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A child with HIV-associated transient cerebral arteriopathy

Transient cerebral arteriopathy (TCA) is a recently described entity that is increasingly recognized as an important cause of arterial ischaemic stroke (AIS) in children. TCA typically causes a lenticulostriate infarct as a result of a non-progressive unilateral intracranial arteriopathy of the distal internal carotid artery (ICA) and proximal middle cerebral artery (MCA) or anterior cerebral artery (ACA) [1]. The diagnosis of TCA requires follow-up vascular imaging, showing stabilization or improvement of the arterial lesions, with or without residual stenosis [1]. The arterial wall is thought to be affected by an inflammatory process. TCA probably represents a homogeneous group of diagnoses with similarities in clinical course and vascular imaging, but different aetiologies [1]. Infectious agents associated with TCA include varicella-zoster virus, enterovirus and *Borrelia burgdorferi*. We here present a patient with HIV-associated TCA.

A 5-year-old boy was admitted with a one day history of headache, speech problems and weakness of his right arm and leg. Neurological examination revealed an expressive aphasia, right facial droop and hemiparesis. The patient had been diagnosed with AIDS (Centers for Disease Control and Prevention classification A3) 5 months earlier and HAART was then initiated, decreasing the viral load from more than 750 000 to 398 copies/ml and increasing the CD4 cell count from 30 to 216 cells/ μ l. Magnetic resonance imaging on the day of admission showed an infarct in the left lenticulostriate MCA territory, and magnetic resonance angiography (MRA) revealed occlusion of the left M1 segment of the MCA

and stenosis of the distal ICA and proximal ACA (Fig. 1, left). Prothrombotic and cardiac evaluation was normal; cerebrospinal fluid showed a normal cell count and protein, polymerase chain reaction was negative for HIV, varicella-zoster virus, herpes simplex virus and enterovirus, and cryptococcal antigen was negative. The patient was given aspirin and improved markedly. Follow-up MRA performed after 2 months showed recanalization of the left MCA and improvement of the ACA and ICA stenosis (Fig. 1, right). Six months later, neurological examination was normal and a follow-up MRA was unchanged.

Cerebrovascular disease in HIV-positive children has an incidence of 1.3–2.6% [2,3], compared with 0.006% in the entire paediatric population [4]. Both ischaemic and haemorrhagic stroke occur, but the latter is uncommon and is often caused by immune thrombocytopenia [2]. The aetiology of AIS in HIV-positive children and adults differs markedly. In adults, stroke is usually caused by opportunistic infections and lymphoma, whereas in children it more often results from coagulopathy, cardioembolism, aneurysms, subacute necrotizing encephalopathy and arteriopathy [5].

A wide range of mechanisms causes arteriopathy in HIV-positive patients. HIV-associated opportunistic infections may cause cerebral vasculitis [6]. Aneurysmal arteriopathy is a frequent cause of AIS in HIV-positive patients, by focal thrombosis or vascular wall distortion compromising the orifice of branching arteries [3]. The most likely cause of non-aneurysmal arteriopathy not

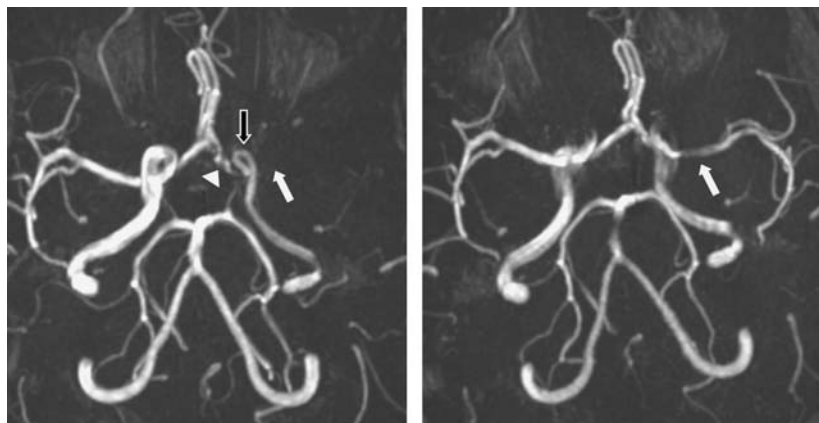


Fig. 1. Magnetic resonance angiography of the intracranial cerebral arteries, one day (left) and 2 months (right) after stroke. The initial occlusion of the middle cerebral artery (MCA, white arrow), and stenosis of the distal internal carotid artery (black arrow) and proximal anterior cerebral artery (arrowhead) significantly improved at follow-up (recanalized MCA, white arrow).

related to opportunistic infections is a secondary HIV-associated vasculopathy [5,7–9]. From the literature it is often unclear whether the HIV-associated vasculopathies are progressive or not [5]. In one cohort study [8] several adult patients had a confirmed non-progressive stenosis of the MCA that was attributed to a non-specific HIV-associated vasculopathy. To our knowledge there are no reports of arteriopathic AIS in HIV-positive children in whom follow-up vascular imaging was performed.

Our report indicates that a transient and improving arteriopathy can be associated with HIV in children. The arteriopathy can be explained either by direct HIV infection of the vascular wall or by an indirect immunological effect of systemic HIV infection. The absence of pleocytosis and the negative polymerase chain reaction for HIV in the cerebrospinal fluid makes the latter mechanism more plausible. The possibility of the stroke being HAART associated, either directly or as a result of immune reconstitution inflammatory syndrome, was considered. A direct effect is unlikely given the 5-month interval between HAART initiation and stroke, and the improvement of the vascular abnormalities despite the unchanged administration of HAART. Immune reconstitution inflammatory syndrome is improbable because no opportunistic infection was detected and the interval exceeded 3 months [10].

In view of the monophasic self-limiting course of TCA, and the low recurrence risk of stroke after its initial phase, we want to emphasize considering this non-progressive arteriopathy as a cause of stroke in HIV-positive children. When vascular imaging reveals an arteriopathy, the possible treatable (infectious) causes should be excluded first, before the diagnosis of TCA is considered. Follow-up vascular imaging should then be performed, according

to previously defined diagnostic guidelines [1]. Moreover, this case report illustrates that HIV infection may be contemplated in children diagnosed with TCA.

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References

1. Sebire G. **Transient cerebral arteriopathy in childhood.** *Lancet* 2006; **368**:8–10.
2. Park YD, Belman AL, Kim TS, Kure K, Llena JF, Lantos G, et al. **Stroke in pediatric acquired immunodeficiency syndrome.** *Ann Neurol* 1990; **28**:303–311.
3. Patsalides AD, Wood LV, Atac GK, Sandifer E, Butman JA, Patronas NJ. **Cerebrovascular disease in HIV-infected pediatric patients: neuroimaging findings.** *Am J Roentgenol* 2002; **179**:999–1003.
4. DeVeber G. **In pursuit of evidence-based treatments for paediatric stroke: the UK and Chest guidelines.** *Lancet Neurol* 2005; **4**:432–436.
5. Narayan P, Samuels OB, Barrow DL. **Stroke and pediatric human immunodeficiency virus infection. Case report and review of the literature.** *Pediatr Neurol* 2002; **37**:158–163.
6. Chetty R. **Vasculitides associated with HIV infection.** *J Clin Pathol* 2001; **54**:275–278.
7. Moriarty DM, Haller JO, Loh JP, Fikrig S. **Cerebral infarction in pediatric acquired immunodeficiency syndrome.** *Pediatr Radiol* 1994; **24**:611–612.
8. Evers S, Nabavi D, Rahmann A, Heese C, Reichelt D, Husstedt IW. **Ischaemic cerebrovascular events in HIV infection: a cohort study.** *Cerebrovasc Dis* 2003; **15**:199–205.
9. Cole JW, Pinto AN, Hebel JR, Buchholz DW, Earley CJ, Johnson CJ, et al. **Acquired immunodeficiency syndrome and the risk of stroke.** *Stroke* 2004; **35**:51–56.
10. Lipman M, Breen R. **Immune reconstitution inflammatory syndrome in HIV.** *Curr Opin Infect Dis* 2006; **19**:20–25.

Discontinuation of non-nucleoside reverse transcriptase inhibitor-based antiretroviral therapy: clinical implications in patients on CD4 T-cell-guided treatment interruption

Ruiz and colleagues [1] found the frequent de novo selection of non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance in the group in their study on guided treatment interruptions (GTI) probably to be driven by the simultaneous interruption of all drugs in patients receiving NNRTI-containing regimens. They discouraged the inclusion of any drug with long plasma or low genetic barriers into GTI regimens because of the risk of the de novo selection of resistant mutations.

Both United States and United Kingdom HIV treatment guidelines acknowledge the difficulty of knowing the exact duration of antiretroviral therapy cover required after stopping NNRTI, and the optimal interval between

stopping efavirenz or nevirapine and other antiretroviral drugs is unknown.

Their prolonged half-life leads to the detection of these drugs for 1–3 weeks after discontinuation [2,3], and the simultaneous interruption of all antiretroviral drugs in a regimen containing these agents may therefore result in functional monotherapy with the NNRTI. This in turn increases the risk of the selection of NNRTI-resistant mutations. Therefore some experts recommend stopping NNRTI while continuing other antiretroviral drugs for 5–7 days [4,5]. We describe our experience in eight patients who simultaneously interrupted all antiretroviral drugs (including NNRTI) during CD4 T-cell GTI and

Table 1. Antiretroviral regimens, date and months of interruption, and time length of undetectable viral load after reintroduction of the same regimen.

Patient	Antiretroviral therapy	Date of interruption	Months of interruption	Date of restarting	Months with HIV-RNA < 50 copies/ml
1	ZDV + ddC + EFV	26.04.04	2	28.06.04	30
2	ZDV + 3TC + NVP	28.04.04	19	11.11.05	13
3	ZDV + 3TC + EFV	14.06.04	11	15.05.05	19
4	d4T + 3TC + NVP	27.05.04	9	31.03.05	21
5	ZDV + ddl + EFV	21.04.04	7	15.11.04	25
6	ZDV + 3TC + NVP	18.05.05	8	11.01.06	11
7	d4T + ddl + EFV	20.10.04	2	13.12.04	24
8	ddl + 3TC + EFV	10.07.05	2	23.09.05	15

ddC, Zalcitabine; ddl, didanosine; d4T, stavudine; EFV, efavirenz; NVP, nevirapine; 3TC, lamivudine; ZDV, zidovudine.

obtained a rapid return of plasma viraemia to undetectable levels after restarting the same regimen.

A total of 46 white European patients were enrolled in the clinical study of CD4 T-cell GTI. Of these, 22 were on NNRTI-based antiretroviral therapy. The criteria used to stop antiretroviral therapy were a CD4 T-cell count of 500 cells/ μ l or greater and a plasma HIV-RNA level of less than 50 copies/ml for at least 3 years. Patients underwent laboratory evaluations 4 and 12 weeks after stopping antiretroviral therapy and then every 3 months. The criteria for restarting HAART were: (i) acute retroviral syndrome; (ii) patient's choice, independent of the CD4 T-cell count; (iii) pregnancy; (iv) the onset of HIV-related systemic symptoms (acute retroviral syndrome) or of opportunistic infections; (v) a CD4 T-cell count decline to less than 250 cells/ μ l.

Four weeks after discontinuation, an antiretroviral genotyping test (TruGene HIV-1 genotyping assay) was performed to assess the presence of NNRTI-related point mutations.

Fifteen patients restarted ART; eight of them (five women, median age 42 years, range 37–55) were on NNRTI (five on efavirenz and three on nevirapine). Four patients were antiretroviral naive and four antiretroviral experienced. Table 1 give details of the antiretroviral regimens, the date and length of interruption, and months of undetectable viral load reached after the reintroduction of the same regimen. Viraemia was undetectable again in all patients within 3 months of the reintroduction of antiretroviral therapy, and continues to be so.

Genotypic resistance tests failed to reveal mutations conferring resistance to NNRTI in all patients (100%). Few data are available on the discontinuation of NNRTI-based therapy in order to avoid a monotherapy state and the potential evolution of drug-resistant variants. Two studies have documented how the duration of detectable levels of these drugs after discontinuation ranges from less than one week [4] to over 3 weeks [5], and the authors suggested continuing the nucleoside reverse transcriptase

inhibitor backbone for a minimum of 5 days to a maximum of 14 days after the discontinuation of NNRTI in order to avoid the emergence of resistance.

Some authors have also shown that certain host genetic polymorphisms, more common among African-American and Hispanic individuals [6,7], result in slower rates of clearance of these drugs. The optimal time sequence for staggered component discontinuation has, however, not been still determined.

Our results, in a small number of individuals, indicate that white European patients with high CD4 T-cell numbers and undetectable plasma HIV-RNA levels can simultaneously discontinue all components of their antiretroviral regimen without developing point mutations conferring resistance to NNRTI. We hypothesized that the 4–7 day delay in viral rebound after therapy interruption, as shown by Neumann *et al.* [8], could explain our results. Perhaps the risk of developing NNRTI resistance when stopping all antiretroviral drugs simultaneously is more common when cessation occurs with a detectable plasma viraemia, as suggested by others [4].

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References

- Ruiz L, Paredes R, Gomez G, Romeu J, Domingo P, Perez-Alvarez N, *et al.* Antiretroviral therapy interruption guided by CD4 cell counts and plasma HIV-1 RNA levels in chronically HIV-1-infected patients. *AIDS* 2007; **21**:169–178.
- Cardiello PG, Hassink E, Ananworanich J, Srasuebku P, Samor T, Mahanontharit A, *et al.* A prospective, randomized trial of structured treatment interruption for patients with chronic HIV type 1 infection. *Clin Infect Dis* 2005; **40**:594–600.

3. Ribaudo HJ, Haas DW, Tierney C, Kim RB, Wilkinson GR, Gulick RM, *et al.* **Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: an Adult AIDS Clinical Trials Group Study.** *Clin Infect Dis* 2006; **42**:401–407.
4. Mackie NE, Fidler S, Tamm N, Clarke JR, Back D, Weber JN, Taylor GP. **Clinical implications of stopping nevirapine-based antiretroviral therapy: relative pharmacokinetics and avoidance of drug resistance.** *HIV Med* 2004; **5**:180–184.
5. Taylor S, Allen S, Fidler S, White D, Gibbons S, Fox J, *et al.* **Stop Study: after discontinuation of efavirenz, plasma concentrations may persist for 2 weeks or longer.** In: *XIth Conference on Retroviruses and Opportunistic Infections*. San Francisco, CA, 8–11 February 2004 [Abstract 131].
6. Haas D, Ribaudo H, Kim R, Tierney C, Wilkinson G, Gulick R, *et al.* **A common CYP2B6 variant is associated with efavirenz pharmacokinetics and central nervous system side effects: AACTG Study NWCS214.** In: *XIth Conference on Retroviruses and Opportunistic Infections*. San Francisco, 8–11 February 2004 [Abstract 133].
7. Ribaudo H, Clifford D, Gulick R, Shikuma C, Klingman K, Snyder S, Acosta E. **Relationships between efavirenz pharmacokinetics, side effects, drug discontinuation, virologic response, and race: results from ACTG A5095/A5097s.** In: *XIth Conference on Retroviruses and Opportunistic Infections*. San Francisco. 8–11 February 2004. [Abstract 132].
8. Neumann AU, Tubiana R, Calvez V, Robert C, Li TS, Agut H, *et al.* **HIV-1 rebound during interruption of highly active antiretroviral therapy has no deleterious effect on reinitiated treatment.** *AIDS* 1999; **13**:677–683.

Fatal myocardial co-infection by *Toxoplasma gondii* and Parvovirus B19 in an HIV patient

Myocarditis has been associated with a wide range of infectious agents. Some microorganisms have a cardio-myocyte tropism, whereas for others, such as Parvovirus B19 (PVB19), the myocardial cellular target is represented by endothelial cells [1,2]. Nevertheless, a vasculitis of the intramural coronary vessels caused by PVB19 has never been described.

We report a case of fatal myocarditis and small vessel vasculitis related to a PVB19-*Toxoplasma gondii* co-infection in an HIV-positive patient, clinically mimicking an acute myocardial infarction.

Case report

A 45-year-old woman was admitted because of chest pain and dyspnoea. Electrocardiogram revealed atrial fibrillation (160 bpm mean rate) with diffuse ST-segment elevation and T-wave inversion. Echocardiography showed moderate dilation with severe global left ventricular hypokinesia (ejection fraction 0.25). The myocardial type isoenzyme fraction of creatine kinase was 223 U/l (normal value < 10 U/l) and the troponin I level was 6 ng/ml (normal value < 0.05 ng/ml). Unpredictably, coronary angiography showed a normal coronary network. On the basis of a persistent marked lymphocytopenia (CD4 cells 76/μl, CD8 cells 1566/μl, CD4:CD8 ratio 0.05) a serological assay for HIV antibodies was performed, which gave a positive result. The HIV-RNA plasma level was greater than 500 000 copies/ml. Serology showed a positivity for PVB19 and *T. gondii* IgG, whereas no evidence of active infection was detected. Despite full medical therapy with diuretics, angiotensin-converting enzyme inhibitors and digitalis, the patient's clinical condition worsened and cardiac function progressively deteriorated (ejection

fraction 0.15). Ten days after admission the patient died as a result of cardiac arrest.

Microscopic evaluation of the heart revealed diffuse lymphocytic myocarditis, with a predominance of CD8 cells, diffusely infiltrating both the myocardium and intramural vessels (Fig. 1a). Cardiomyocytes were focally swollen by intracytoplasmic cysts containing trophozoites of *T. gondii* (Fig. 1b). Polymerase chain reaction analysis confirmed the presence of *Toxoplasma* DNA and demonstrated the presence of the PVB19 genome (Fig. 1c), whereas it was negative for other cardiotropic viruses. Polymerase chain reaction on blood samples was negative for PVB19 and *T. gondii*. Immunohistochemistry for PVB19 antigen showed the presence of granular intracytoplasmic staining in the endothelial cells of intramural vessels, more evident in those surrounded by severe inflammatory infiltrates (Fig. 1d), and was negative in other cell types.

A previously undescribed myocardial inflammation caused by *T. gondii* and PVB19 co-infection has been reported. The major contribution of *T. gondii* was related to its direct mechanism of cardiomyocyte damage, as documented by the presence of intracytoplasmic cysts. Conversely, PVB19 infection affected endothelial cells, as shown by their strong positivity for viral antigens, leading to vasculitis of the intramural vessels. The synergic action of the two agents caused a severe and rapidly progressive myocardial inflammation, mimicking the clinical phenotype of an acute myocardial infarction.

T. gondii is the most frequent protozoan causing opportunistic infections in immunocompromised individuals, and has been observed in up to 12% of HIV patients at necropsy [3]. Occasionally, *Toxoplasma* myocarditis can occur in association with other infectious

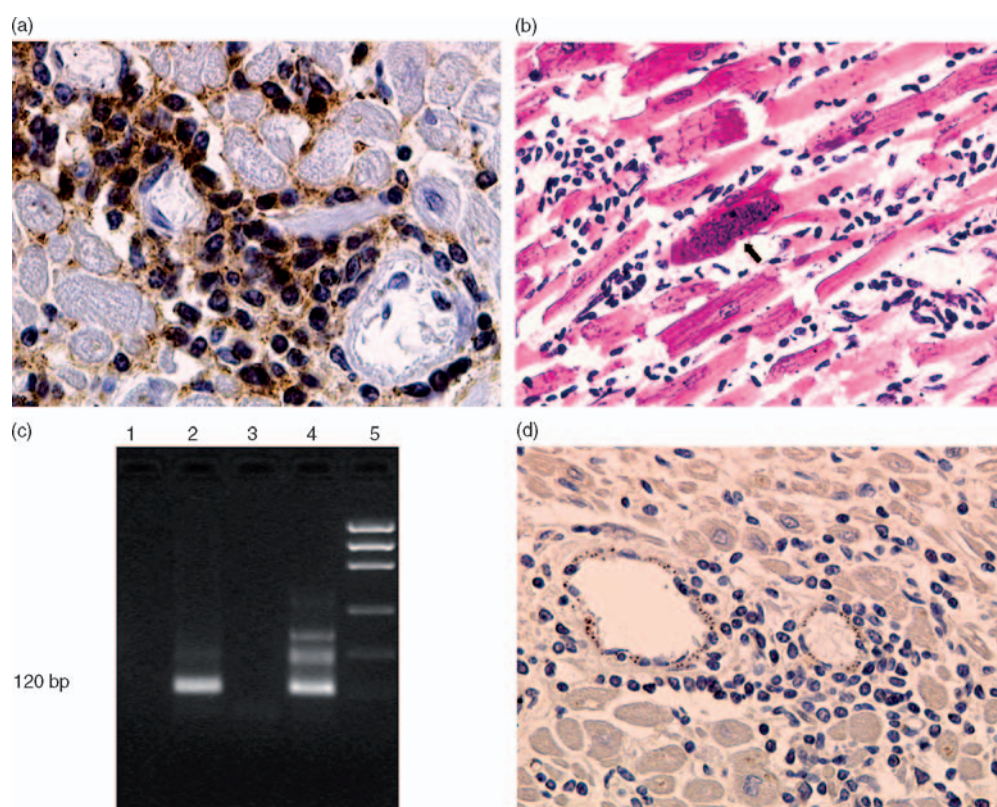


Fig. 1. Microscopic evaluation and polymerase chain reaction analysis of the heart, from the patient. (a) Cardiomyocytes and intramyocardial vessel infiltration by CD8 lymphocytes (immunoperoxidase, 400 $\times$). (b) Prominent lymphocytic infiltrates around myocytes that focally contain cysts of *Toxoplasma gondii* (arrow; haematoxylin and eosin 250 $\times$). (c) Nested polymerase chain reaction analysis using primers designed to amplify a 102 base pair sequence of PVB19. Lane 1, serum from the patient; lane 2, myocardium; lane 3, negative control (no template); lane 4, PVB19-positive control; lane 5, molecular weight marker. (d) Granular intracytoplasmic positivity of VP1 and VP2 PVB19 antigens (cloneR92F6; Novocastra, Newcastle, UK) in endothelial cells of intramyocardial vessels, surrounded by inflammatory infiltrates (immunoperoxidase 250 $\times$).

agents, but, as far as we know, co-infection with PVB19 has never been described.

Human PVB19 is a DNA virus member of the family of *Parvoviridae*, and is emerging as a new agent associated with myocarditis, dilated cardiomyopathy and isolated diastolic dysfunction [1,2]. This agent has also been detected in serum and tissue samples of patients with systemic vasculitis syndromes [4]. In our patient, the presence of the PVB19 genome in the myocardium associated with the positivity for viral antigens on endothelial cells and the absence of serological evidence of an acute PVB19 infection suggests that the virus entered the cells during a previous asymptomatic infection and persisted in the latent phase for many years, until unknown factors, possibly immunodepression, induced transcriptional activity of PVB19, resulting in acute symptomatic disease. The immunocompromised state could explain the occurrence of a previously undescribed severe vasculitis of the intramyocardial vessels, leading to endothelial dysfunction and impairment of coronary microcirculation simulating ischaemic heart disease. Moreover, PVB19 antigens can induce an

immune-mediated reaction with the release of proinflammatory cytokines and secondary myocyte necrosis and inflammation [5]. The prominent lymphocytic infiltrates of both myocytes and vessels, which are unusual for isolated *Toxoplasma* myocarditis, support this hypothesis and a relevant pathogenetic role for PVB19 infection.

Toxoplasmosis infection can receive a specific treatment with confirmed clinical good results even in immunocompromised patients [3]. Moreover, PVB19-associated vasculitis has been successfully treated with intravenous immunoglobulin therapy [4].

An early diagnosis by endomyocardial biopsy and molecular biology studies, with the prompt institution of a specific anti-infectious treatment, might have prevented the fatal course of myocardial infection.

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References

1. Bultmann BD, Klingel K, Sotlar K, Bock T, Baba HA, Sauter M, Kandolf R. **Fatal parvovirus B19-associated myocarditis clinically mimicking ischemic heart disease: an endothelial cell-mediated disease.** *Hum Pathol* 2003; **34**:92–95.
2. Tschope C, Bock CT, Kasner M, Noutsias M, Westermann D, Schwimmbeck PL, et al. **High prevalence of cardiac parvovirus B19 infection in patients with isolated left ventricular diastolic dysfunction.** *Circulation* 2005; **111**:879–886.
3. Montova JG, Liesenfeld O. **Toxoplasmosis.** *Lancet* 2004; **363**: 1965–1976.
4. Finkel TH, Torok TJ, Ferguson PJ, Durigon EL, Zaki SR, Leung DY, et al. **Chronic parvovirus B19 infection and systemic necrotizing vasculitis: opportunistic infection or aetiological agent?** *Lancet* 1994; **343**:1255–1258.
5. Nigro G, Bastianon V, Colloridi V, Ventriglia F, Gallo P, D'Amati G, et al. **Human parvovirus B19 infection in infancy associated with acute and chronic lymphocytic myocarditis and high cytokine levels: report of 3 cases and review.** *Clin Infect Dis* 2000; **31**:65–69.

Successful long-course after failure of short-course desensitization in a patient with severe hypersensitivity reaction to enfuvirtide

Enfuvirtide is a milestone in antiretroviral salvage therapy. When combined with a backbone regimen optimized through results of HIV drug resistance testing, it allows virological control to be regained even after many failures. Rare cases of hypersensitivity reactions (HSR) to enfuvirtide have been described, which may limit the possibility to benefit from this drug. Less than 1% patients in the TORO trials experienced HSR to enfuvirtide [1]. Two male patients have so far been reported in the literature, both developing a mild skin reaction, whose recurrence was successfully prevented by short desensitization procedures [2,3]. It is currently unknown what is the actual pathogenesis of the event and whether a short desensitization procedure could be applied successfully to more severe HSR.

Case report

A 34-year-old white woman who failed all three classes of antiretroviral drugs (nucleoside and non-nucleoside reverse transcriptase inhibitors and protease inhibitors) was prescribed tenofovir/emtricitabine with darunavir and enfuvirtide on the basis of resistance testing results when her CD4 T-cell count was 306 cells/ μ l (14.6%) and HIV plasma viral load was 63 000 copies/ml. Her nadir CD4 T-cell count was 126 cells/ μ l (9%). After 5 weeks from starting the new combination, she developed a maculopapular generalized rash and described symptoms possibly related to laryngopharyngeal oedema, with breathing difficulties. Enfuvirtide was stopped and prednisone was started at a dose of 25 mg a day. One week later, the patient recovered from HSR.

For the sake of the diagnosis and trying to elucidate the possible pathogenesis of this phenomenon, a skin test evaluation was performed. In particular, we tested the hypothesis of an IgE-mediated mechanism responsible for the skin eruption. This hypothesis was formulated because IgE-mediated reactions are held to be responsible for clinical symptoms on the second administration of allergen or during the first administration after at least 10 days of continuous treatment, as was the case with our patient. A skin prick test was performed either with enfuvirtide alone or with enfuvirtide previously incubated with albumin, thanks to its ability to link to plasma proteins, particularly albumin. Albumin preincubation served to increase the molecular weight of enfuvirtide, should it work as an aptenic drug. We performed a skin prick test with enfuvirtide or enfuvirtide plus albumin at the following dilutions 1:100, 1:10, and with the undiluted drug. In the first instance, the skin prick test turned out to be negative but after 2 h a pruritic diffuse wheal eruption appeared. Therefore, we decided to start a desensitization protocol.

This was initiated one week later according to protocols described in the literature [2], starting with 0.00625 mg of enfuvirtide, with subsequent dose increases at 1 h intervals so as to reach the undiluted dose after 11 h. Two hours after the highest dose, itching red lesions appeared at the sites of the current and all the previous injections in the abdomen and legs, notwithstanding the administration of hydroxyzine cloridrate (25 mg three times a day) during the entire procedure (see Fig. 1). A punch biopsy was performed at one of the abdominal lesions and stained with haematoxylin-eosin. Mixed lymphocytic infiltrate with granulocytic and eosinophil cell infiltrations were demonstrated, resembling the

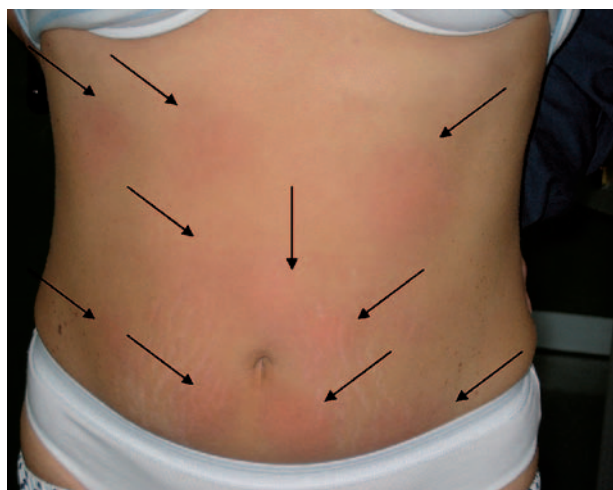


Fig. 1. Pruriginous and erythematous indurations developed at the previous enfuvirtide injection site after the short desensitization schedule (injection site reactions are indicated by the arrows).

histological characteristics of an acute urticaria-like pattern.

During the subsequent week, the patient recovered from the nodular exanthema. At that time, a new protocol was applied with a more gradual increase in dosages of enfuvirtide (see Table 1). Hydroxyzine cloridrate was continued. Through this protocol, the patient was successfully desensitized and was continued on the previously described combination. Ten weeks later, the patient's HIV-1 load was less than 50 copies/ml and the CD4 T-lymphocyte count was 368 cells/ μ l (22.9%).

Enfuvirtide is an antiretroviral drug, electively used in rescue regimens. Hypersensitivity reactions occur rarely, but result in a treatment-limiting effect [1–4]. Our patient experienced a diffuse cutaneous reaction. The pathogenic mechanisms are unclear. A negative skin

prick test did not corroborate the hypothesis of an immediate IgE-mediated immune reaction. Nevertheless, after a latency of 2 h from the skin testing, the patient developed a diffuse pruritic skin eruption, suggesting a close link between the administration of enfuvirtide and clinical symptoms. Because of the known rapid development of resistance to this drug [5,6], we started a literature-derived short course desensitization protocol that turned out to be ineffective. Interestingly, skin reactions developed at all previous injection points. The local reactivation at the previous injection sites of subcutaneous administration may have been caused by cytokine and histamine releasing from infiltrating cells reactivated by an endocrine and paracrine mechanism [7].

In conclusion, this case demonstrates that short desensitization procedures for HRS to enfuvirtide may be ineffective and a more prolonged and cautious desensitization protocol may be needed. Our protocol demonstrated a favourable tolerance and outcome. Therefore we propose an alternative protocol to short course desensitization procedures after more severe skin reactions occur.

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Table 1. Prolonged enfuvirtide desensitization protocol (dose increase was performed at intervals of 2 h).

Dose no.	Enfuvirtide (mg)	Dose no.	Enfuvirtide (mg)
1	0.0009	16	4.5
2	0.0045	17	5.4
3	0.009	18	6.3
4	0.027	19	7.2
5	0.045	20	8
6	0.09	21	9
7	0.18	22	18
8	0.27	23	27
9	0.45	24	36
10	0.54	25	45
11	0.72	26	54
12	0.9	27	63
13	1.8	28	72
14	2.7	29	81
15	3.6	30	90

References

1. Clotet B, Raffi F, Cooper D, Delfraissy JF, Lazzarin A, Moyle G, et al. Clinical management of treatment-experienced HIV-infected patients with the fusion inhibitor enfuvirtide: consensus recommendations. *AIDS* 2004; **18**:1137–1146.
2. DeSimone JA, Ojha A, Pathak R, Cohn J. Successful desensitization to enfuvirtide after a hypersensitivity reaction in an HIV-1-infected man. *Clin Infect Dis* 2004; **39**:e110–e112.
3. Shahar E, Moar C, Pollack S. Successful desensitization to enfuvirtide-induced skin hypersensitivity reaction. *AIDS* 2005; **19**:451–452.
4. Maggi P, Ladisa N, Cinori E, Altobella A, Pastore G, Filotico R. Cutaneous injection site reactions to long-term therapy with enfuvirtide. *J Antimicrob Chemother* 2004; **53**:678–681.
5. Lu J, Deeks SG, Hoh R, Beatty G, Kuritzkes BA, Martin JN, et al. Rapid emergence of enfuvirtide resistance in HIV-1-infected patients: results of a clonal analysis. *J Acquir Immune Dis Syndr* 2006; **43**:60–64.
6. Wei X, Decker JM, Liu H, Zhang Z, Arani RB, Kilby JM, et al. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. *Antimicrob Agents Chemother* 2002; **46**:1896–1905.
7. Martinez-Moczygemba M, Huston DP, Lei JT. JAK kinases control IL-5 receptor ubiquitination, degradation, and internalization. *J Leukoc Biol* 2007; E-pub ahead of print DOI 10.1189/JLB.0706465.

Misleading results of screening for illicit drugs during efavirenz treatment

We investigated in 12 healthy subjects the influence of efavirenz, a non-nucleoside reverse transcriptase inhibitor, on the pharmacokinetics of midazolam, which served as a marker for CYP3A4 activity. The study was authorized by the Ethics Committee of the Medical Faculty (University of Heidelberg, Germany) as well as the Competent Regulatory Authority. All study participants gave their written informed consent. They received 3 mg midazolam by mouth together with the first dose of efavirenz. Thereafter, efavirenz was administered in a daily dose of 400 mg by mouth for 14 days. On day 8, a safety check including a routine screening for illicit drugs was performed, in which 11 out of 12 subjects screened positive for benzodiazepines and eight for tetrahydrocannabinol using the Triage 8 (Biosite, San Diego, California, USA) single-use, rapid immunochemical assay. In the product information of efavirenz an interference of efavirenz with the CEDIA-DAU multi-level tetrahydrocannabinol assay is mentioned [1]. Earlier reports characterized the interference by efavirenz in an estradiol enzyme-linked immunosorbent assay [2] and with several tetrahydrocannabinol immunoassays [3]. We therefore suspected that efavirenz or its metabolites may also cause false-positive results for benzodiazepines through crossreactivity.

To verify our results we evaluated the urine samples collected on day 14 before the last administration of efavirenz again using the Triage 8. All 12 subjects tested positive for benzodiazepines, 11 for tetrahydrocannabinol.

It is believed that positive tetrahydrocannabinol immunoassays are caused by an interference with efavirenz-8-glucuronide formed during efavirenz treatment [3]. Using the Triage 8, we also obtained false-positive tetrahydrocannabinol results, which confirmed the data reported for a test by the same manufacturer [3]. The newly described positive benzodiazepine immunoassay prompted us to characterize the occurrence of efavirenz crossreactivity regarding benzodiazepines with several commercial reagents used to screen for benzodiazepine exposure.

The urine samples of the 12 subjects from day 14 were subjected to six different commercially available single or multiple drug screening assays. To ensure that in these samples no midazolam was present after the administration on day 1, the urine and serum samples collected before the intake of efavirenz on day 14 were analysed by liquid chromatography-mass spectrometry-mass spectrometry for midazolam and its metabolite 1-OH-midazolam. Both substances could not be detected (< 0.2 ng/ml for midazolam, < 0.3 ng/ml for 1-OH-midazolam).

Triage 8 utilizes a patented two-phase competitive binding immunochemistry assay in which a chemically labelled drug (drug conjugate) competes with drugs present in the urine for antibody binding sites. After a brief

incubation time (10 min) the reaction mixture is transferred to the membrane. The residue of free drug conjugate that results from specific antibody binding to drug present in the urine sample binds to a zone of monoclonal antibody that is immobilized on the membrane in the solid phase. After the unbound drug conjugate is removed by a washing solution, a positive specimen produces a distinct coloured bar in the drug detection zone [4].

The other five tests used were the immunochromatographic assays DrugControl (Ultimed, Ahrensburg, Germany), Mahsan Kombi/DOA6-Rapidtest (Mahsan Diagnostika, Reinbek, Germany), the multi drug test (von Minden GmbH, Moers, Germany), HITADOMulti 10a test (Gabmed GmbH, Nettetal, Germany) and Syva Rapid d.a.u. tetrahydrocannabinol and benzodiazepine single test (Dade-Behring Ltd., Milton Keynes, UK). These tests contain either drug conjugate or monoclonal antibodies immobilized on the membrane. If the drug conjugate is immobilized on the membrane, the drug in question binds to the marked antibody and prevents the binding of this antibody to the immobilized drug conjugate. The detection line stays invisible. If the monoclonal antibody is immobilized on the membrane, the drug in question binds to the unmarked antibody on the detection line, the marked drug conjugate is washed away. The detection line also remains invisible. In the case of a negative urine sample, the marked counterpart binds to the membrane and the detection line appears. All six tests have cutoff levels of 50 ng/ml for 11-nor- Δ^9 -THC-9-COOH and 300 ng/ml for oxazepam. Midazolam is only tested positive above 10 000–12 500 ng/ml depending on the test system.

Triage 8 results were confirmed with all subjects being positive for tetrahydrocannabinol and benzodiazepines. All other tests but DrugControl screened positive for tetrahydrocannabinol, but not for benzodiazepines (Table 1). Besides the tetrahydrocannabinol and the benzodiazepines tests none of the other drug screens were positive (cocaine, methadone, morphine, amphetamines, barbiturates, methamphetamines, opioids, tricyclic antidepressants, phencyclidine).

The interferences with immunoassays for tetrahydrocannabinol with efavirenz have been documented in the literature [3]. In contrast to the reported negative results for the instrument-based Dade-Behring test system [3], all urine samples tested positive with the Syva RapidTest d.a.u. (Dade-Behring). DrugControl was the only test showing no crossreactivity with respect to tetrahydrocannabinol testing.

The crossreaction with benzodiazepine drug screening tests during efavirenz treatment in human urine has not yet been described, but positive benzodiazepine results did occur using Triage 8. A careful selection of drug abuse

Table 1. Immunoassay results of six different drug screens from different vendors with respect to benzodiazepines and tetrahydrocannabinol.

Subject	Triage 8		DrugControl		Mahsan Kombi/ DO A6-Rapidtest		Drug screen multi test		HITADO multi 10a		Syva RapidTest d.a.u.	
	BZD	THC	BZD	THC	BZD	THC	BZD	THC	BZD	THC	BZD	THC
Control	–	–	–	–	–	–	–	–	–	–	–	–
1	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
2	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
3	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
4	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
5	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
7	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
8	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
9	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
10	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
11	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.
12	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	–	–	Pos.
13	Pos.	Pos.	–	–	–	Pos.	–	Pos.	–	Pos.	–	Pos.

BZD, Benzodiazepine; THC, tetrahydrocannabinol.

screening is thus recommended during HIV therapy regimens including efavirenz to avoid false-positive results.

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References

1. Product information Sustiva. Bristol-Myers Squibb Company, Princeton, USA; 2006.
2. Sinicco A, Raiteri R, Rossati A, Savarino A, Di Perri G. **Efavirenz interference in estradiol ELISA assay [Letter]**. *Clin Chem* 2000; **46**:734–735.
3. Rossi S, Yaksh T, Bentley H, van den BG, Grant I, Ellis R. **Characterization of interference with 6 commercial delta9-tetrahydrocannabinol immunoassays by efavirenz (glucuronide) in urine [Letter]**. *Clin Chem* 2006; **52**:896–897.
4. Package insert, Triage 8. Biosite, San Diego, USA; 2007.

Alopecia associated with ritonavir-boosted atazanavir therapy

Alopecia may complicate the course of antiretroviral therapy, especially when HIV-1 protease inhibitors are used [1]. Here we report the first case of hair loss associated with ritonavir-boosted atazanavir therapy in an antiretroviral-naïve patient with HIV infection.

A 39-year-old Hispanic man with a history of cryptosporidiosis presented with hair loss 3 weeks after commencing combination antiretroviral therapy. He was on prophylactic therapy for *Pneumocystis jirovecii* pneumonia with trimethoprim–sulfamethoxazole and *Mycobacterium avium* complex with azithromycin for several weeks when a daily regimen of atazanavir (300 mg), ritonavir (100 mg), and emtricitabine/tenofovir (300/200 mg, fixed-dose combination) was initiated. The physical examination was remarkable only for diffuse hair loss (Fig. 1a) without skin lesions. Laboratory evaluation included a complete blood cell count and chemistry, serum folate, serum vitamin B₁₂, serum testosterone, follicle-stimulating hormone, luteinizing hormone, and thyroid-stimulating hormone, and was only remarkable for

mild anemia (hemoglobin 13.5 g/dl) and low folate levels (4.5 ng/ml). The CD4 T-lymphocyte count was 10 cells/μl, with an HIV-RNA viral load of more than 750 000 copies/ml upon initiation of antiretroviral therapy. Folate replacement therapy was started, with resolution of the anemia. The hair loss worsened, however, and folate was discontinued after 2 weeks of therapy. Alopecia caused by ritonavir-boosted atazanavir therapy was suspected, with progressive hair regrowth noted one month after the replacement of protease inhibitors by efavirenz (Fig. 1b). Emtricitabine/tenofovir was continued unchanged. After one month on the new regimen, the CD4 T-lymphocyte count was 203 cells/μl, and the HIV-RNA viral load became undetectable (< 400 copies/ml).

Antiretroviral-induced alopecia has been reported with the use of protease inhibitors, particularly with indinavir therapy given alone [1,2] or in combination with ritonavir [3]. Other drugs or regimens associated with this adverse event include lopinavir/ritonavir [4], zidovudine [5] and lamivudine [6]. Hair loss associated



Fig. 1. Photos of the patient showing diffuse hair loss (a), and progressive regrowth one month after the replacement of protease inhibitors by efavirenz (b).

with ritonavir-boosted atazanavir therapy has not been reported to our knowledge.

Alopecia occurs mostly during the first 6 months of starting protease inhibitors (median of 50 days), with some cases reported as soon as 2–3 weeks after beginning treatment [3,4]. This complication could be accompanied by other cutaneous manifestations, such as hair loss in eyelids, eyebrows, beard, axilla and pubic areas, and body hair, skin dryness, cheilitis, and paronychia [1,2]. The temporal relationship of the initiation of protease inhibitors and this patient's hair loss suggests that ritonavir-boosted atazanavir induced this abnormality. The alopecia reversed after a protease inhibitor-sparing regimen was initiated. A rechallenge would be necessary to confirm causality.

The mechanism of antiretroviral-induced hair loss is unknown. In some cases, the alopecia observed in patients receiving HIV-1 protease inhibitors is indistinguishable from the toxic effects of systemic retinoid therapy [1,2]. Homologies between the C-terminal region of the cytoplasmic retinoic-acid binding protein type 1 and the

catalytic site of protease inhibitors have been noted. It has been hypothesized that protease inhibitors bind to this homologous region within cytoplasmic retinoic-acid binding protein type 1 and inhibit the binding of retinoic acid [7]. Some protease inhibitors enhance the activity of retinal dehydrogenase increasing retinoic acid concentrations, which might be responsible for the retinoid-like side effects [8]. The role of immune reconstitution in this setting cannot be excluded. Alopecia usually resolves over time with discontinuation of the offending antiretroviral agent. Interestingly, hair regrowth has occurred after the replacement of indinavir by ritonavir plus saquinavir or nelfinavir [1], and of lopinavir/ritonavir by nelfinavir [4]. Therefore, protease inhibitor-induced alopecia seems to be a drug-specific rather than a drug class side effect.

In summary, patients receiving ritonavir-boosted atazanavir therapy have to be monitored for alopecia, and this combination may have to be discontinued if this complication occurs and other options are available. A switch to a non-protease inhibitor regimen may not necessarily be required.

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References

1. Bouscarat F, Prevot MH, Matheron S. **Alopecia associated with indinavir therapy.** *N Engl J Med* 1999; **341**:618.
2. D'Arminio Monforte A, Testa L, Gianotto M, Gori A, Franzetti F, Sollima S, et al. **Indinavir-related alopecia.** *AIDS* 1998; **12**:328.
3. Ginarte M, Losada E, Prieto A, Lorenzo-Zuniga V, Toribio J. **Generalized hair loss induced by indinavir plus ritonavir therapy.** *AIDS* 2002; **16**:1695–1696.
4. Bongiovanni M, Chiesa E, Monforte A, Bini T. **Hair loss in an HIV-1 infected woman receiving lopinavir plus ritonavir therapy as first line HAART.** *Dermatol Online J* 2003; **9**:28.
5. Geletko SM, Segarra M, Mikolich DJ. **Alopecia associated with zidovudine therapy.** *Pharmacotherapy* 1996; **16**:79–81.
6. Fong IW. **Hair loss associated with lamivudine.** *Lancet* 1994; **344**:1702.
7. Carr A, Samaras K, Chisholm DJ, Cooper DA. **Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance.** *Lancet* 1998; **351**:1881–1883.
8. Toma E, Devost D, Chow Lan N, Bhat PV. **HIV-protease inhibitors alter retinoic acid synthesis.** *AIDS* 2001; **15**:1979–1984.

Efavirenz: innocent bystander or direct nephrotoxin?

Barbour and colleagues [1] report an interesting case of minimal change disease occurring in the setting of HIV infection and antiretroviral therapy. The authors implicate the non-nucleoside reverse transcriptase inhibitor efavirenz, based on the clinical resolution of proteinuria after discontinuation. This is the first suggestion of direct nephrotoxicity associated with efavirenz, which has previously been associated with a single case of interstitial nephritis in the setting of systemic hypersensitivity [2]. Potential efavirenz nephrotoxicity would have particularly important implications for the safety of the fixed-dose combination with emtricitabine and tenofovir, which already requires enhanced monitoring for tubular toxicity associated with tenofovir [2,3].

Although this case suggests a need for increased vigilance and further studies, the authors have not established a causal relationship between efavirenz and the development of glomerular disease in their patient. HIV infection itself has been associated with minimal change disease [4,5], and early focal segmental glomerulosclerosis can be misdiagnosed as minimal change disease as a result of biopsy sampling error. As noted by the authors, the diagnosis of HIV-associated focal segmental glomerulosclerosis is less likely in the setting of non-black race; however, the development of nephrotic syndrome after a period of viremia and the gradual response to restored virological suppression are compatible with HIV-associated nephropathy [1]. Finally, spontaneous remission of minimal change disease may occur [6], and the discontinuation of efavirenz may have been merely coincidental.

Expert guidelines currently recommend biannual monitoring for kidney function and urinary abnormalities in tenofovir-treated patients with risk factors for kidney disease or reduced kidney function [3]. Until further studies clarify the potential for efavirenz-associated glomerular injury, it seems prudent to apply these recommendations to patients without additional risk factors who are receiving combination therapy with

efavirenz. Patients who develop proteinuria on therapy with either of these agents should be referred for nephrology evaluation and possible biopsy, which may identify an alternative etiology and avoid unnecessary interruptions in antiretroviral therapy.

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References

1. Barbour TD, Furlong TJ, Finlayson RJ. **Efavirenz-associated podocyte damage.** *AIDS* 2007; **21**:257–258.
2. Wyatt CM, Klotman PE. **Antiretroviral therapy and the kidney: balancing benefit and risk in patients with human immunodeficiency virus infection.** *Expert Opin Drug Saf* 2006; **5**:275–287.
3. Gupta SK, Eustace JA, Winston JA, Boydston II, Ahuja TS, Rodriguez RA, et al. **Guidelines for the management of chronic kidney disease in HIV-infected patients: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America.** *Clin Infect Dis* 2005; **40**:1559–1585.
4. Singer DR, Jenkins AP, Gupta S, Evans DJ. **Minimal change nephropathy in the acquired immunodeficiency syndrome.** *BMJ* 1985; **291**:868.
5. Lopes GS, Marques LP, Rioja LS, Basilio-de-Oliveira CA, Oliveira AV, Nery AC, Santos O da R. **Glomerular disease and human immunodeficiency virus infection in Brazil.** *Am J Nephrol* 1992; **12**:281–287.
6. Tse KC, Lam MF, Yip PS, Li FK, Choy BY, Lai KN, Chan TM. **Idiopathic minimal change nephrotic syndrome in older adults: steroid responsiveness and pattern of relapses.** *Nephrol Dial Transplant* 2003; **18**:1316–1320.