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Acceptance by Belgian physicians of in-vitro fertilization treatment in women with HIV infection

In the 1980s, the course of HIV disease was so often fatal that infected women were unlikely to be considered for assisted-reproduction techniques [1]. The development of new antiretroviral drugs has dramatically improved life expectancy [2]. Moreover, caesarean section combined with antiviral therapy has considerably reduced the rate of mother-to-child transmission of HIV [3]. Subsequently, HIV patients are seeking help in order to procreate [1]. We conducted a survey in order to evaluate how a request for medical assistance to procreate from an HIV-infected woman is accepted by Belgian physicians.

We described the following clinical scenario: A 30-year-old woman has been infected by HIV. She has never developed complications related to the HIV infections. She is treated by antiviral therapy and her medical condition is stable. Her immunological status is normal and the viral load undetectable. She leads a normal life. One can hope that her life expectancy will be satisfying. Her partner is not infected. After great hesitation, the couple express the desire to have a child and request in-vitro fertilization (IVF), which will also solve tubal sterility. In this situation, it can be estimated that antiviral treatment and a caesarean section will reduce the risk of vertical transmission to a rate as low as 2%. We compared the attitude of physicians towards the above-described case to that of two other cases. The first describes a woman in an identical situation, except that she had been infected by hepatitis C instead of HIV. The second also concerns a patient infected by hepatitis C, but in a less favourable situation: Her liver enzymes are elevated, despite antiviral treatment. A hepatic biopsy documents chronic active hepatitis without cirrhosis. We indicated that her life expectancy might be reduced, and that the expected vertical transmission rate is estimated to be 7%.

We sent at random (computer drawing), one of the three cases to a total of 3450 different gynaecologists, paediatricians and internists, ensuring that only one case was sent to each physician. They were asked to choose between the following answers: (i) I think that the patient should not be admitted for an IVF; (ii) I think that the patient should be admitted for an IVF; (iii) I have no clear opinion.

We obtained 1175 analysable responses (35% response rate after one mailing). The response rates were similar for the three cases and also among the different specialities (see Table 1). For the HIV case, the percentages of physicians in favour of an IVF, varied between 40.2% (for internists) and 59.8% (gynaecologists and paediatricians). This relatively high percentage is much lower than that for the hepatitis C in similar clinical conditions, which obtained favourable rates of between 73.3 and 86%. Also in this survey, approximately 50% of the responders were in favour of an IVF in a hepatitis C patient with a poor prognosis.

Uncomplicated and well controlled HIV infection, much more than hepatitis C, is still viewed by approximately half of the surveyed physicians as a sufficiently severe disease to avoid considering pregnancy. Different hypotheses may explain our results: physicians may feel that HIV infection is a more fatal disease than hepatitis C, despite the recent improvement in life expectancy of HIV patients and the reduction in vertical transmission rates. Furthermore, until recently, most IVF centres excluded HIV-infected patients from their programmes [1,4]. It is also possible that the physicians interrogated were not only influenced by the true risks of a pregnancy associated with seropositivity for HIV or hepatitis C, but that the word

Table 1. Percentages of physicians in favour, not in favour or without opinion for in-vitro fertilization in a woman infected either by HIV (with good health indicators), or by hepatitis C (with good health indicators), or by hepatitis C with evolution to chronic hepatitis.

	HIV	Hepatitis, good prognosis	Chronic hepatitis
All			
Not in favour of IVF	35.1	10.9	34.8
In favour of IVF	53.6	79.0	51.0
No clear opinion	11.3	10.1	14.2
Obstetrician-gynaecologists			
Not in favour of IVF	29.5	5.1	37.4
In favour of IVF	59.8	86.2	52.0
No clear opinion	10.6	8.7	10.6
Paediatricians			
Not in favour of IVF	29.5	15.7	37.5
In favour of IVF	59.8	74.5	45.8
No clear opinion	10.7	9.8	16.7
Internists			
Not in favour of IVF	48.0	11.6	32.0
In favour of IVF	40.2	73.3	50.6
No clear opinion	11.8	15.1	16.5

IVF, In-vitro fertilization.

'HIV' has a much stronger (negative) impact than the words 'hepatitis C'.

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Exclusive breast-feeding and HIV transmission

We presented first in an early report [1] and then in full [2] our findings that mother-to-child HIV transmission is substantially less among those breast-feeding mothers who maintain exclusive breast-feeding until the child is 3 months of age or older than that among other breast-feeding mothers. The transmission rate observed among exclusively breast-feeding mothers is similar to that observed among mothers who do not breast-feed at all. Our findings opened a new avenue for research, which is important because the avoidance of all breast-feeding is not a realistic option for most HIV-infected women in southern Africa. Failure to breast-feed substantially increases morbidity and mortality rates from other diseases in early childhood [3]. In many circumstances, breast milk substitutes are unavailable, are prohibitively expensive, or cannot be prepared hygienically. In many societies, breast-feeding is culturally entrenched and deeply valued, and failure to breast-feed may be a tacit disclosure of HIV status in situations in which breast-feeding is near universal. The importance of breast-feeding for maternal and child health in developing countries demands of us the cautious and critical appraisal of any practices that may undermine it. We thus appreciate the serious attention that has been given to our findings by Forsyth [4] and Walker *et al.* [5].

The first concern raised by Forsyth [4] about the 'remarkably low' rate of transmission over the first few weeks of life in the exclusive breast-feeding group is, however, based on a misunderstanding of the presentation of our results. The first protocol-scheduled HIV test in the child after the first week test was at 6 weeks. At 6 weeks, the rate of transmission in the exclusive breast-feeding group (15%) was remarkably similar to that observed in the never breast-fed group (18%). Given our method of analysis, it is not possible to make good inferences about the transmission rates in the time periods when testing was not scheduled to take place.

The second concern raised by Forsyth [4], which might be described as a 'healthy survivor bias' warrants careful analysis. We investigated whether or not child morbidity preceded shifting away from exclusive breast-feeding to mixed breast-feeding. It did not. We investigated whether or not markers of the severity of maternal HIV disease were associated with the ability to maintain exclusive breast-feeding to 3 months. They were not. We investigated whether or not intrauterine transmission rates were different in the groups. They were not. In sum, we did not observe associations consistent with this hypothesized bias. To investigate further a possible 'survivor bias', we would like to present the results on only those children who survived to 3 months of age, thus excluding those children hypothesized to bias the results (Fig. 1). Consistent with the other data we have already presented, transmission rates in this sub-group of children surviving to 3 months were highest in mixed breast-fed infants, and similar between exclusively breast-fed and never breast-fed infants up until approximately the age at which no more exclusive breast-feeding continued.

In our paper we presented two complementary analyses. The first was based on Kaplan–Meier curves with a fixed covariate for feeding practice and the second was based on a Cox proportional hazards model with a time-dependent covariate for feeding practice. Contrary to the assertion by Walker *et al.* [5], this second analysis (which they consider the 'correct' one) generated almost exactly the same findings as the first analysis (which they consider the 'inappropriate' one). Exclusive breast-feeding was associated with almost half the instantaneous risk of HIV transmission (hazard ratio 0.56, 95% confidence interval 0.32–0.98) compared with mixed breast-feeding. The magnitude of this hazard ratio was unchanged after adjusting for many covariates. Only considerably larger studies would be able to separate out age-specific risks.

Our findings raise many questions in need of further research beyond the simple repetition of the main findings. These include investigation of how to support exclusive breast-feeding among HIV-infected women who elect to breast-feed. Exclusive breast-feeding is very rare in most parts of Africa, even in those regions where breast-feeding is almost universal and of long duration. For instance, DHS surveys estimate the

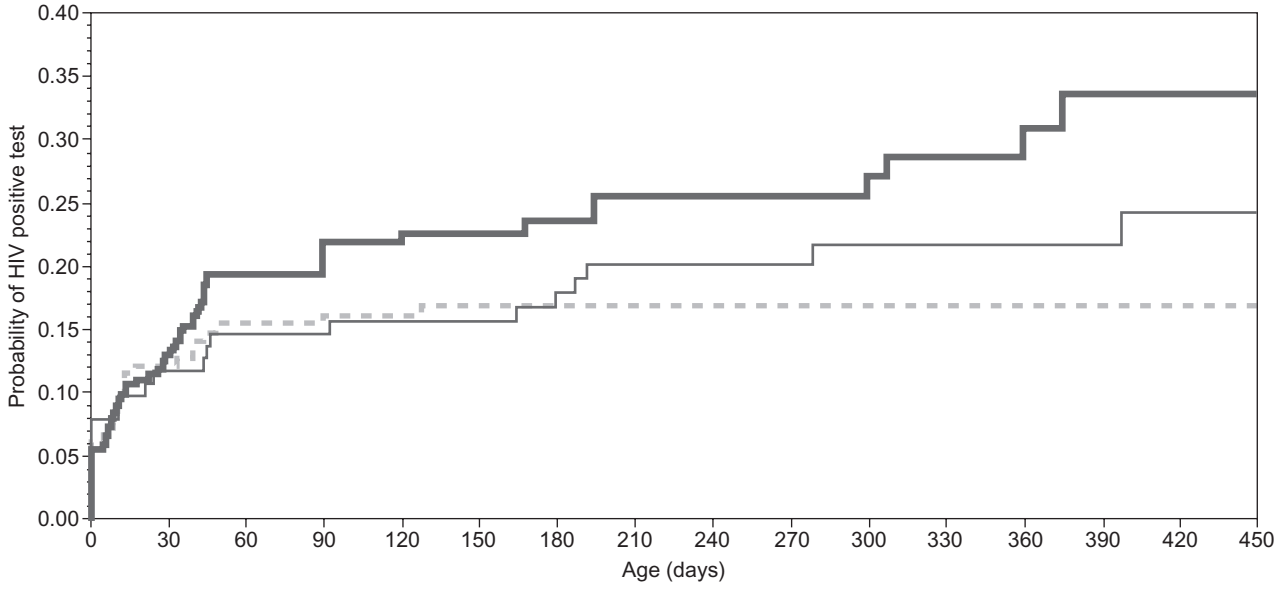

Fig. 1. Cumulative probability of detecting HIV infection over time among 150 children who were never breast-fed, 118 exclusive breast-feeders and 261 mixed breast-feeders surviving to 3 months. – – – Never breast-fed; —— exclusively breast-fed; ▬▬ mixed breast-feeding.

prevalence of exclusive breast-feeding among children of 2–4 months of age to be 27% in Zambia, 17% in Kenya, and 11% in Malawi [6]. We need to obtain a better understanding of the relationships between exclusive breast-feeding and breast pathology, including sub-clinical mastitis; factors that appear to play an important role in post-natal HIV transmission [7,8]. We need to evaluate better options for mothers and babies after 6 months of age when breast-feeding can no longer be exclusive. A polarized argument in support of breast-feeding or in support of formula feeding offers little in the way of scientific insight into how best to minimize the risks of post-natal HIV transmission through breast-feeding, without creating new problems for maternal and child health.

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Modelling HIV incidence in gay men: increased treatment, unsafe sex and sexually transmissible infections

Several authors have now used mathematical models to assess the effect that combination antiretroviral treatments might have on the incidence of HIV by decreasing the HIV viral load and therefore, presumably, reducing the risk of HIV transmission [1–3]. These different models have produced qualitatively similar results, indicating that apparently very large reductions in the risk of HIV transmission in individuals receiving antiretroviral treatments can be counterbalanced in terms of new HIV infections by more modest, albeit quite substantial, increases in risk behaviours. Our model suggested that, among homosexual men in

Australia, two-, five- and 10-fold decreases in infectiousness as a result of antiretroviral treatment could be counterbalanced in terms of new HIV infections by increases in unprotected anal intercourse (UAI) between HIV-negative and positive men of approximately 40, 60 and 70%, respectively.

One aspect that none of these models has investigated is that any increases in unprotected intercourse could lead to increased rates of sexually transmissible infections (STI), which independently increase the risk of HIV sexual transmission [4]. In Australia, there is continuing evidence of increases in UAI among HIV-infected and uninfected homosexual men [5]. There has also been evidence of increases in the rates of gonorrhoea among homosexual men in Sydney [6], and a doubling in the reports of rectal gonorrhoea among men in New South Wales [7].

To investigate how increased STI rates (as a result of increased UAI) among homosexual men in Australia might independently effect HIV transmissions, we extended the mathematical model described previously [1]. Data from the Sydney Men and Sexual Health Study, a large cohort study of homosexual men in Sydney, indicated that before the availability of effective antiretroviral treatment in Australia, self-reported gonorrhoea 6 month period prevalence rates in homosexual men were approximately 2% (unpublished data on file). Since the widespread availability of combination antiretroviral treatments in mid-1996, the prevalence rates of rectal gonorrhoea in men in New South Wales have at least doubled [6,7]. The increase in prevalence rates of all STI, including gonorrhoea, were therefore simulated from a uniform distribution corresponding to an increase of 2% of homosexual men (range 1–3%). The increase in STI rates was assumed to occur in all homosexual men, regardless of HIV infection status or disease stage. This magnitude of increase in the percentage of homosexual men with STI was assumed to be 100%, correlated with the magnitude of the simulated increases in UAI. The effect of being infected with an STI was assumed to correspond to a 3.5-fold (range two- to fivefold) increase in the risk of HIV transmission [4]. A combination of these parameters resulted in the effect of STI on increasing HIV transmission being 73%, correlated with increases in UAI. In all other respects the model was the same as previously described [1]. As before, the effects of antiretroviral treatment and increases in UAI were assumed to occur instantaneously. One thousand simulations of the model were run over a time-horizon of a single year, and as before the model-predicted rate of HIV incidence was compared

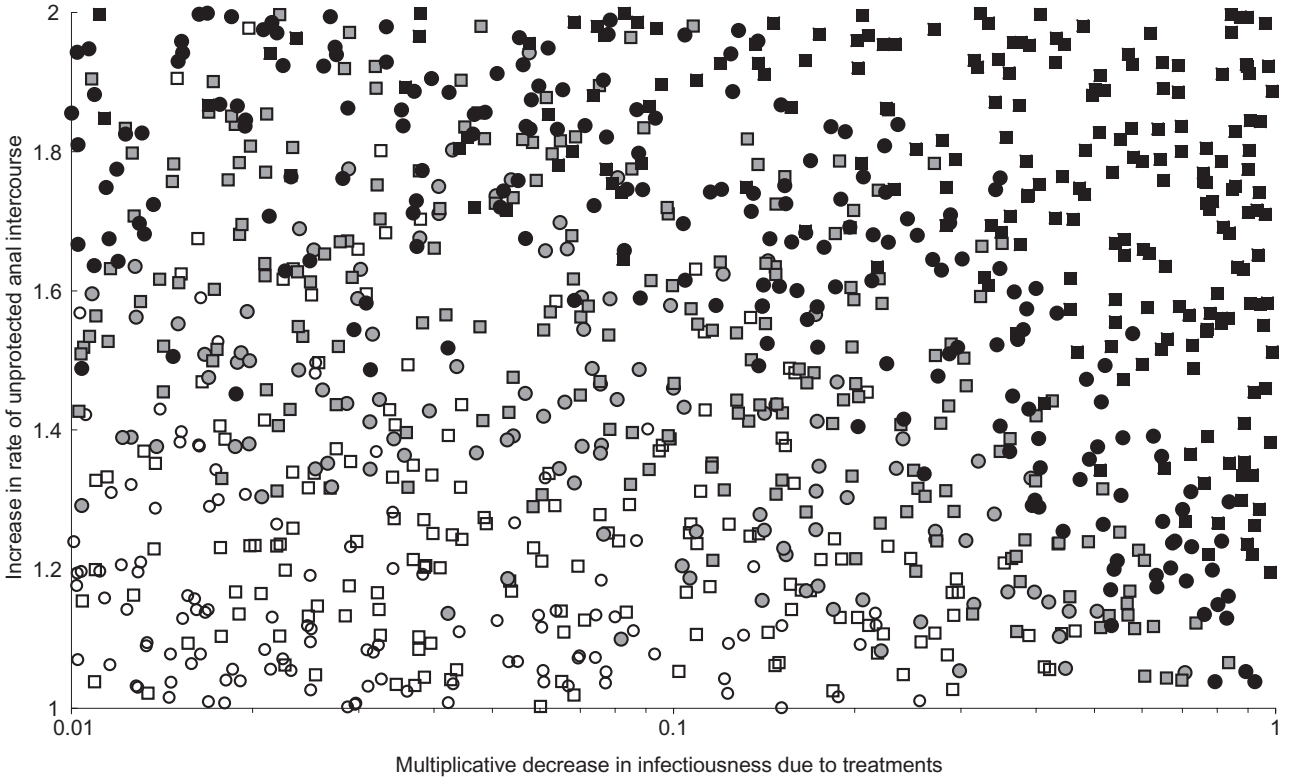

Fig. 1. Change in annual HIV incidence by increase in the rate of unsafe sex and decrease in infectiousness as a result of treatments. ○ ⩽ 38%; □ ⩽ 255%; ○ ⩽ 15%; □ < 0%; ● < 20%; ■ > 20%.

with a null model in which there was no effect of antiretroviral treatment on reducing HIV infectiousness, and no increase in UAI.

With this model, the dynamic competing effects of reduced HIV infectiousness as a result of antiretroviral treatment and the increased rates of UAI and STI on HIV incidence are illustrated in Fig. 1. In this figure, black dots and black squares correspond to simulations in which the incidence of HIV was found to increase by 0–20%, and over 20%, respectively, compared with the null model. This figure suggests that two-, five- and 10-fold decreases in infectiousness as a result of antiretroviral treatment could be counterbalanced in terms of new HIV infections by increases in UAI of approximately 30, 50 and 65%, respectively.

These models suggest that even very modest increases in STI, as a result of increased UAI, could have an important multiplicative effect increasing the incidence of HIV. Our results serve to underscore that the dynamic between interventions that may decrease HIV transmissions, such as combination antiretroviral treatment or vaccines, and changes in risk behaviours, which may increase HIV transmission, is complex and may not be easily predictable.

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Cross-neutralizing antibodies against primary isolates in African women infected with HIV-1

In previous studies performed among patients seen at the HIV clinic, the Institute of Tropical Medicine, Antwerp, Belgium, a disproportionate number of African HIV-1-infected women were shown to have circulating antibodies that could neutralize different subtypes of primary HIV-1 isolates. However, some relevant factors were confounded because few Europeans were included in the first study [1], and most of the women in the second study were Africans [2]. The question is whether the disproportionate number of Africans occurs by chance or is a function of their sex or origin.

In this study, 168 plasma samples of African and European men and women (42 of each group) were screened in a randomized manner against primary CA4 (*env* subtype F), CA13 (*env* subtype H) from group M and VI686 from group O [1]. Sixteen plasma samples that cross-neutralized all three viruses were further tested against nine other primary isolates, belonging to group M (*env* A–H) and two of group O.

Neutralization indices (NI) were calculated for each plasma/isolate combination, i.e. the infectious virus titre, expressed in $\log_{10}$, in phytohaemagglutinin-stimulated peripheral blood mononuclear cell cultures divided by the titre after incubation in the presence of plasma. An NI greater than 1.0 (90% reduction) is taken as an indicator for neutralization.

The proportion of NI greater than 1.0 for each isolate tested was used in a binomial expansion to calculate the expected numbers of plasma samples that would neutralize none, one, two and three isolates. The expected values were compared with their observed values for each neutralization pattern and were then compared by χ^2 analysis.

Sixteen out of 168 plasma samples (9.5%) neutralized the three isolates. Of these, 15 were Africans ($P = 0.006$) and one was European. Of the Africans, 10 were women ($P = 0.009$); the European was a woman.

From the 16 plasma samples that were able to neutralize the three isolates, only six of the African

women, one African man and the European woman were able to neutralize 10 or 11 out of 11 of the wider range of isolates ($P = 0.06$; not statistically significant) (data not shown).

Although plasma from Africans showed a disproportionate number that could neutralize the three isolates, this phenomenom was also observed with the neutralization of one or two isolates (Table 1). The frequencies of neutralizing none, one, two or three isolates by plasma from Europeans showed a good fit with expected frequencies, indicating that the neutralization of one isolate did not influence the neutralization of the others. In contrast, for Africans there was a significant difference between expected and observed frequencies, and this is particularly evident in women.

Cross-neutralization is particularly apparent in the plasma of African women. When plasma were screened against three HIV-1 isolates, no African women had antibodies that were specific for the group O virus nor doubly specific group O and subtype H, although given the numbers of plasma samples that could neutralize these two isolates, seven or eight of the 42 plasma samples should have fallen into one of these two categories.

In contrast, 10 African women were able to neutralize all three isolates against an expected number of two or three. Although there was a similar trend among the African men, the distortion was not so evident because there were six plasma samples that were specific for either the subtype H or group O isolates, such that any difference from an assumed random distribution did not reach statistical significance (Table 1).

These observations are compatible with the idea that neutralization of the subtype H and group O isolates are not independent parameters in African women: the same set of antibodies may be able to neutralize both viruses. This resolves some of the anomalies obtained with previous results.

The disproportionate number of women with broad cross-neutralizing antibodies was probably a result of their being African, rather than because of their sex. In a previous study, we concluded that the neutralizing activity was antibody mediated [2]. Interestingly, Burrer *et al.* [3] also observed antibody-mediated neutralization, particularly in African women.

Our results show that there is a difference in neutralization patterns between African and European plasma, especially in African women. An attempt to generate human monoclonal antibodies from African women with broad cross-neutralizing capacity is ongoing.

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Table 1. Comparison between expected and observed values for different neutralization profiles CA4 (*env* F)/CA13 (*env* H)/VI686 (*env* O).

African											
Spectrum	−/−/−	+/−/−	−/+/−	−/−/+	+/+/−	+/−/+	−/+/+	+/+/+			
Expected frequency	21.76	18.84	7.73	11.46	6.69	9.92	4.07[a]	3.52[a]			
Observed frequency	38	10	5	2	2	12	0[a]	15[a]	N = 84		
χ^2	12.120	4.148	0.964	7.809	3.88	0.436	−7.234[a]−		Total χ^2 = 35.999	$\gamma = 6$	$P < 0.01$
European											
Spectrum	−/−/−	+/−/−	−/+/−	−/−/+	+/+/−	+/−/+	−/+/+	+/+/+			
Expected frequency	53.310	12.552	5.617	8.066	1.318[a]	1.892[a]	0.847[a]	0.199[a]			
Observed frequency	56	10	4	8	3[a]	2[a]	0[a]	1[a]	N = 84		
χ^2	0.230	0.519	0.465	0.00054		−0.715[a]−			Total χ^2 = 1.929	$\gamma = 4$	$P > 0.05$ (NS)
African woman											
Spectrum	−/−/−	+/−/−	−/+/−	−/−/+	+/+/−	+/−/+	−/+/+	+/+/+			
Expected frequency	8.836	9.723	3.539[a]	5.439[a]	3.896[b]	5.987	2.178[b]	2.398[b]			
Observed frequency	19	5	1[a]	0[a]	1[b]	6	0[b]	10[b]	N = 42		
χ^2	11.692	2.297		−7.089[a]−		0.000282	−0.754[b]−		Total χ^2 = 21.832	$\gamma = 4$	$P < 0.01$
European man											
Spectrum	−/−/−	+/−/−	−/+/−	−/−/+	+/+/−	+/−/+	−/+/+	+/+/+			
Expected frequency	26.321	7.166	3.555[a]	2.763[a]	0.968[a]	0.752[a]	0.373[a]	0.102[a]			
Observed frequency	26	7	4[b]	3[a]	1[a]	1[a]	0[a]	0[a]	N = 42		
χ^2	0.00396	0.00394				−0.026[a]−			Total χ^2 = 0.0339	$\gamma = 2$	$P > 0.05$ (NS)

− Neutralization indices (NI) < 1.0. + NI > 1.0. Spectrum −/−/−, NI CA4 < 1.0/NI CA13 < 1.0/NI VI686 < 1.0. Expected frequency −/−/−, P(NI *CA4* < 1.0) × P(NI CA13 < 1.0) × P(NI VI686 < 1.0) × N. N, Number of individuals. [b/a], Categories are taken together to give expected values ⩾ 5. Neutralization assay: 1 h incubation, 2 h adsorption, 7 days culture, two duplicates.

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Reduction of HIV-1 viral load in saliva by indinavir-containing antiretroviral regimen

Epidemiological studies and AIDS surveillance data showed that HIV transmission depends on contact with infected body fluids, primarily blood and semen [1]. These compartments are established as reservoirs of HIV-1 infection [2,3], and monitoring the HIV-1 viral load in compartments is essential for assessing transmission risk, disease progression, and response to antiretroviral regimens.

Recent evidence suggests that the oral cavity may actually be an HIV-1 reservoir. Indeed, Freel *et al.* [4] documented one case of discordant HIV-1 subpopulations in peripheral blood and in the oral cavity, suggesting that selected individuals may harbour different viral populations in the blood and saliva, as has been widely documented for blood and other body fluids [5].

Therapeutic regimens including HIV protease inhibitors greatly reduce the viral load in plasma [6]. To gain insights into the efficacy of antiretroviral therapy in reducing the seminal viral load, we evaluated in a longitudinal study the effects of three antiretroviral regimens on changes of viral load in plasma and semen of HIV-1-infected individuals, and demonstrated that highly active antiretroviral therapy (HAART) can dramatically reduce the viral load in the semen of HIV-1-infected subjects [7].

In the present study, we aimed at evaluating the effects of indinavir-containing regimens on HIV-1-RNA levels in saliva.

Seventeen anti-HIV-1-positive, antiretroviral-naive subjects provided paired (i.e. collected on the same day) saliva and plasma specimens at the beginning of the study and between 4 and 12 weeks later. All patients were men, and their ages ranged between 24 and 45 years. None had received previous lamivudine or protease inhibitor treatment. The median plasma HIV-1-RNA level at enrolment was 59 260 copies/ml, and the CD4 cell count was 234/mm^3. All patients received 300 mg zidovudine or 40 mg stavudine with 150 mg lamivudine, given twice a day in combination with 800 mg indinavir three times a day.

The presence in the plasma and saliva of specific HIV-RNA sequences was first assessed by qualitative reverse transcriptase–polymerase chain reaction (RT–PCR). A competitive RT–PCR-based assay was used to quantify cell-free HIV-1-RNA molecules in plasma and saliva samples; the limit of detection of competitive RT–PCR is one copy per 100 μl plasma [2].

At baseline, HIV-1 RNA was detectable in 14 out of the 17 samples of saliva (82.4%), but only five out of 17 patients showed HIV-1-RNA levels greater than 500 copies/ml. By contrast, HIV-1 RNA in plasma was greater than 500 copies/ml in all patients. The mean concentration was 247 copies/ml (range 0–86 240 copies/ml) in saliva compared with 59 260 copies/ml (1034–886 780) in plasma. After a period of treatment ranging between 8 and 12 weeks, 13 subjects (76.5%) were free of HIV-1 RNA in saliva when tested by RT–PCR, and the HIV-RNA level in saliva was less than 100 copies/ml in the remaining four patients. By contrast, HIV-1 RNA in plasma was less than 100 copies/ml in only eight out of 17 patients (47.1%), although all experienced at least a 1.5 log reduction (Table 1).

The transmission of HIV-1 through the oral cavity may occur during breastfeeding [8,9], oral–genital sex [10], and direct deposition in macaques [11], despite the observed risk being low.

Therefore, oral sexual activities that entail a massive exchange of saliva cannot be considered 'safe sex' even if the risk of infection is exceedingly small. In some patients, the HIV-1-RNA level in saliva was found to be higher than that in semen [2]. This is very telling, because within the framework of the sexual transmission of HIV, semen is considered to be highly infective. Moreover, the medium level of viral load in saliva, measured using competitive RT–PCR, in subjects with CD4 cell counts of less than 200/mm^3, was higher than that found in maternal milk [8]. This finding is also relevant because breastfeeding is thought to play an important role in the increased transmission rate of HIV-1 from mother to infant [8,9].

Table 1. Absolute number of cell-free HIV-1 genomes as calculated by using data from competitive reverse transcriptase–polymerase chain reaction analyses in plasma and saliva from 17 patients examined before and after starting highly active antiretroviral therapy at various timepoints.

Patient no.	Time-points (weeks)	HIV-RNA copies/ml	
		Plasma	Saliva
X01a	0	124 890	5700
X01b	4	47 000	95
X01c	8	53	0[a]
X02a	0	104 712	9780
X02b	4	63 679	1210
X02c	8	145	0[a]
X03a	0	56 790	230
X03b	8	2300	54
X03c	12	21	0[a]
X04a	0	114 814	86 240
X04b	4	54 678	1289
X04c	12	1670	98
X05a	0	1034	0[a]
X05b	8	50	0[a]
X06a	0	886 780	148
X06b	4	230 900	0[a]
X06c	8	24 780	0[a]
X06d	12	1300	0[a]
X07a	0	35 678	229
X07b	4	1300	87
X07c	12	45	0[a]
X08a	0	125 121	225
X08b	4	34 900	85
X08c	8	190	0[a]
X09a	0	43 308	113
X09b	4	2790	67
X09c	12	85	54
X10a	0	59 260	162
X10b	4	3890	120
X10c	8	90	79
X11a	0	91 730	398
X11b	4	3450	85
X11c	12	3230	0[a]
X12a	0	12 800	0[a]
X12b	12	80	0[a]
X13a	0	17 320	4770
X13b	8	1345	245
X13c	12	120	0[a]
X14a	0	35 890	710
X14b	4	1900	0[a]
X14c	8	230	0[a]
X15a	0	98 500	402
X15b	8	255	79
X16a	0	54 350	21
X16b	4	1390	0[a]
X16c	8	12 430	0[a]
X16d	12	1700	0[a]
X17a	0	95 860	265
X17b	8	2450	250
X17c	12	89	0[a]

[a]Free of HIV-RNA when tested by reverse transcriptase–polymerase chain reaction.

Antiretroviral therapy has a powerful effect on reducing HIV-1-RNA levels in blood [6] and semen [7]. Our data demonstrated that HAART can also constantly reduce the HIV-RNA level in saliva, achieving very low levels in all patients analysed and undetectable levels in the majority of patients, also considering that the more sensitive qualitative method was used. The proportion of patients reaching an undetectable RNA level in saliva was considerably higher than that in plasma. This result was probably related to the baseline RNA levels in saliva, which were generally 1–2 logs lower than in plasma.

Our findings, observed in 8–12 weeks of HAART in naive patients, imply that saliva is virtually risk free, with regard to HIV transmission.

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Probable interaction between efavirenz and cyclosporine

In vitro, efavirenz induces and inhibits cytochrome p450 isoenzymes, with induction effects being predominant [1]. Currently, data on the interaction between efavirenz and immunosuppressants are lacking. We would like to report an apparent drug interaction between efavirenz and cyclosporine in an HIV-infected patient.

The patient was a 39-year-old, newly diagnosed HIV-positive man whose previous medical history included depression, hypertension, pernicious anaemia, urinary tract infections, gout, and end-stage renal disease secondary to IgA nephropathy, requiring peritoneal dialysis in March 1995 and a living-related, human leukocyte antigen-identical kidney transplant in February 1996. The course of his post-transplant care was unremarkable, with no complications or rejection episodes. For the past few years, the patient had been receiving nifedipine XL 60 mg in the morning and 30 mg in the evening, with metoprolol 100 mg twice a day for hypertension and nefazodone 50 mg a day for depression. One month before admission, he was started on allopurinol 100 mg a day for gout. For rejection prophylaxis, he was maintained on prednisone 2.5 mg a day and cyclosporine (neoral), most recently at a dose of 200 mg twice a day since March 1999. The mean cyclosporine drug level using the fluorescence polarization immunoassay at this dose was 328 μg/l.

On 17 August 1999, the patient was admitted to our institution for fever of unknown origin, 20 lb weight loss, general malaise, and fatigue that had progressively worsened over the previous 6 months. A diagnosis of HIV was made shortly after admission. The patient's baseline CD4 cell count and viral load at that time was 352×10^6 and 5.38 $\log_{10}$, (Chiron 3 assay). On admission, the cyclosporine level was 307 μg/l. All standard medications were continued, and two doses of intravenous ciprofloxacin 500 mg every 12 h and two daily doses of prednisone 20 mg were given. Three days after admission, the patient's cyclosporine level was elevated at 372 μg/l. The cyclosporine dose was subsequently reduced to 175 mg twice a day and prednisone was changed to 2.5 mg a day. As a result, the cyclosporine levels gradually declined to an average of 203 μg/l (Fig. 1).

On 25 August, nefazodone was discontinued and efavirenz 600 mg at bedtime, zidovudine 300 mg twice a day and lamivudine 150 mg twice a day were initiated. An initial rise in the cyclosporine level was noted for 2 days after antiretroviral initiation. The levels then proceeded to decline to 80 μg/l approximately 7 days after the first dose of efavirenz. In response, his cyclosporine was temporarily increased to 200 mg twice a day, and the patient was discharged on 4 September. Four days later, his cyclosporine was changed back to a

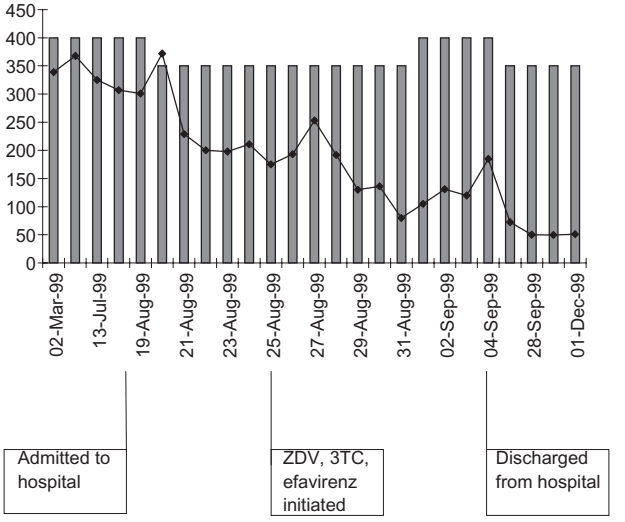

Fig. 1. Cyclosporine concentrations in relation to antiretroviral administration. ■ Cyclosporine dose (mg/day); —◆— cyclosporine levels (μg/l). 3TC, Lamivudine; ZDV, zidovudine.

maintenance dose of 175 mg twice a day. One month after antiretroviral initiation, his cyclosporine level had reached a nadir of 50 μg/l. At 2 years follow-up, the patient remains on the same medications, and is doing extremely well. His viral load has been continuously suppressed (< 50 copies/ml), his CD4 cell count is over 900 cells/mm^3, and his renal function remains excellent (serum creatinine 119 μmol/l).

Cyclosporine is extensively metabolized by CYP3A4 isoenzyme, and is also a substrate and inhibitor of P-glycoprotein [2]. Drugs that are enzyme inducers or inhibitors would be expected to decrease or increase cyclosporine levels, and numerous interactions have been documented in the literature [3,4]. For instance, rifampin, phenytoin, and phenobarbital reduced cyclosporine concentrations by 39, 70, and 68.2%, respectively [5–7].

However, interaction data regarding the concomitant administration of cyclosporine and antiretroviral drugs are scarce. There has been one case report of an HIV-positive renal transplant patient whose cyclosporine levels tripled 3 days after the initiation of saquinavir; the postulated mechanism was competition for CYP3A metabolism and P-glycoprotein drug transport by saquinavir [8]. To date, there have been no other interaction reports involving antiretroviral agents and cyclosporine.

Efavirenz is a substrate of CYP3A4, and it predominantly exerts cytochrome p450 3A4 enzyme induction effects [9–13]. Although the initial effects of enzyme induction may be noticeable within a few days, the

maximal effects would be expected after a few weeks, because of the time required for the synthesis of new metabolizing enzymes and the long half-life of efavirenz [14]. In our patient, cyclosporine levels began to decrease 5 days after efavirenz was added to a stable cyclosporine regimen, and reached a nadir (representing an 75% decrease from baseline) one month later.

In conclusion, we describe an apparently significant interaction between efavirenz and cyclosporine. Close monitoring of cyclosporine levels and serum creatinine is recommended when these agents are co-prescribed, and cyclosporine dosage adjustment may be required to maintain desired therapeutic levels in previously stable patients. The potential for a similar interaction may exist with nevirapine, which is also a potent enzyme inducer.

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Gynaecomastia without lipodystrophy in HIV-1-seropositive patients on efavirenz: an alternative hypothesis

Caso *et al.* [1] recently reported three cases of anatomopathologically confirmed gynaecomastia without evidence of lipodystrophy syndrome in HIV-infected male patients treated with efavirenz. Although some cases of breast hypertrophy had been reported on efavirenz therapy, some with the lipodystrophy syndrome, none had anatomopathological confirmation [1]. We have previously shown that true gynaecomastia could be effectively differentiated from breast hypertrophy secondary to lipodystrophy syndrome (lipomastia) by breast ultrasonography [2], and that true gynaecomastia could occur with the use of all currently available classes of antiretroviral medications, including efavirenz and nevirapine and not just protease inhibitors and nucleoside reverse transcriptase inhibitors, as other authors have previously reported. Breast ultrasonography was furthermore shown to be a sensitive method of differentiating true gynaecomastia, in which there is an increase in ductal tissue and periductal stroma, from lipomastia (pseudogynacomastia) associated with the lipodystrophy syndrome [2,3].

A total of 11 HIV-1-infected patients with true gynaecomastia treated with different antiretroviral combinations including efavirenz [2] were investigated for other causes of gynaecomastia by complete hormone profiles, biochemical profiles, serum cholesterol, triglyceride and tumour markers. A complete drug history excluded concomitant medications as a potential cause of gynaecomastia [2]. All patients achieved an HIV-1-RNA load of less than 50 copies/ml with the current antiretroviral regimen, as found in the cohort described by Caso *et al.* [1], and achieved an excellent increase in CD4 cell counts. Since then we have extended our original cohort to 15 patients with true gynaecomastia, which developed after the initiation of effective highly active antiretroviral therapy, with similar overall findings [4]. In addition, 12 out of the 15 patients described had complete resolution of the gynaecomastia after a mean period of 2 months without any specific therapy. We believe that in view of the rapid increase in the CD4 cell count, the reduction in HIV-1-RNA load to less than 50 copies/ml, the absence of other pathological conditions, and the rapid resolution with no specific treatment, the true gynaecomastia described by Caso *et al.* [1] was a manifestation of immune restoration

disease and not just a specific effect of efavirenz-containing antiretroviral regimens.

The mechanism underlying the development of true gynaecomastia is unclear. However, after the commencement of highly active antiretroviral therapy, there is an improvement in the T helper cell cytokine response, specifically an increased production of IL-2 [5]. IL-2 has been shown to increase the proliferation of human breast carcinoma cells *in vitro* [6]. In addition, IL-6 has been shown to increase aromatase activity in breast tissue [7], with a consequent increase in oestrogen available to stimulate breast growth. This evidence suggests that cytokine perturbations occurring with immune restoration may result in altered breast tissue oestrogen availability, which ultimately causes true gynaecomastia. Once immune restoration has occurred, the levels of these cytokines fall leading to restoration of the oestrogen : testosterone ratio, with the resolution of gynaecomastia without therapy.

We believe that immune restoration is a plausible unifying mechanism to explain the development of true gynaecomastia that occurs in patients treated with different classes of antiretroviral medications. Clearly, this hypothesis needs further investigation.

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Transmission of HIV-1 variants resistant to the three classes of antiretroviral agents: implications for HIV therapy in primary infection

Over the past 3 years, the transmission of multidrug-resistant HIV has been detected in few cases [1–4]. Recently, a trend towards an increase in the incidence of multidrug-resistant HIV from 0.4 to 5.8% in US cities was highlighted [5]. The slightly increasing frequency of this transmission can be related to the sequential use of several antiretroviral drug regimens and the incomplete suppression of viral replication. Consequently, the transmission of drug-multiresistant variants could impair the success of the management of antiretroviral therapy in newly infected patients.

We report a new case of transmission of an HIV-1 variant with multiple mutations associated with resistance to protease and both nucleoside and non-nucleoside reverse transcriptase (RT) inhibitors. The index patient was a 54-year-old heterosexual woman, who had been tested negative for HIV-1 antibodies 9 months before visiting. She reported having had, 3 months before visiting, a preservative accident during sexual intercourse with her HIV-1-infected partner. One month later, she noted an episode of fever. At visiting time, in November 1999, two enzyme immunosorbent assays for HIV-1 antibodies (Axsym HIV1/2gO, Abbott GmbH Diagnostika, Wiesbaden-Delkenheim, Germany; Access HIV-1/2 New, Bio-Rad, Marnes La Coquette, France) were strongly reactive; Western blot was complete (New Lav Blot I, Bio-Rad); p24 antigen (VIDAS HIV P24 II, BioMérieux sa, Marcy l'Etoile, France) was undetectable; the plasma HIV-1-RNA level was 6.0 $\log_{10}$ copies/ml (COBAS Roche Amplicor HIV-1 Monitor test version 1.5, Roche Diagnostics, Meylan, France); the CD4 cell count was 597 cells/μl; and physical examination was normal. A diagnosis of primary HIV-1 infection was made. The patient insisted on the immediate initiation of antiretroviral therapy, and received zidovudine plus lamivudine (300/150 mg × 2/24 h by month), and nevirapine (200 mg × 2/24 h by month), which was continued without interruption for 15 months. Genotypic analysis in the RT and protease genes from plasma HIV-1 RNA by *pol* gene sequencing [6] was performed just before the initiation of therapy and showed multiple mutations associated with antiretroviral resistance to the three classes of drugs (Table 1).

Table 1. Genotypic analysis of the drug-resistance mutations of HIV-1 from the index and source patients at baseline and during follow-up.

	Amino acid substitutions			
	Source patient		Index patient	
Enzyme	21/04/1999	31/05/2000	16/11/1999	27/11/2000
Reverse transcriptase	M41L E44D K103N L210W T215Y	M41L E44D K103N L210W T215Y	M41L E44D K103N L210CG T215Y	M41L E44D D67DN K103N M184V L210W T215Y
Protease	L10I L24I M36I M46L I54V L63P A71V V82T I84V	L10I L24I M36I M46L G48EQ I54V L63P A71V V82T I84V L90LF	L10I L24I M36I M46L I54V L63P A71V V82T I84V	L10I L24I M36I M46L I54V L63P A71V V82T

After 15 months of triple therapy, a poor virological response was noted: the viral load did not fall below 2.98 $\log_{10}$ copies/ml (detection limit of the assay 200 copies/ml) despite good adherence to treatment. CD4 cell counts were constantly higher than 350 cells/μl, and no opportunistic infection occurred.

Genotypic testing performed from the source patient's plasma HIV RNA 3 months before transmitting virus to the index patient showed the same genotypic resistance profile in the RT and protease genes that were later found in the index patient (Table 1). Genotypic analysis performed during a follow-up of 12 months showed only slight differences in both individuals. Phylogenetic analysis of the RT and protease genes indicated that the viral sequences from the source patient and index patient clustered together, with bootstrap values of 100%. A high similarity (97.7%) within the RT sequences was found in the source and index patient's sequences (data not shown).

This case report confirms previous cases of transmission at the time of primary HIV infection of multiple-drug genotypically resistant HIV-1, which showed an impaired effect of antiretroviral therapy on viral replication. For example, in one index case, the HIV-1-RNA level remained at 3.3 $\log_{10}$ copies/ml 7 months after the initiation of triple therapy [3]. In another case, there was only a slower decrease in the number of copies of plasma HIV-1 RNA, contrasting with the undetectable levels of HIV RNA within 12 weeks after starting treatment in 36 other patients treated with similar drug combinations [1]. Conversely, another study [7] reported the case of a patient who chose to defer therapy: at month 5 after diagnosis, the multi-drug-resistant strain was replaced by a more susceptible population of viruses; the HIV-1-RNA level that had a spontaneous decrease showed a 10-fold increase; an increase in the CD4 cell count of 67 cells/μl (17%) was noted between baseline and month 5, suggesting that the absence of treatment had no marked deleterious effect. It thus remains unclear whether altering treatment to replace a poorly fit resistant virus population by a replication-competent wild-type virus is a relevant therapeutic strategy in the setting of primary HIV infection.

The implications of these data for the management of antiretroviral therapy in patients who acquire multi-drug-resistant HIV-1 are unknown, as well as the clinical, virological and immunological evolutions in such patients. Therefore, sequential follow-up in the same situations either in the presence or in the absence of highly active antiretroviral therapy pressure is needed.

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Rhodococcus equi pneumonia: highly active antiretroviral therapy helps but does not cure lung infection

Rhodococcus equi pneumonia is a rare condition first reported in HIV-infected patients 15 years ago [1]. The cases described come from the era when highly active antiretroviral therapy (HAART) was not available [2–4], or there is no reference to the concomitant use of this therapy. Here we describe a case of *R. equi* pneumonia in a patient with HIV infection, emphasizing the implications of HAART.

A 34-year-old man was admitted to the hospital suffering from fever and cough lasting for 3 weeks, with occasional bloody sputum. He was a heavy smoker without any other toxic behaviour. He was febrile (38°C) with a good general condition, but some crackles were heard in the right lower lung. Laboratory tests were within the normal ranges. Chest X-ray showed an alveolar infiltrate, with cavitation and gas–liquid level in the right lower lung, confirmed by computed tomography. Blood cultures, sputum stain for acid-fast bacilli (AFB) (× 5) and sputum cytology were all negative. A fibrobronchoscopy disclosed multiple endobronchial lesions in the right bronchial branches, with non-specific changes in bronchial biopsy. No AFB were seen in the bronchial aspirate or biopsy, and bacterial culture was negative. A partial clinical improvement was documented after intravenous amoxycillin–clavulanic therapy, but no radiological changes were observed. A Löwenstein culture from previous sputum and bronchial aspirate was reported as being contaminated.

One month later a second fibrobronchoscopy showed similar endobronchial lesions. Bronchial biopsy disclosed a lung parenchyma with numerous macrophages containing bluish rounded inclusions, suggestive of malacoplakia. No AFB were seen in the bronchial aspirate, but a Löwenstein culture grew some colonies identified as *R. equi*. Anti-HIV antibodies (enzyme-linked immunosorbent assay, Western blot) were positive and the CD4 cell count was 15/μl, with a viral load of 104 863 (5.02 log) HIV-RNA copies/ml.

No clinical/X-ray changes were reported after therapy with ciprofloxacin by mouth (substituted for rifampin, because of dermal toxicity), erythromycin by mouth and vancomycin intravenously for 6 weeks. Antiretroviral therapy (zidovudine, lamivudine and indinavir) was introduced, together with continued oral antibiotic therapy (ciprofloxacin, erythromycin and trimethoprim/sulfamethoxazole). Two months later the CD4 cell count was 56/μl and the viral load was less than 200 HIV-RNA copies/ml.

During the next 6 months, clinical recurrent impairment appeared, with an intensification of the cough and fever, but no significant changes in the right lower lung infiltrate. *R. equi* was repeatedly isolated in sputum cultures, which was susceptible to erythromycin, ciprofloxacin, rifampin, vancomycin and imipenem, adding imipenem or teicoplanin (3 weeks to 4 months) to oral antibiotics, with partial clinical improvement, and a recurrence of respiratory symptoms on parenteral antibiotic withdrawal.

Twelve months after the diagnosis of pneumonia was made, the patient presented once more with intermittent low-grade fever. A chest X-ray showed the total collapse of the right lower lobe. Fibrobronchoscopy disclosed a polypoid occlusion of the right lower lobe bronchus, with microscopic changes consistent with malacoplakia in bronchial biopsy. At this time the CD4 cell count was 110/μl and the viral load was less than 50 HIV-RNA copies/ml. A right lower lobectomy was performed without complications (Fig. 1). *R. equi* was isolated from the surgical specimen culture, and combined antibiotic therapy (erythromycin by mouth, ciprofloxacin by mouth, and teicoplanin intramuscularly) was maintained for 1 month, together with the same antiretroviral regimen.

During the 18 month period post-surgery, the patient has been asymptomatic and currently continues in an excellent clinical condition. The last CD4 cell count was 234/μl and the viral load was less than 50 HIV-

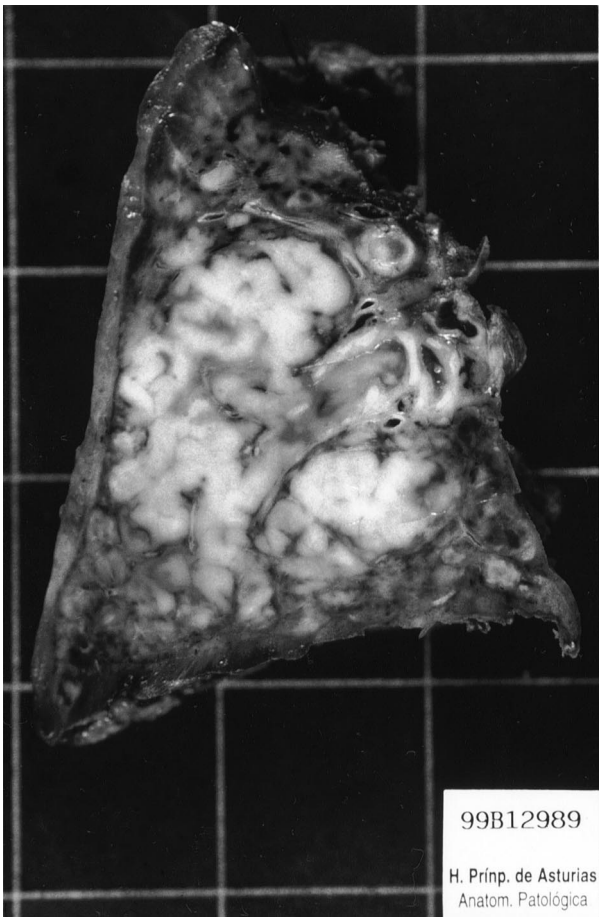

Fig. 1. Gross appearance of a section of the right lower lung surgically resected.
The whole lung tissue is extensively filled up with a dense, caseous-like material, where *Rhodococcus equi* was isolated.

RNA copies/ml. Chest X-ray shows post-surgical changes without any lung infiltrate. Trimethoprim/sulfamethoxazole has been withdrawn and efavirenz substituted for indinavir.

Infections produced by *R. equi* have been described most commonly in deeply immunosuppressed HIV patients (CD4 cell count $< 50/\mu l$) [2], although some cases have been reported in immunocompetent individuals [5,6]. The presence of malacoplakia from the first stages of pneumonia in the patient reported represents a failure in intracellular bacterial digestion by histiocytes. As has been described, this is a feature clearly linked, but not pathognomonic, to HIV infection [7,8].

Before HAART was available, the management of *R. equi* pneumonia with continued combined antibiotic therapy provided a poor outcome in HIV-positive patients, sometimes requiring a surgical approach, with a short survival and a mortality rate greater than 50% [2], in sharp contrast to the results of immunocompetent individuals (survival rate 90%) [6].

By itself, or added to specific anti-infectious therapy, HAART has allowed the resolution of otherwise fatal opportunistic infections in HIV-positive patients [9,10]. It could be hoped that immune reconstitution derived from HAART would resolve the lung infection when added to combined antibiotic therapy addressed to rhodococcal infection.

Without HAART, the short-term outcome of the patient reported here would probably have been fatal. Antiretroviral therapy has provided a significant immunological response and a consistent inhibition of viral replication. However, after 10 months of uninterrupted combined antibiotic therapy (through the oral and parenteral route), together with HAART, a failure to erradicate bacterial infection was demonstrated, allowing progressive lung abscess organization (Fig. 1), which finally needed surgical resection.

To our knowledge, this is the first case reported that clearly shows the effects of HAART on *R. equi* pneumonia in an HIV patient: the immune reconstitution derived from the therapy allowed us to keep the patient alive, but failed to resolve the bacterial infection, in spite of concomitant, intense and continuous antibiotic therapy.

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