Correspondence

Acute respiratory manifestations of the abacavir hypersensitivity reaction

Abacavir, a carbocyclic nucleoside analogue reverse transcriptase inhibitor, is used in combination with other antiretroviral agents for the treatment of HIV-1 infection. The major toxicity associated with therapy is a potentially life-threatening hypersensitivity reaction occurring in approximately 3–5% of patients, usually during the first 6 weeks of treatment, with a median time to onset of 8 days [1,2]. Clinical manifestations most frequently include fever, rash, fatigue, gastrointestinal symptoms such as nausea, vomiting, diarrhea and abdominal pain, and respiratory symptoms such as pharyngitis, dyspnea and cough. Although the described respiratory symptoms are usually mild, we report here the case of a man who developed acute, severe pneumonitis leading to acute respiratory distress syndrome after 11 days of therapy with abacavir.

The patient was a 57-year-old man with HIV who came to medical attention in October 2002 as a result of 2 days of subjective fever, malaise and diarrhea. Approximately 6 days before the onset of symptoms he was seen in the infectious disease clinic, where his antiretroviral regimen was changed from sustiva and lamivudine–zidovudine to zidovudine, abacavir and lopinavir–ritonavir. The change was prompted by a drop in his CD4 cell count from 164 to 128 cells/ml over the previous 3 months. Six days after the initiation of this abacavir-containing regimen, he began to describe profound fatigue, loose stools, along with episodic sweats and chills, which worsened over the next 2 days, requiring hospitalization. He denied dyspnea or cough at that time and a chest X-ray was negative for acute disease (Fig. 1a).

Within 72 h of admission, his initial symptoms were followed by acute dyspnea, along with severe hypoxemia, hemoptysis and new diffuse bilateral pulmonary infiltrates on a chest X-ray (Fig. 1b). Abacavir was promptly discontinued because of the suspicion of a hypersensitivity drug reaction. The patient was then placed on mechanical ventilation, underwent diagnostic bronchoscopy, and was started on empiric antibiotic therapy for a possible infectious etiology of symptoms. Transthoracic echocardiography revealed normal left ventricular wall motion and a normal ejection fraction. After 3 days of abacavir discontinuation, the patient's pulmonary status improved dramatically, mechanical ventilation was terminated, and the patient's chest films showed almost complete resolution of the infiltrative disease (Fig. 1c). Electron microscopy of the bronchial washings revealed
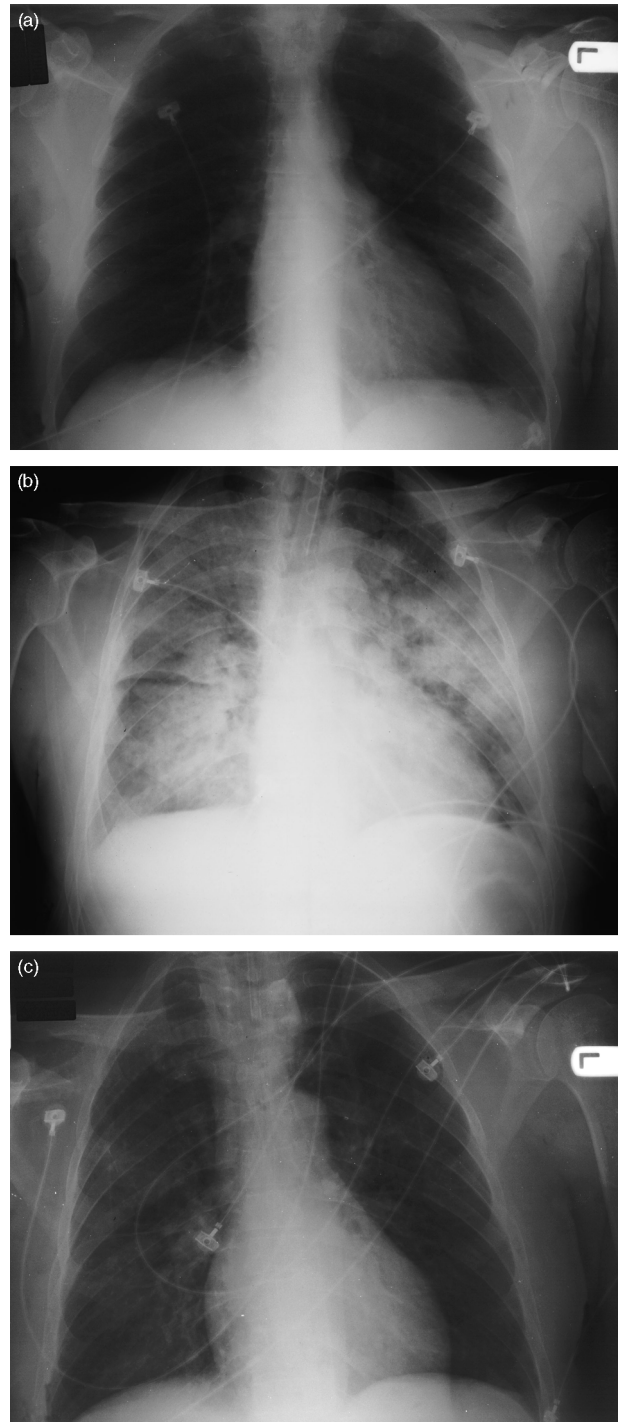

Fig. 1. Chest radiographs (anteroposterior views) of the patient. (a) On admission; (b) 11 days after the initiation of abacavir therapy; and (c) 3 days after abacavir discontinuation.

blood, mucus, benign respiratory epithelium, macrophages and neutrophils. No microorganisms or malignant cells were seen. Cultures (blood and bronchial lavage fluid) remained negative.

The hypersensitivity reaction described here highlights the potential aggressive respiratory manifestations associated with the use of abacavir. Although the clinical presentation of our patient at first suggested an infection causing his symptoms, tests for infectious diseases were negative, and in particular we witnessed a dramatic improvement within 72 h after the withdrawal of abacavir. Approximately 30% of life-threatening hypersensitivity cases involve a respiratory symptom, the most frequently reported of which are dyspnea, cough or pharyngitis [3]. Our observation is remarkable because of the unusual presentation of acute respiratory distress syndrome as the manifestation of a hypersensitivity reaction to abacavir.

Although the pathogenesis of abacavir hypersensitivity is not entirely clear, data suggest that in certain individuals, the creation of a chemically reactive metabolite leads to immune activation and, ultimately, cellular damage [1]. The reactive intermediate acts as a hapten that covalently binds to autologous proteins, creating an immunologically reactive metabolite−protein adduct. On interaction of the metabolite−protein adduct with cells of the immune system, the release of cytokines results in the development of symptoms. The exact metabolite responsible for hypersensitivity is unknown; however, the generation of a carboxylate derivative after oxidation may be implicated as the causative agent [4].

The key to appropriate clinical management is early and accurate recognition of abacavir hypersensitivity to avoid the symptoms described. The only established treatment is discontinuation of the drug. Rechallenge with abacavir after a hypersensitivity reaction typically results in the recurrence of symptoms within hours, with the potential to induce a more severe clinical syndrome with an increased risk of life-threatening hypotension and death [3]. Given the possibility of severe lung involvement, clinicians need to monitor their patients on abacavir closely and understand the need for the rapid discontinuation of the drug, as this is the only established treatment, if symptoms arise.

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Ritonavir/saquinavir safety concerns curtail antiretroviral therapy options for tuberculosis−HIV-co-infected patients in resource-constrained settings

Tuberculosis is the most common serious opportunistic infection associated with HIV infection in sub-Saharan Africa, and a strong case has been made for integrating tuberculosis and HIV care [1]. In those settings where the tuberculosis and HIV epidemics converge, existing tuberculosis programmes provide an opportunity for efficiently identifying those HIV-infected patients who are eligible for antiretroviral therapy (ART), as well as for initiating this therapy in order to utilize the existing tuberculosis directly observed therapy infrastructure. This approach is, however, dependent on the availability of effective, safe and affordable antiretroviral regimens that are compatible with the standard treatments for tuberculosis, including rifampicin. As a result of cost considerations, the widespread use of alternative rifamycins, such as rifapentine or rifabutin, is not feasible in resource-constrained settings.

Current guidelines, such as those from the US Department of Health and Human Services, suggest that regimens based on the non-nucleoside reverse transcriptase inhibitor efavirenz be used as first-line choices in patients receiving concomitant rifampicin [2]. Therapy choices become far more difficult in the face of treatment-limiting toxicities, virological failure or pregnancy. In these cases, a protease inhibitor-based regimen is needed as nevirapine may not be an appropriate option because of its interaction with rifampicin and additive hepatic toxicity. The guidelines suggested the use of ritonavir-boosted saquinavir [2]. Although the ability of ritonavir to boost plasma concentrations of saquinavir is well described, there is only limited evidence that this combination is adequate to counteract the enzyme induction caused by rifampicin and is clinically effective [3,4]. The issue of a 'Dear Health Care Provider' letter by Roche Pharmaceuticals on 7 February 2005 has now caused considerable additional uncertainty [5]. The manufacturers have informed the US Food and Drug Administration of problems experienced in a phase I, randomized, open-label, multiple-dose clinical pharmacology study in

healthy volunteers. Of 28 patients given rifampicin 600 mg once a day together with ritonavir 100 mg and saquinavir 1000 mg twice a day, 11 (39.3%) had developed significant hepatocellular toxicity during the 28-day study period. In the light of this evidence, the continued use of this combination cannot be supported. As a consequence, the US Food and Drug Administration Advisory has now removed the only protease inhibitor-containing regimen recommended for use concurrently with rifampicin containing tuberculosis treatment, and has thereby reduced the therapeutic options available for those requiring ART beyond standard first-line therapy options. In the absence of direct advice to the contrary, two options are being explored that we feel to be questionable. Some have argued that the exact doses of ritonavir and saquinavir used in the pharmacokinetic study (100 mg and 1000 mg, respectively) were different from those used in practice (400 mg and 400 mg, given twice a day), thus justifying thte continued use of this combination. Others have argued that adding additional ritonavir to co-formulated lopinavir–ritonavir would be sufficient to overcome the hepatic enzyme induction caused by concomitant rifampicin. However, in an open-label, randomized trial of two such dosing regimens in health volunteers, 12 out of 32 subjects withdrew from the study [6]. For nine of these, the co-administration of lopinavir–ritonavir and rifampicin was associated with elevations in liver enzyme levels. The similarity to the outcome of the saquinavir–ritonavir pharmacokinetic study cannot be ignored. The remaining options are, thus, either to switch to a triple nucleoside regimen or to cease further antiretroviral therapy until the completion of the rifampicin-containing tuberculosis treatment.

Until recently, the multitude of studies on alternative treatment regimens has focused on the needs of the developed world where ART has been widely available and switching options are increasingly important. The Global Fund Against AIDS, TB and Malaria, the WHO's 3-by-5 programme, the US President's Emergency Program For AIDS Relief (PEPFAR) and local efforts are slowly increasing access to ART in resource-constrained regions such as sub-Saharan Africa. The newly described toxicity of the rifampin, saquinavir, ritonavir regimen thus vividly highlights the great need for research, particularly pharmacokinetic studies, also to address the ART options appropriate for resource-limited settings, and in particular, in this instance, for co-administration with rifampicin-containing tuberculosis treatment.

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Appendix

The Starting Tuberculosis and Anti-Retroviral Therapy (START) project team includes: Salim Abdool Karim, Quarraisha Abdool Karim, Susan Brobst, Gavin Churchyard, Wafaa El-Sadr, Gerald Friedland, Clive Gray, Andy Gray, Rene Gonin, Richard Hafner, Scott Hammer, Rodney Hoff, Munira Khan, Mark Lurie, Terence Moodley, Marita Muurman, Kogieleum Naidoo, Gonasagrie Nair, Nesri Padayatchi, Diane Pizzano, Moussa Sarr, Aarthi Singh and Douglas Wilson.

Care should be taken when promoting microbicide use among sex workers who are able to use condoms consistently: response to Smith *et al.* (2005)

In a recent paper in *AIDS*, Smith *et al.* [1] used mathematical modelling to explore the issue of condom migration, or 'condom replacement', on the potential impact of microbicide use by female sex workers. Similar to an earlier paper that we published [2], they developed a static model of the risk of HIV acquisition, and used this to

obtain a threshold level of microbicide efficacy and use required to offset condom replacement. Although there are some differences in the mathematics used to formulate the risk of HIV infection and the scenarios considered, in most respects our two analyses yielded similar conclusions. In particular, both concluded that condom migration/replacement is not a substantial concern in populations that have low levels of condom use (as did Karmon *et al.* [3] in a similar analysis).

However, we are concerned that only the positive policy conclusions are presented by Smith *et al.* [1] with regard to condom replacement among sex workers: 'For low/ moderate efficacy microbicides, the risk of HIV acquisition in FSWs will be reduced – even if complete condom abandonment occurs – if prior condom use was low'.

This conclusion ignores the fact that, with proper counselling and support, sex workers can achieve a high rate of consistent condom use with their paying clients. A recent review of surveys of sex workers in Asia generally found that the median percentage reporting using a condom with their clients in the last sex act was over 75% [4,5]. There is also growing evidence across different settings that, following intervention, sex workers frequently achieve high levels of reported condom use with their clients [6–11], with as many as 96% of male military conscripts reporting using a condom at last commercial sex in northern Thailand, for example [7]. Although there exist many sex worker populations who have low levels of condom use with their clients [5,12–15] (and many more that have not been studied), it is important to acknowledge when considering microbicide introduction scenarios that in some settings sex workers have attained high levels of condom use.

Our analysis suggests that among groups of sex workers with high levels of condom use, migration may be of concern. We found that if sex workers originally used condoms in over 53% of sex acts but abandoned condoms altogether after the introduction of a 50% efficacious microbicide, then the risk of HIV will increase even if microbicides are used in every sex act [2]. If Smith *et al.* [1] had considered higher levels of condom use, they would have reached similar conclusions. For example, if Fig. 3 (b) had been extrapolated for higher levels of condom use, then a threshold for a 50% efficacious microbicide can also be seen [1]. From this, it appears that if sex workers had originally used condoms in over 62% of sex acts but abandoned condoms altogether after microbicide introduction, then the risk of HIV will increase even if microbicides are used in every sex act.

Our analysis also showed that if a microbicide of 50% efficacy against HIV and sexually transmitted infections is used in 50% of sex acts not protected by condoms then high-consistency condom users (who use condoms in 90% of sex acts) could only reduce condom use to 86% without increasing the risk of HIV [2]. However, this 'high-consistency condom-user' scenario was not discussed in the paper by Smith *et al.* [1].

The papers highlight the fact that further research is needed to explore the issue of microbicide introduction to sex workers. If women are able consistently to negotiate condom use with their clients, both models suggest that there may be risks associated with microbicide introduction if it weakens women's ability or resolve to negotiate condom use. At the same time, this result should not be used to withhold or limit the ability of sex workers to access microbicides [16]. Clearly, there is an urgent need for microbicides, to provide additional protection to the many sex workers who are unable to negotiate consistent condom use with their clients [5,12–15], and to the many more who cannot use condoms in their non-commercial relationships (despite achieving high levels of use with clients) [4,5,9,11,17–19]. Instead, such studies raise important programmatic challenges about how best to promote microbicide use in a way that does not undermine consistent condom use, recognizing that there are small margins for error. For, although mathematics can provide a quantification of the risks and benefits, operational and social science research is needed to identify how best to respond.

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Syphilis does not seem to involve virological and immunological course of concurrent HIV disease

In their interesting report, Buchacz and coworkers [1] claim that novel syphilis infection is significantly linked to an increase in viraemia and a drop in the CD4 lymphocyte count, in their series of 52 HIV-infected men with primary of secondary syphilis, but an antiretroviral treatment rate limited to 58% of cases. Although statistically significant or tending to statistical significance, the mean variations in $\log_{10}$ HIV-RNA copies/ml observed before and after syphilis were limited to a very restricted range, from +0.22 to −0.10, and the modification of the mean absolute CD4 cell count ranged from −62 to +33 cells/μl, respectively, before and after the concurrent syphilitic infection [1]. These limited variations in laboratory data are known to be of nearly negligible clinical value, especially when considering the retrospective, non-controlled study design, laboratory data evaluated by different hospital facilities, and the natural evolution of HIV disease, particularly when a substantial rate of affected patients (42%) did not take advantage of anti-retroviral drugs [1], and for the remaining 52% of patients detailed data on HAART administration and efficacy are lacking [1]. Therefore, we strongly believe that generalizations cannot be made on the grounds of the presented data only.

After the recent concerns about the increase in sexually transmitted diseases among HIV-infected individuals [2–4], a prospective observational survey was undertaken at our reference centre in a homogeneous cohort of over 1000 HIV-infected patients followed since the year 2001.

Thirty-eight patients (25 homo−bisexual men, and 13 heterosexuals) were identified as novel cases of syphilis (secondary syphilis in 31 episodes), according to clinical features and standardized laboratory testing, received appropriate treatment, and were followed up for 18 consecutive months. Virological and immunological testing were available for 36 patients (33 men and three women, aged 29−58 years), including at least the 6 months preceding the diagnosis of syphilis, and the 9 months after disease treatment (Table 1). Only five out of the evaluable 36 did not take any antiretroviral therapy, but it was not indicated according to current international guidelines [5]; all other subjects received various combinations of HAART containing at least three drugs, and therapy was adjusted according to the present recommendations in this field [5]. As summarized in Table 1, no statistically significant trend of both virological and immunological parameters was observed among the HIV−syphilis co-infected patients, using either a plain Student *t*-test, Wilcoxon rank sum test, or more sophisticated statistical analysis.

Although the reciprocal relationship between syphilis and HIV has not been extensively investigated from an immunological and especially a virological point of view, it is well known that the HIV-associated quantitative and functional impairment of cell-mediated immune response, could possibly affect the course of syphilis during HIV disease, as suggested by appropriate animal models and ex-vivo studies [6,7]. Impaired cellular immunity and

Table 1. Temporal trend of mean CD4 lymphocyte count and mean $\log_{10}$ HIV-RNA copies/ml in 36 evaluable HIV-infected patients who developed a novel episode of syphilis since the year 2001.

	−6 months	−3 months	Diagnosis of syphilis	+3 months	+6 months	+9 months
Mean CD4 lymphocyte count (cells/μl)	555 ± 132	573 ± 141	589 ± 120	573 ± 133	598 ± 139	587 ± 131
Mean $\log_{10}$ HIV-RNA level (copies/ml)	3.1 ± 0.8	3.3 ± 0.4	3.2 ± 0.7	3.5 ± 0.8	3.2 ± 0.5	3.3 ± 0.7

Patients were followed before and after the occurrence of syphilis infection with at least quarterly laboratory examinations. No statistically significant variation occurred through time (from 6 months before to 9 months after syphilis).

blunted cytokine networks were clearly demonstrated during the different stages of syphilis, leading to altered migration and clearance mechanisms [7,8].

On the other hand, it is quite difficult to expect that a non-opportunistic occurrence such as syphilis super-infection may trigger potent pathogenetic pathways, able to influence the course of HIV disease significantly, especially when an effective control of immune recovery can be obtained by HAART administration. The data presented by Buchacz *et al.* [1] are also confusing, because 42% of their patients did not receive HAART, and the authors did not explain who and why. The last, large patient group might have experienced a greater worsening of their HIV disease course during and after syphilis [1]. As a consequence, the entire evaluation of laboratory markers of HIV progression in this case series becomes absolutely unpredictable, and the perceived, minimal variations in the mean CD4 lymphocyte count or mean viral load cannot, in our opinion, be attributed to an incidental syphilitic infection. Another relevant bias may stem from the confounding definition of laboratory examinations obtained 'during' syphilis, which included determinations performed from 6 months before to 2 months after (an overall duration of up to 8 months 'around' the syphilis episode) [1]. In our series, a single determination was made during the diagnosis and treatment of syphilis, so that more affordable laboratory analyses were performed during previous and subsequent quarterly examinations.

Whereas we completely agree with the authors' concerns regarding the recent outbreaks of sexually transmitted diseases in patients with HIV disease or exposed to HIV [2−4], their arguments related to a possible, aspecific activation of the immune system during concurrent infections, which might be lead to enhanced viral replication, were commonly claimed for the majority of HIV-associated opportunistic disorders (i.e. cytomegalovirus, Epstein−Barr virus, tuberculosis, atypical mycobacteriosis, haematological malignancies, and so on), but cannot be extrapolated to syphilis, because neither updated literature data (all reported quotations are 10−15 years old), nor pathogenetic hypotheses are still available. On the other hand, there is recent, mounting evidence that syphilis is involved in immune dysfunction [8,9], as well as in the apopotosis of lymphoid cells [9], instead of their presumed activation.

Although healthcare givers have to take into careful account all sexually transmitted diseases in patients having HIV or at risk of being HIV infected [3,4], at this time only extensive, prospective, case−control studies, matched according to the use and effectiveness of antiretroviral therapy, might answer some questions regarding the eventual existence of bidirectional pathogenetic interactions between HIV infection and syphilis.

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Human placental transfer of atazanavir: a case report

Antiretroviral drugs, particularly protease inhibitors, cross the placenta with variable efficiency. There are no published data describing the human placental transfer of atazanavir, but this has been documented in animal studies (Bristol-Myers Squibb, unpublished data).

We describe a case of atazanavir use in pregnancy.

A 24-year-old Caucasian lady was diagnosed HIV-1 positive through antenatal screening at 14 weeks' gestation with a baseline CD4 cell count of 22 cells/μl and HIV viral load of 348 928 copies/ml. She commenced highly active antiretroviral therapy with tenofovir, lamivudine and nevirapine. Difficulties with tolerability and adherence culminated in her commencing tenofovir, lamivudine and boosted atazanavir at 30 weeks' gestation when her HIV viral load was 18 822 copies/ml. Two weeks later she was well but jaundiced, with a bilirubin level of 43 μmol/l, normal liver function and an HIV viral load of 88 copies/ml. At delivery, the cord blood atazanavir level was 362 ng/ml. A maternal atazanavir level taken just before delivery was 1515 ng/ml with an HIV viral load of less than 50 copies/ml. The baby was clinically well, with a bilirubin level of 122 μmol/l at birth not requiring intervention, and at 6 weeks had a negative HIV DNA polymerase chain reaction and HIV viral load.

Protease inhibitors are highly protein bound, and in pregnancy increased maternal protein levels may reduce free drug levels. In addition, high p-glycoprotein levels in the placenta may limit placental drug transfer. This is reflected in studies demonstrating the poor human placental transfer of nelfinavir, ritonavir, saquinavir and lopinavir [1]. The placental transfer of atazanavir has been demonstrated in rat studies (Bristol-Myers Squibb, unpublished data), but human data are lacking.

Atazanavir inhibits hepatic glucuronyl transferase, leading to unconjugated hyperbilirubinemia in 22−47% of patients taking atazanavir [2]. The degree of hyper-bilirubinemia appears to be dose dependent, and work has been done to establish an efficacious yet safe therapeutic range for the drug [3]. If atazanavir crosses the placenta, neonatal hyperbilirubinemia could result, with the risk of neonatal neurotoxicity and kernicterus. On the other hand, placental transfer of atazanavir may be of benefit in terms of pre and postexposure prophylaxis for the infant.

This is the first report documenting the human placental transfer of atazanavir. There were no significant clinical sequelae for the infant. The drug was well tolerated and adequate maternal levels were achieved at standard dosing, resulting in a viral load decay of 2.33 log within 2 weeks.

Physicians prescribing atazanavir to women who are or who may become pregnant need to be aware that placental transfer does occur and that the baby should be monitored appropriately. Further work is needed.

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Stopping antiretroviral therapy in 'prematurely treated' HIV-1-infected children with full viral supression is safe

Unfortunately antiretroviral treatment (ART) cannot eradicate HIV-1 and infected individuals are faced with lifelong therapies. This problem is worse in vertically infected children. In the long run, long therapies favour reduced adherence to therapy, leading to incomplete viral suppression and the emergence of drug-resistant viruses. Furthermore, continued exposure to ART may produce adverse effects, toxicities and metabolic complications [1−3]. Several strategies have been explored to help in the long-term management of HIV infection, among them various types of ART interruptions in adults [4], and recently in children with virological failure in a retrospective study [5]. However, no longitudinal studies regarding how 'prematurely' treated and well-controlled children may respond to those strategies exist.

Many currently infected children have been treated according to the 1996 guidelines that recommended early treatment. However, the therapeutic management of HIV infection in children has changed; current guidelines (2004) are more cautious regarding the initiation of ART.

Our objective was to address the safety of stopping ART in early treated children. For this, we have studied four children in the context of a treatment interruption programme guided by the CD4 T-lymphocyte counts. Their pre-treatment characteristics would not meet the current guidelines for starting therapy. The inclusion criteria were: (i) stable highly active antiretroviral therapy (HAART) for at least 6 months; (ii) CD4 cell count greater than 25% and viral load below detectable limits (≤ 50 copies/ml) during the previous 12 months; despite being grown-up children they were vertically infected and the percentage CD4 cell count (%CD4) better reflects the immunological status than the absolute number; (iii) clinically asymptomatic and never having had AIDS. The CD4 cell count and plasma HIV-RNA level were measured monthly by flow cytometry and Roche assay (50 copies/ml), respectively. HIV-related diseases and adverse events were systematically monitored during all follow-up. The criterion for re-introducing HAART was the appearance of clinical symptoms or a drop in the %CD4 of 15% or less.

Child 1, an 8-year-old girl who had been 6 years on ART had a nadir (defined as the lowest %CD4 that each child previously had) of 20.5% CD4, and was followed for 56 weeks. At entry, she had 25% (1345 cells/mm^3) CD4, which dropped to 17% (428 cells/mm^3) during the first 4 weeks associated with a viral load increase (1150 copies/ml). However, the percentage and absolute numbers of CD4 cells increased in the next 4 weeks and always remained higher than 20% (653 cells/mm^3; Fig. 1a). The

viral load remained between less than 50 and 1630 copies/ml during follow-up (Fig. 1a).

Child 2 (16 years), who had been 10 years on ART with a nadir of 25.8% CD4, was followed for 40 weeks. At entry he had 33% (517 cells/mm^3) CD4 and did not experience a decrease, rather an increase in %CD4 and absolute counts (Fig. 1b). The viral load remained between 50 and 3500 copies/ml during follow-up (Fig. 1b).

Child 3 (14 years), who had been 8 years on ART with a nadir of 13% CD4, was followed for 80 weeks. At entry he had 33% (856 cells/mm^3) CD4. During the first 12 weeks his CD4 cell count decreased to 22.5% (642 cells/mm^3) associated with an increase in the viral load (147 000 copies/ml). Although the CD4 cell count never reached the initial figure it always remained greater than 15% (> 300 cells/mm^3; Fig. 1c). Although the viral load ranged between 4230 and 147 000 copies/ml during the follow-up, it was lower in the last 20 weeks (4230–19 900 copies/ml) than during the first 36 weeks (22 900–59 500 copies/ml; Fig. 1c).

Child 4 (15 years), who had been 10 years on ART with a nadir of 13% CD4, was followed for 52 weeks. At entry, he had 33.6% (1141 cells/mm^3) CD4. During the first 8 weeks without treatment his CD4 cell count decreased to 25% (820 cells/mm^3) and the viral load increased (84 500 copies/ml). During the follow-up he always had greater than 25% CD4 (> 505 cells/mm^3; Fig. 1d). Despite this, the viral load was always relatively
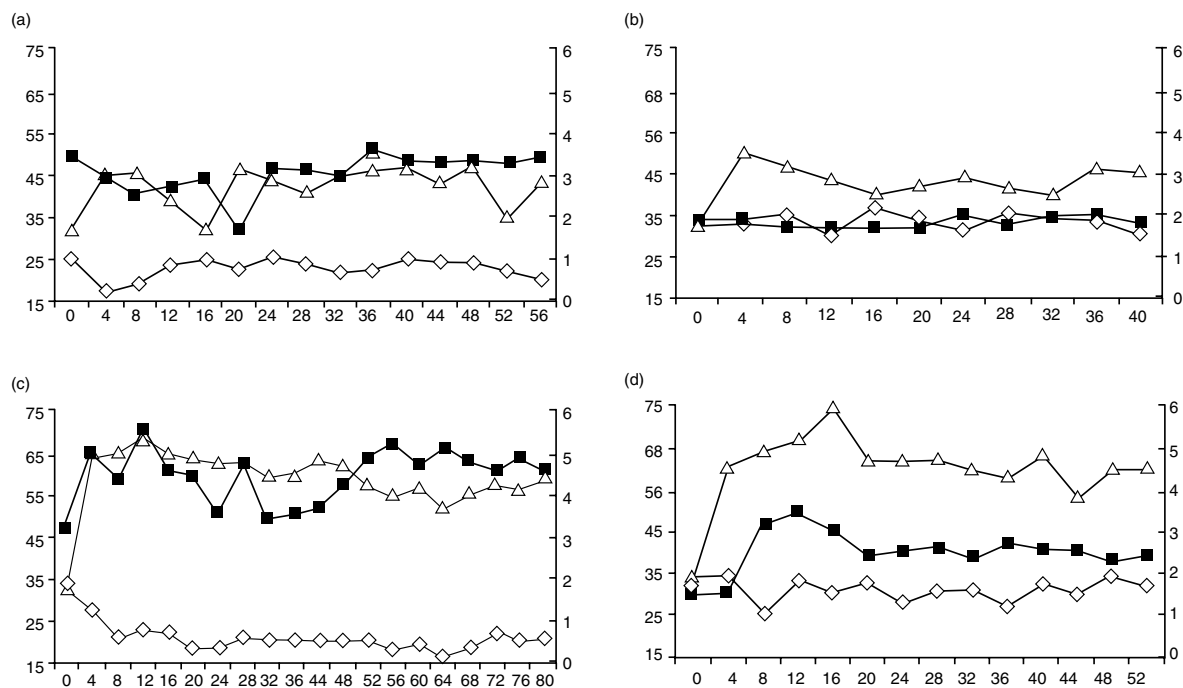

Fig. 1. Evolution of the percentage of CD4 T lymphocytes, the percentage of CD8 T lymphocytes and log$_{10}$ of the viral loads of the four children in the study. (a) Child 1; (b) child 2; (c) child 3; and (d) child 4. $-\diamond-$ % CD4 T lymphocytes; $-\blacksquare-$ % CD8 T lymphocytes; $-\triangle-$ log$_{10}$ viral loads.

high, ranging between 33 500 and 908 000 copies/ml (Fig. 1d).

Some viral load rebound was observed during interruption, and although those values may seem high, children normally have higher viral loads than adults, even after HAART. Rebound of the viral load was more pronounced in children 3 and 4, with CD4 cell nadirs below 15% in comparison with those children who had CD4 cell nadirs above that number, suggesting some influence of previous CD4 cell numbers on the control of viral replication. Nonetheless, all children always presented with good %CD4 and remained asymptomatic through all the interruption period. Also, the quality of their lives was improved by being without HAART for many weeks.

Our results demonstrate the safety of stopping ART in HIV-infected children (prematurely treated according to current guidelines). Those results are consistent with previous reports in HIV-infected adults. Moreover, children have a more functional thymus than adults, which may allow a better recovery of their immune system and they probably respond better to treatment interruptions.

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What do HIV-infected patients become after an opportunistic infection?

Although antiretroviral therapy has demonstrated its effectiveness [1,2] many patients are still admitted to hospital as a result of an AIDS-defining opportunistic infection (mainly toxoplasmosis and *Pneumocystis carinii* pneumonia). Several studies [3–5] have underlined the fact that over 50% of deaths in HIV-infected patients are currently related to such AIDS-defining infections, and of these latecomers 13% die within 2.5 years in spite of the initiation of adequate antiretroviral therapy. However, few studies have focused on the epidemiological and sociodemographic characteristics of these patients. The objective of this survey was to study survival among patients presenting with an opportunistic infection in the era of highly active antiretroviral therapy (HAART) and to identify predictive factors for mortality.

We analysed epidemiological, clinical and biological characteristics of all HIV-infected patients admitted to hospital for an AIDS-defining opportunistic infection in the Nice University Hospital and the district hospitals of Fréjus and Cannes between January 1997 and January 1999. Patients were asked to complete a questionnaire during a face-to-face interview at the end of their hospital stay. Questions included sociodemographic data (circumstances of HIV screening and subsequent medical follow-up) and details of treatment [6]. The same questionnaire was submitted to patients 30 months after the diagnosis of the inaugural opportunistic infection.

For patients lost to follow-up, we questioned official registries at their place of birth to find out whether any deaths had been registered.

Statistical analysis was conducted using SPSS software. Chi-square and non-parametric Kruskall–Wallis test results are given with a 5% level of significance. Survival rates were calculated using Kaplan–Meier curves.

Eighty-one HIV-infected patients were included in this study. Among these, 26 (32%) were unaware of their HIV-positive status and therefore discovered this upon admission to hospital (group 1); and 25 patients (31%) knew they were infected but had received no care before diagnosis (group 2); 30 patients (37%) had already had one or several irregular contacts with the healthcare system before diagnosis (group 3). There was no statistically significant difference for the median CD4 T-cell count and viral load between the three groups (CD4 T-cell counts 50, 65 and 60 cells/mm^3 and viral loads 5.5, 5.5 and 5.1 log copies/ml for groups 1, 2 and 3, respectively). There was a larger proportion of intravenous drug users (IVDU) in group 3 (53% versus 18% group 1; and 20% group 2; $P < 0.05$). After a mean delay of 30 months (range 7–60 months) after the diagnosis of opportunistic infection, 20 of the 81 patients had died: four patients in group 1, [one cytomegalovirus infection, one laryngeal cancer, one HIV-related encephalopathy, one hepatitis C
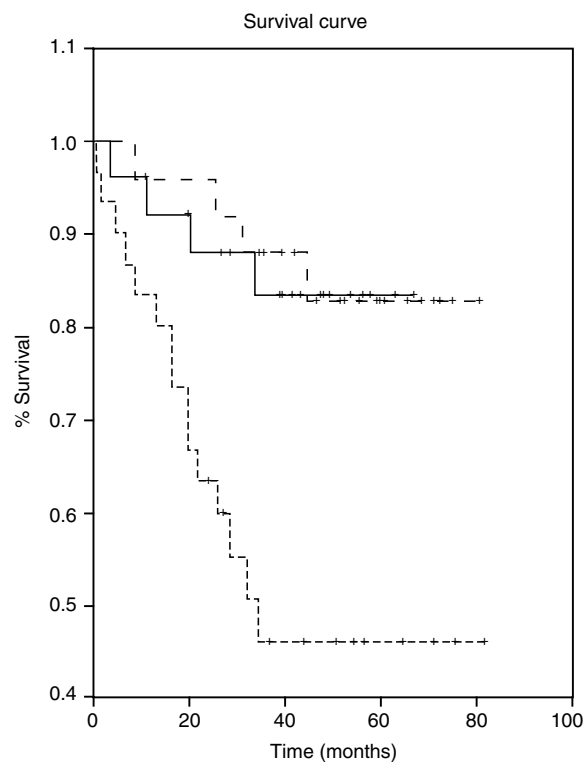
Survival curve

Fig. 1. Survival curve. The risk of death is significantly higher in G3 (non-compliant patients or irregular follow-up) than in G1 (non tested patients prior to admission) and G2 (patients tested but no follow-up). ---------- Non-compliant patients; – – – – patients with no follow-up; ———— non tested patients.

virus (HCV) infection], three in group 2 (two *P. carinii* infections, one Hodgkin's disease), and 13 in group 3 (three HIV-related wasting syndrome, one *P. carinii* infection, one HIV-related encephalopathy, four HCV infections, one suicide, and three of unknown cause). The risk of death was significantly higher in group 3 than in groups 1 and 2 (Fig. 1, log-rank test $P = 0.0012$). Among the 61 patients remaining alive at month 30, the course was favourable in terms of clinical condition (normal Karnofski score in all patients by the end of the year after diagnosis, no recurrence of opportunistic infection), viral load (in 80% of patients the viral load was below 400 copies/ml, regardless of group) and immunological markers [ΔCD4 T-cell count $+ 279$ (group 1), $+ 240$ (group 2) and $+ 183$ (group 3)]. Our results are in accordance with those described in other recent studies [7,8].

Our study suggests that in the era of HAART, among patients who present with an opportunistic infection in the context of severe immune depression (CD4 T-cell count < 200 cells/ml), non-adherent patients have a higher mortality risk than patients in whom care is delayed because of a lack of screening or a complete lack of previous follow-up. The latter already have a higher

risk of dying than patients who benefit from medical follow-up sooner, because the risk is 16-fold higher during the first 6 months and fourfold higher within the following 3 years [9].

Our results are in line with those of other studies [10,11], in which non-adherence was shown to multiply the risk of death by four to sixfold. Irregular follow-up generates difficulties in adherence to antiretroviral treatment, with well-known clinical, virological and immunological consequences [12]. However, it also complicates surveillance of the immune status and identification of any requirement for prophylactic treatment.

The proportion of IVDU was higher in group 3 than in the other groups in our study. The fact that IDVU were more often in a precarious professional situation [13] would suggest personal difficulties in complying with regular treatment and follow-up schedules in relation to overwhelming socioeconomic concerns. Moreover, deaths related to HCV infection are more frequent in group 3, which reflects a larger proportion of co-infected patients in this group. It has recently been suggested that early treatment initiation with HAART could slow the progression of hepatic fibrosis resulting from HCV infection [14]. However HCV infection cannot solely explain the high mortality rate observed in this group; the difference in mortality between the three groups remains significant ($P = 0.01$) even when HCV-related deaths are excluded.

Irregular care thus appears to be an essential feature, which should be a cause of concern before opportunistic infections arise. This makes adherence reinforcement strategies a matter of urgency, particularly at certain key points during the course of treatment: reluctance upon initial treatment prescription, the prospect of prolonged and highly demanding treatment regimens, major side-effects. ... During these critical situations, discontinuation of care might be avoided by an improved patient–physician relationship and by a multidisciplinary approach (treatment adherence counsellors, health educators, psychologists, social workers...). Finally, improving networking among the various medical and non-medical facilities would ease the re-integration of patients into an adequate care-providing framework.

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Response to: 'Carotid intima-media thickness: assessment of sub-clinical atherosclerosis in HIV-infected patients'

We appreciate the comments from Coll and Alonso-Villaverde. As they appropriately point out, there are many reasons why the literature is not uniformly consistent with regard to the association between HIV infection, antiretroviral therapy and atherosclerosis. However, on the basis of the extensive data that exists in the area of atherosclerosis imaging, particularly with the intima–media thickness (IMT) endpoint, it is unlikely that the methodology we used for assessing carotid IMT is the major explanation for the differences among studies. Studies such as ours that have carefully controlled for known cardiovascular risk factors tend to find no association between HIV status and atherosclerosis. The matched design of our study was the first to control prospectively for major cardiovascular risk factors. We acknowledged in our discussion that the patients included in our study as a group may have been at a lower risk of atherosclerosis than those included in other reports; none had a family history of premature coronary heart disease, diabetes, or previous cardiovascular disease, and only 22% were current smokers. This is a new area of investigation and all possibilities need to be determined, including potential differences in the atherothrombotic clinical event with only marginally detectable differences in atherosclerosis. Our study has recently concluded 96 weeks' of follow-up. As we proceed with the final analysis of our study, we will examine the differences among groups in the rate of progression of carotid IMT. The progression of subclinical atherosclerosis among the different groups of participants may be more revealing than discerning differences cross–sectionally. As concerns the issues surrounding the use of protease inhibitors and atherosclerosis, it is imperative that this relationship is well understood and that prospective studies are conducted before public health statements are made about whether these agents are atherogenic, because these are evidence-based life-saving medications for patients with HIV infection.

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A case of HIV-associated fever of unknown origin: deficit of IL-1β antagonistic activity and resolution with monocyte–granulocyte apheresis

Fever of unknown origin (FUO) is considered to be one of the major diagnostic and therapeutic challenges in medicine. We report here a case of HIV-associated FUO, which displayed a selective deficit in IL-1β antagonistic activity and was successfully treated with monocyte–granulocyte cell apheresis (GCAP).

In August 2002, a 46-year-old male patient came to our observation with a positive HIV test. In January 2003, he began antiretroviral treatment with lopinavir, ritonavir, zidovudine and lamivudine. His CD4 T-cell count was 281 cells/μl (14.8%) and his HIV-1 viral load was 27 719 RNA copies/ml before treatment. He achieved a negative viral load (< 50 copies/ml) and 456 CD4 T cells/μl by November 2003, and continues at the time of writing to respond well to this same regimen. In February 2003, the patient began to report daily episodes of fever characterized by two peaks, one late morning and the second at nighttime. Both episodes were typically of 1 h duration, with fever reaching 40°C and followed by asthenia, with difficulty executing daily activities. These episodes persisted on a daily basis for 80 consecutive weeks without interruption. The patient reported a history of similar unexplained febrile episodes in his early 20s, which resolved spontaneously. To identify the cause of the fever the patient was subjected to extensive clinical and laboratory analysis, which failed to provide any evidence of concomitant infectious (human T-cell leukaemia/lymphoma virus type II, hepatitis C virus, hepatitis B virus, human herpesvirus 8, Epstein–Barr virus, cytomegalovirus, *Leishmania*, *Brucella*, *Plasmodium malariae*, *Mycobacterium tuberculosis*, *Borrelia Burgdorferi*),

non-infectious inflammatory (rheumatoid factor, antibodies specific for nucleic acids, endomisium, platelets), or neoplastic diseases (total body computed tomography, scintigraphy with labelled leukocytes, positron emission tomography, colonscopy). Additional investigations included abdomen and heart ultrasound scan, chest X-ray, brain magnetic resonance imaging and bone marrow biopsy. On the basis of this we classified the fever as HIV-associated FUO [1].

TNF-α and IL-1β are pyrogenic cytokines that have been suggested to play a role in FUO [2,3]. We measured their levels in plasma samples collected at regular intervals during the febrile episodes. All samples had very low or undetectable levels of TNF-α. In contrast, the levels of IL-1β were higher compared to those of a panel of 14 healthy donors (Fig. 1a). We then measured the IL-1 receptor antagonist (IL-1ra) and the decoy soluble receptor II (IL-1sRII). IL-1ra and IL-1sRII are two molecules that counteract IL-1β activity, the first by binding with high affinity to the IL-1β receptor I, and the second by binding to IL-1β and thereby preventing binding to its receptor [3]. The levels of IL-1ra were comparable to those of controls, whereas the IL-1 sRII levels were significantly lower (Fig. 1a). These data suggested that the patient had developed a selective deficit in IL-1sRII activity.

As both TNF-α and IL-1β are monocyte-derived cytokines, we subjected the patient, after signature of an informed consent form, to six consecutive weekly GCAP following a protocol already established in our
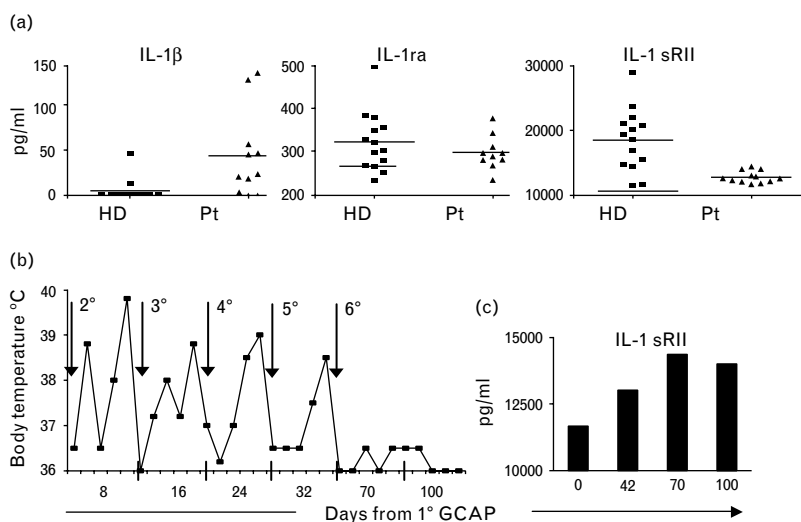

Fig. 1. Effects of monocyte–granulocyte cell apheresis on cytokines levels and body temperature. (a) Plasma levels of IL-1β, IL-1 receptor antagonist (IL-1ra) and the decoy soluble receptor II (IL-1 sRII) in 14 healthy donors (HD) and 11 patient samples (Pt) collected during the febrile episodes. The horizon lines indicate mean values. (b) Body temperature of patient monitored on different days after first monocyte–granulocyte cell apheresis (GCAP). The arrows indicate the day of execution of GCAP. (c) Patient's plasma levels of IL-1sRII on different days after last GCAP.

institute for HIV-infected patients [4–6]. GCAP was performed using an adsorptive type extracorporeal device (Adacolumn, JIMRO, Takasaki, Japan). The device contains a mono-use polycarbonate column with a capacity of approximately 335 ml, filled with 220 g of cellulose acetate beads (carriers) of 2 mm diameter, bathed in sterile saline. The device has been developed for selective absorption of activated leukocytes by apheresis and it specifically absorbs monocytes and granulocytes, with lymphocytes remaining substantially untrapped. Previous studies have shown that GCAP can have several immunomodulatory effects, including changes in cytokine production and the regulation of leukocyte homing receptors [6,7]. Each GCAP session was of 1 h duration. We monitored body temperature at regular intervals during and after GCAP (Fig. 1b). At day 8, corresponding to the second GCAP, there was no change in the fever episodes, with the patient having the day and night peaks of fever as reported during the past 2 years. After the third GCAP (day 16), the day peak began to reduce in intensity and gradually disappeared. At the fifth GCAP (day 32) the night peak was reduced in intensity and then gradually disappeared. Four weeks after the last GCAP the patient became apyretic. After 8 months of follow-up he is still apyretic without any pharmacological support. Interestingly, his blood levels of IL–1sRII have begun to increase since the last GCAP (Fig. 1c).

These data suggest that a deficit in IL-1β antagonistic activity can be associated with FUO and that GCAP can normalize body temperature, possibly restoring the homeostasis of the IL-1β system.

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Nodular regenerative hyperplasia of the liver after IL-2 therapy in an HIV-infected patient

IL-2 is a potent stimulator of T-cell proliferation and maturation, used as intermittent therapy of HIV-infected patients in combination with antiretroviral treatment to increase the CD4 T-cell count [1–5]. Acute IL-2 therapy has a dose-dependent hepatotoxicity estimated at between 20 and 33% of cases [1,4]. We report the case of an HIV-infected patient who developed liver nodular regenerative hyperplasia (NRH), revealed by anicteric cholestasis and portal hypertension 33 months after the beginning of six courses of IL-2 therapy and 2 months after rechallenge.

A 65-year-old man, seropositive for HIV-1, was referred to our liver unit in July 2003 for chronic anicteric cholestasis. He had no history of illicite drug use, no viral co-infection and no excessive alcohol consumption. HIV infection discovered in 1995 was treated with azidothymidine monotherapy. In 1996, treatment including didanosine, lamivudine and saquinavir was started for a low CD4 T-cell count (Fig. 1). In February 2000, with the emergence of viral escape mutant, lamivudine was stopped, abacavir and ritonavir were introduced. Viral load dropped and remained definitely under the detection level. He received six courses of IL-2 (4.5 MIU twice a day, subcutaneously) for 5 days, stopped in September 2001 for adverse events ('flu-like syndrome, asthenia, rash). He progressively developed an anicteric cholestasis. In July 2003, a first liver biopsy showed mild portal fibrosis without inflammation. Sinusoids were enlarged in the mediolobular region. IL-2 therapy was re-introduced
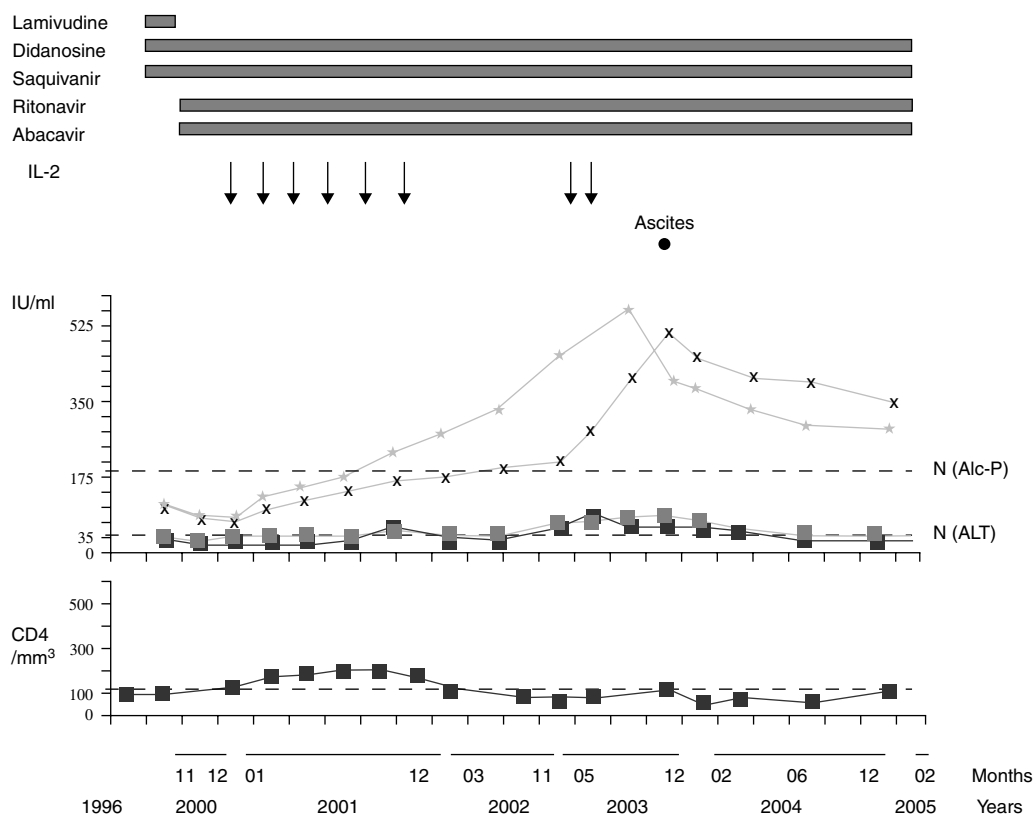
Fig. 1. Antiretroviral treatment, hepatic tests, and CD4 cell count during the follow-up period. —■— Alanine aminoaspartate; —■— Alanine aminotransferase; —■— gamma-glutamyl transferase; —×— Alkaline phosphatase.

for a persistent low CD4 T-cell count. Two months later, he a developed ascites, and the spleen was enlarged without clinical signs of hepatic insufficiency. Endoscopy revealed oesophageal varices (grade I–II). Liver function tests, including albumin, prothrombin time and bilirubin, remained within the normal range. Liver imaging showed liver dysmorphy, without vascular obstruction, or liver tumour. Transjugular liver biopsy with haemodynamic evaluation showed portal hypertension (10 mmHg, N < 4 mmHg), defined as the difference between wedge hepatic venous pressure (18 mmHg) and free hepatic pressure (4 mmHg) [6]. Liver histology showed no cirrhosis, but modified liver architecture with atrophic and hypertrophic hepatic cells, sinusoidal distension, suggestive of NRH, confirmed by reticuline staining. Eighteen months later the patient remained stable under beta-blocker therapy.

Liver NRH is thought to result from occlusion of the terminal branches of the hepatic arterioles and portal veinules, secondary to endothelial cell damage [7]. In the present case, the diagnosis of NRH was assessed by the following considerations. First, the classic conditions associated with liver NRH, including systemic and vascular disorders, coagulopathies, myelo and lympho-proliferative diseases, drug-induced endothelial toxicity and diabetes [7], were ruled out. Second, our observation clearly shows a temporal relationship between IL-2

therapy, the progressive development of an anicteric cholestasis, and the appearance of portal hypertension with oesophageal varices revealed by ascites 2 months after IL-2 rechallenge. Third, the diagnosis of liver NRH suggested by the presence of (grade I–II) oesophageal varices despite a modest elevation of the hepatic venous pressure gradient (below 12 mmHg), in the absence of hepatocellular insufficiency was confirmed by sequential liver biopsies showing the progressive appearance of intrahepatic veinule abnormalities followed by remodelling of liver architecture and fibrosis.

High dose of IL-2 induced severe systemic toxicity with a capillary leakage syndrome characterized by a loss of intravascular fluids, leading to generalized oedema and haemodynamic instability and organ dysfunction [8]. Elevated transaminases and bilirubin are commonly observed with subcutaneous IL-2 therapy (3–15 MIU/day) [1,4]. However, hepatic tests are not fully described in long-term follow-up studies [2]. Of note was the fact that one series mentioned the occurrence of liver NRH in a patient [3].

In our case, liver toxicity related to highly active antiretroviral therapy could not be excluded, but does not provide an explanation for portal hypertension in the absence of cirrhosis. In animal models, pharmacological doses of IL-2 ranging from 4×10^6 to 36×10^6 IU/kg

per day induced vascular leak syndrome associated with increased transaminases, hyperbilirubinemia, lymphocytic infiltration in the livers of C57BL/6 mice as a consequence of an increased production of TNF-α and chemokines by activated Kupffer cells, increased adhesion molecules by endothelial cells (interstitial cell adhesion molecule 1, vascular cell adhesion molecule 1) [9], leading to a decreased hepatic sinusoid blood flow [10,11]. Prolonged studies support a fibrogenic property of IL-2, in rat with human recombinant IL-2 injection after 4 months [12], or in the schistosomia model in mice [13].

The diagnosis of liver NRH needs a careful examination of liver tests, mainly anicteric cholestasis. Our observation clearly shows a temporal relationship between IL-2 therapy and the occurrence of liver NRH, revealed by portal hypertension in an HIV-infected patient.

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