although the clamp revealed a significantly different reduction in insulin sensitivity between atazanavir/ritonavir and lopinavir/ritonavir, the OGTT did not (their table 2). The authors claimed that this discrepancy was due to the fact that the OGTT reflects both liver and peripheral insulin sensitivity, whereas the glucose clamp only reflects the latter component. This argument is questionable for the following reasons.

First, a patent contradiction immediately emerges. If the OGTT reflects both liver and peripheral insulin sensitivity, whereas the clamp only reflects the latter, the OGTT would be expected to be more capable than the clamp of detecting differences in insulin sensitivity among the study groups. This contradicts the experimental evidence, which shows the opposite.

Second, the claim that the clamp only reflects peripheral insulin sensitivity has no grounds. The index of clamp-based insulin sensitivity used by Noor *et al.* [1] is the ratio M/I, where M is the exogenous glucose infusion rate and I is the insulin concentration, both measured at the end of the clamp. It is worth emphasizing that the glucose infused during the clamp allows a constant plasma glucose concentration to be maintained because it balances exactly the increase in glucose disappearance (Rd) and the reduction in endogenous glucose production (EGP). Thus, the M-value measures the ability of insulin to stimulate peripheral glucose uptake, as well as to inhibit glucose production ($M = \Delta Rd - \Delta EGP$, where Δ is the change from pretest level) [5]. In the study by Noor *et al.* [1], the inhibition of endogenous glucose production could not be assumed to be identical in the study groups because protease-inhibitors are known to affect liver insulin sensitivity [6]. The contribution of glucose uptake can only be isolated from that of endogenous glucose production if a glucose tracer is concurrently infused along with unlabeled glucose [5], but Noor *et al.* [1] did not use a tracer. It can be concluded that the true reason for the discrepancy between the clamp and OGTT results remains to be elucidated.

In conclusion, we hope that the above considerations may prove useful to clinicians in the interpretation of studies assessing the effects of antiretroviral drugs on carbohydrate metabolism using methods such as the euglycemic hyperinsulinemic clamp, the HOMA, or the OGTT.

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