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Declining number of patients with cryptococcosis in the Netherlands in the era of highly active antiretroviral therapy

Cryptococcosis is caused by the encapsulated yeast-like fungus *Cryptococcus neoformans*. It is the most common life-threatening fungal infection in individuals with compromised cellular immunity, such as patients with malignancies, with AIDS, or receiving immunosuppressive therapy. Since the beginning of the AIDS epidemic, major changes in the epidemiology of cryptococcosis have occurred. In France, an increase in the number of cases was reported because of an increase in HIV-related cases [1]. The introduction of highly active antiretroviral therapy (HAART) in 1996 has changed the natural history of HIV infection by reducing the incidence of AIDS-related opportunistic disorders [2,3].

Before 1986, the annual incidence of cryptococcosis in the Netherlands varied from 0.2 to 0.5 per 1 million inhabitants [4]. To gain insight into the epidemiological spectrum of this disease during the following years we analysed cases of cryptococcosis retrospectively. Here we report the changes in the rate of new cases of cryptococcosis in the Netherlands between 1986 and 2000.

The patient population described here was identified in two different ways. The majority was recovered from the registers of the Netherlands Reference Laboratory for Bacterial Meningitis in Amsterdam. Almost all clinical microbiology laboratories in the Netherlands collaborate by sending their results from patients with bacterial meningitis or cryptococcosis to the Netherlands Reference Laboratory for Bacterial Meningitis. Moreover, the medical microbiology departments of five major university hospitals were contacted for additional cases of cryptococcosis, because most patients with cryptococcosis are treated at university hospitals.

Epidemiological data (age, sex, hospital) and relevant medical history (underlying disease, medication) for all patients were obtained through their treating physicians, or by reviewing their charts. The results of our study were compared with the AIDS Therapy Evaluation Netherlands (ATHENA) database. That nationwide multi-centre clinical cohort study started in 1998 and aims to monitor the use and effectiveness of HAART.

A total 268 patients were identified with cryptococ cosis. HIV infection was present in 203 patients (76%). Other predisposing factors included: the use of immunosuppressive medication for various reasons (14 patients, 5%), including organ transplantation (seven patients, 2.5%) and connective tissue disorders (two patients, 1%); haematological malignancy (16 patients, 6%), and in two patients (1%) a rare hereditary case of impaired cellular immunity. In nine cases (3%) no predisposing factors could be detected despite an extensive evaluation. In 24 cases (9%), no information about an underlying condition was available. Most of the HIV-infected patients had advanced disease, with a median CD4 positive T cell count of 35 cells/μl (range 3–240).

A sharp increase in the number of patients with cryptococcosis was seen in 1987, as a result of the increasing number of HIV-infected individuals. During the first 10 years, the mean annual rate of cryptococcosis was 21.3 cases per year. Interestingly, this declined by 55% to only nine and 10 cases per year in 1996 and 1997, respectively; and more or less stabilized at 13 patients over 1998 and 1999. This decrease was mainly caused by the reduced occurrence of cryptococcosis in HIV-infected individuals (Fig. 1).

The annual number of new AIDS cases in the Netherlands has decreased [5]. Although efforts have recently been made to estimate the incidence of HIV-infected

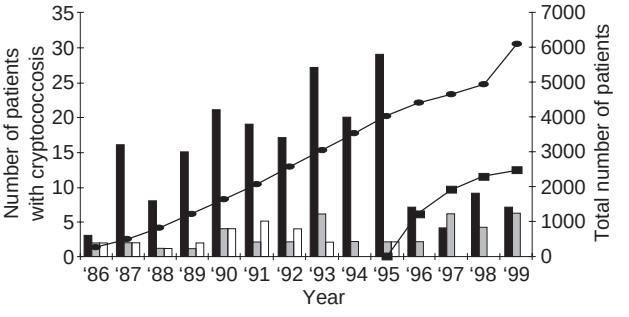

Fig. 1. Number of patients with cryptococcosis during 1986–1999, accumulating estimated AIDS diagnoses and total number of patients on highly active antiretroviral therapy included in the AIDS Therapy Evaluation Netherlands (ATHENA) study cohort. ■ HIV-infected patients; ▒ non-HIV-infected patients; □ unknown; —■— total of HIV-infected patients included in ATHENA; —●— accumulating AIDS diagnosis.

individuals and monitor these patients on a regular basis [6], to our knowledge no published data exist that substantiate the direct relationship between the decline of cryptococcosis and the use of different treatment regimens for advanced HIV infection in the Netherlands. To strengthen further the suggestion that the decline of cryptococcosis is correlated with the introduction of HAART, we have looked at the ATHENA database. By the end of 1999, 1235 antiretroviral-naive and 1218 pretreated HIV-infected patients were enrolled. This representative cohort of 2500 HIV-infected patients shows that a large population of patients with AIDS before 1996 switched to HAART, and that newly diagnosed patients are offered HAART as the standard therapy for advanced HIV infection (Fig. 1).

Other authors have reported a similar decrease in HIV-infected patients with cryptococcal meningitis up to 1997, and questioned whether this decrease would be sustained because of virological failure on the new antiretroviral drug regimens [7]. In our study, none of the HIV-infected individuals were on HAART in 1996 and 1997. However, four patients used HAART in 1998 and 1999, of which one was failing therapy.

This retrospective analysis of cryptococcosis cases demonstrates changes in the occurrence of cryptococcosis that are mainly caused by changes in the epidemiology of AIDS in the Netherlands and are temporally associated with the introduction of HAART. To evaluate their course further in HIV-infected patients, continuous monitoring of opportunistic infections is essential.

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CCR5 Δ32 deletion and response to highly active antiretroviral therapy in HIV-1-infected patients

Patients bearing a Δ32 deletion on one allele of the gene coding for the CCR5 chemokine receptor progress less rapidly to AIDS and death than do wild-type (wt) patients. CCR5 expression at the cell surface is reduced in Δ32 heterozygous patients and the plasma viral load is lower in these patients. The use of highly active antiretroviral therapy (HAART) has brought substantial clinical, virological and immunological benefits to HIV-infected patients. However, concern still exists about the occurrence of HAART failure in a large number of patients. The objective of this study is to investigate whether the Δ32 deletion has an impact on response to HAART in protease inhibitor (PI)-naive patients followed in the SEROCO and HEMOCO cohorts.

We selected all patients (N = 166) from these cohorts who were prescribed a triple-drug therapy including a PI after March 1996, and who had had at least 6 months of laboratory follow-up after the initiation of HAART. All patients had to be PI-naive at HAART initiation. A 6 month response to HAART was defined by both a virological response (at least one plasma HIV-1 RNA measurement below the threshold of 500 copies/ml or a 2 log HIV-1 RNA reduction over the 6 month laboratory follow-up) and an immunological response (an increase of at least 50 CD4 cells/μl at 6 months). The independent role of the Δ32 deletion was assessed using a logistic regression model. The dependent variable was the 6 month response to HAART, as previously defined.

Twenty-two patients in the study population (13%) were heterozygous for the CCR5 Δ32 deletion. All patient characteristics at the initiation of HAART were similar in the heterozygous and wild-type patients. The median viral loads were equal to 4.7 and 4.5 log copies/ml in heterozygous and wild-type patients, respectively, whereas the median CD4 cell counts/μl were equal to 162 and 190, respectively. The year of HAART initiation, the type of PI, the number of new nucleoside analogues with respect to treatment history of the patient, and the type of nucleoside analogues included in HAART were similar in heterozygous and wild-type patients.

Eighty-eight patients in the study population (53%) responded to HAART. An immunological response was noted in 116 patients (70%) and a virological response in 114 (69%): 104 patients (63%) were below the threshold of 500 copies/ml and 72 (43%) had a 2 log plasma HIV-1 RNA decrease. We observed a significantly higher response rate in heterozygous than in wild-type patients whatever the type of response: an immunological and a virological response (82 versus 49%, $P = 0.004$), at least an immunological response (91 versus 67%, $P = 0.021$) or at least a virological response (91 versus 65%, $P = 0.016$). The median plasma HIV-1 RNA decrease over the 6 month period was also significantly more important in Δ32 heterozygous than in wild-type patients (2.1 $\log_{10}$ copies/ml versus 1.1 $\log_{10}$ copies/ml, $P = 0.003$).

In the multivariate analysis (Table 1), the CCR5 genotype remained significantly predictive of the 6 month response to HAART, even after adjustment for well-known confounding factors such as the type of PI and nucleoside analogues included in HAART and the number of previous antiretroviral regimens: the response rate was significantly higher in heterozygous than in wild-type patients [Δ32/wt versus wt/wt: adjusted odds ratio (OR) = 4.7; 95% confidence interval 1.4–15.5, $P = 0.011$].

We also studied whether the Δ32 deletion remained predictive of the response to HAART at 12 months. A total of 127 out of 166 patients had available plasma HIV-1 RNA and CD4 cell count measurements at month 12 (with a window interval of months 9–15). The criteria used to define 12 month response to HAART were a plasma HIV-1 RNA level below the threshold of 500 copies/ml and at least a 100 CD4 cell/μl increase between the initiation of HAART and month 12. Forty-one patients (32%) responded to HAART, 73 patients (57%) had at least an immunological response, and 65 patients (51%) had at least a virological response. Of the 86 non-responders (68%), 32 had previously been classified as 6 month responders. Again, we observed a significantly higher response rate to HAART in heterozygous than in wild-type patients (56 versus 28%, $P = 0.023$) even after adjusting for all covariables analysis (Δ32/wt versus wt/wt, adjusted OR = 3.7, $P = 0.019$).

In summary, the CCR5 Δ32 deletion was found to be predictive of a higher immunological and virological response rate to HAART at 6 and 12 months in this population of HIV-1-infected PI-naive patients with advanced disease. This result was obtained independently of the two known predictive factors of response to HAART, the type of PI included in HAART and the number of previous antiretroviral regimens before

Table 1. Predictors of 6 month immunological and virological response to highly active antiretroviral therapy.

Variables	Crude odds ratio (95% CI)	*P*	Adjusted odds ratio (95% CI)[a]	*P*
CCR5				
wt/wt	1		1	
Δ32/wt	4.8 (1.5–14.8)	0.007	4.7 (1.4–15.5)	0.011
Number of previous antiretroviral regimens				
3 or more	1		1	
0–2	1.4 (0.8–2.7)	> 0.2	2.1 (1.0–4.2)	0.048
Baseline HIV-1 RNA[b]	1.1 (0.8–1.6)	> 0.2	1.4 (0.9–2.0)	0.096
Baseline CD4 cell count[c]	10.1 (0.9–1.3)	> 0.2	1.1 (0.9–1.4)	> 0.2
Protease inhibitors included in HAART				
Saquinavir	1		1	
Nelfinavir, indinavir or ritonavir	4.6 (2.0–10.7)	$< 10^{-3}$	4.6 (1.9–11.4)	$< 10^{-3}$
Nucleoside analogues included in HAART				
ZDV/3TC	1		1	
D4T/3TC	1.5 (0.7–3.2)	> 0.2	1.1 (0.5–2.6)	> 0.2
Other	0.6 (0.3–1.3)	> 0.2	0.6 (0.3–1.6)	> 0.2

CI, Confidence interval; D4T, stavudine; 3TC, lamivudine; wt, wild-type; ZDV, zidovudine.
[a]Adjusted for CCR5 genotype, number of previous antiretroviral therapy regimens, baseline plasma HIV-1 RNA and CD4 cell count, protease inhibitor and nucleoside analogues included in highly active antiretroviral therapy (HAART).
[b]For a $\log_{10}$ decrease.
[c]For a 100 cell/μl decrease.

the initiation of HAART (adjusted OR = 4.7, $P = 0.011$).

Some findings from the literature could contribute to explain the predictive effect of Δ32 deletion on the response to HAART: CCR5 expression is diminished in Δ32 heterozygous patients [1], the switch from non-syncytium inducing (NSI) to syncytium inducing (SI) is delayed in heterozygous patients [2], HAART was found to be associated with a change in viral phenotype from SI/X4 to NSI/R5 within the first year after the initiation of HAART in at least 70% of children infected with SI variants [3], and a reduction of CCR5 expression at the protein or messenger RNA level was observed after several months of HAART [4]. Recently, Valdez *et al.* [5] reported that the CCR5 Δ32 deletion was also associated with a greater likelihood of virological success after the initiation of HAART. Similarly, O'Brien *et al.* [6] found that CCR5 Δ32 heterozygous patients tended to have lower rates of virological failure than wild-type patients, but the finding was not statistically significant.

To conclude, heterozygous patients possibly revert under HAART to the lower viral replication rate described in the natural history of HIV infection. However, we cannot exclude the alternative hypothesis that the pharmacokinetics of potent antiretroviral regimens differ between Δ32 heterozygous and wild-type patients. Further studies including data on adherence and viral phenotype should be carried out in order to confirm the results of this study and provide a better understanding of the mechanism of the association between CCR5 genotype and the response to HAART.

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Acceleration of confirmed coronary artery disease among HIV-infected patients on potent antiretroviral therapy

Potent antiretroviral combination therapy has occasionally been related to the aggravation of coronary artery disease and other vascular complications [1–9]. However, the incidence of the induction of cardiovascular events among HIV-infected individuals with known or unknown coronary artery disease still needs to be determined. Protease inhibitors have been suggested to cause a lipodystrophy syndrome [10–12], which resembles syndrome X, consisting of central or visceral obesity, hyperlipidaemia, insulin resistance, hypertension, hyperuricaemia, and which confers an increased risk of cardiovascular disease [13]. Other drugs, including efavirenz, nevirapine or nucleoside reverse transcriptase inhibitors, which can affect mitochondrial function and prolong the survival of HIV-infected patients, have been proposed to be possible co-factors in the development of the metabolic syndrome [11,12,14].

We describe the course of confirmed coronary artery disease in 14 patients with HIV infection on antiretro-

viral therapy. Patients were identified from databases of the Divisions of Cardiology and Infectious Diseases at our University Hospital between 1991 and 1999. Coronary artery disease was regarded as confirmed if patients had a definite myocardial infarction (five patients) or angiographically significant (> 50%) stenosis of a major epicardial coronary artery (nine patients). One-vessel disease was present in five, two-vessel disease in one and three-vessel disease in three patients. Deterioration of coronary artery disease was defined as aggravation of angina, or the occurrence of a myocardial infarction. At the time of analysis all patients were on antiretroviral therapy consisting of at least three drugs containing either a protease inhibitor or non-nucleoside reverse transcriptase inhibitor. All patients had other cardiac risk factors, of which smoking and hyperlipidaemia were the most prevalent (Table 1).

The lipid profiles before, during antiretroviral therapy and at follow-up are shown in Table 1. During antiretroviral therapy, hyperlipidaemia was present in all but one patient. The increase in triglyceride levels was especially pronounced, with levels up to 17.4 mmol/l (normal 0.5–1.6 mmol/l), the cholesterol levels increased up to 10.3 mmol/l (normal < 5.2 mmol/l). LDL and HDL levels, however, were not significantly affected.

In six out of the 14 patients, coronary artery disease was known before the initiation of antiretroviral therapy. Of these, while on antiretroviral therapy, three patients remained stable, one experienced a second myocardial infarction, and two had unstable angina. In eight of the 14 patients, coronary artery disease became symptomatic only after the initiation of antiretroviral therapy. One of these eight patients had unstable angina and seven experienced an acute myocardial infarction. Coronary angiography was performed in nine of the 14 patients, with subsequent revascularization and clinical improvement in all. No patient died.

Table 1. Course of 14 HIV-infected individuals with confirmed coronary artery disease.

	At or before initiation of antiretroviral therapy	Peak values	Last follow-up
Patient baseline characteristics[a]			
No. of patients	14 (100)		
Male	12 (86)		
Smoking	13 (93)		
Family history of cardiovascular events	4 (29)		
Hypertension	4 (29)		
Diabetes mellitus	2 (14)		
Total cholesterol > 6.5 mmol/l	0 (0)[b]		
Age, median (range)	50 (35–69)		
HIV infection			
Previous AIDS	7 (50)		
CD4 lymphocytes, median (range)	312 (10–745)		693 (115–1115)
HIV-1 RNA, median copies/ml (range)	69 344 (1583–2 054 889)		< 50 (< 50–3691)
HIV-1 RNA < 50 copies/ml (no. of patients)	0		12 (86)
Antiretroviral therapy			
Nucleoside reverse transcriptase inhibitors	14 (100)		14 (100)
Non-nucleoside reverse transcriptase inhibitors	1 (7)		2 (14)
Protease inhibitors	10 (71)		14 (100)
Follow-up, median (range), months			
On antiretroviral therapy			40.5 (8–91)
On potent antiretroviral 3 or 4 drug regimens			34.5 (8–58)
Lipid levels, median (range), mmol/l			
Triglycerides (normal: 0.5–1.6 mmol/l)	2.3 (0.5–6.1)[b]	4.5 (0.6–17.4)	2.7 (0.8–10.9)
Total cholesterol (normal: < 5.2 mmol/l)	4.2 (3.8–5.4)[b]	6.7 (3.8–10.3)	5.9 (4.4–7.1)
HDL cholesterol (normal: 1.0–1.3 mmol/l)	0.7 (0.7–1.3)[b]	1.3 (0.8–4.9)	1.05 (0.7–1.4)
LDL cholesterol (normal: 2.9–4.9 mmol/l)	3.0 (2.3–4.7)[b]	4.1 (1.9–6.7)	3.2 (0.9–4.6)
CAD (cumulative number of patients with cardiovascular events)			
Myocardial infarction	5 (36%)		12 (85%)
Unstable angina pectoris	1 (7%)		4 (28%)
Median time to new event, months (range)			16 (1–58)
Total of patients with unstable CAD	NA		11 (79%)
Stable CAD on potent antiretroviral therapy	NA		3 (21%)[c]
Interventions (cumulative number of patients)			
Surgical revascularization	1 (7%)		2 (14%)
Percutaneous transluminal coronary angioplasty	2 (14%)		8 (64%)

CAD, Coronary artery disease.
[a]If not otherwise stated, values indicate number of patients (%).
[b]Values at initiation of antiretroviral therapy available for five patients. All patients had lipids assessed during antiretroviral therapy.
[c]One of these patients developed symptomatic peripheral vascular occlusive disease.

The median time on protease inhibitors was 8 months (range 1–28 months) (Table 1). Overall, 11 out of 14 patients experienced a deterioriation of coronary artery disease during antiretroviral therapy.

As we did not prospectively collect data on cardiovascular events before potent antiretroviral therapy became available in 1995, a causal relationship between treatment and the deterioration of coronary artery disease is not established by our case observations. Nevertheless, our findings of a definite deterioriation in 11 out of 14 patients with confirmed coronary artery disease while on potent antiretroviral therapy parallel to an unfavourable change in lipid profile remains a worrying observation. Hyperlipidaemia, which is associated with antiretroviral therapy, may help to explain the deteriorating course of coronary artery disease in these patients. A subgroup of patients with triglyceride levels of over 2.3 mmol/l and an LDL : HDL ratio of more than 5 : 1 have a significantly higher risk of cardiac events [15]. However, it remains unknown whether other pathogenetic co-factors might have influenced the course of coronary artery disease.

Since the introduction of triple-drug regimens in HIV patients at our clinic in 1995, we have cared for a total of 2423 different individuals on potent antiretroviral therapy followed prospectively within the Swiss HIV Cohort Study. As a result of the prolongation of life expectancy with antiretroviral therapy in HIV patients, the aggravation or induction of coronary artery disease may have a significant impact on life expectancy in this population. The effectiveness of primary and secondary prevention of coronary artery disease through the modification of cardiac risk factors is effective in the general population [16,17]. In patients with HIV and coronary risk factors undergoing antiretroviral therapy, pathological lipid profiles should be treated aggressively according to national guidelines, taking into consideration the possible interactions between lipid-lowering agents and protease inhibitors or non-nucleoside reverse transcriptase inhibitors, which are mainly metabolized in the cytochrome P450 3A4 enzyme system.

Controlled, prospective studies examining the incidence and the course of coronary artery disease, its association with antiretroviral therapy, and the effectiveness of primary and secondary prevention as well as the long-term success of coronary revascularization are needed.

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Carbamazepine toxicity after starting combination antiretroviral therapy including ritonavir and efavirenz

Drug–drug interactions complicate antiretroviral therapy. We report here a case of carbamazepine toxicity after starting an antiretroviral treatment regimen including ritonavir. A dose reduction from 600 to 100 mg was required to achieve a therapeutic carbamazepine concentration. Ritonavir blocks the metabolism of carbamazepine and can cause carbamazepine toxicity.

A 49-year-old woman was hospitalized with a one day history of worsening ataxia, resulting in two falls. She had a long history of HIV infection, with a recent increase in HIV RNA and a decrease in CD4 cell count. Four days before admission her antiretroviral therapy was changed from zidovudine, lamivudine, and indinavir to a regimen of ritonavir (400 mg twice a day), saquinavir (400 mg twice a day), and efavirenz (600 mg once a day at bedtime). Her past medical history was notable for a generalized seizure disorder as a result of a previous right thalamic infarction, successfully treated with carbamazepine (600 mg/day). The serum carbamazepine concentration just before the change in antiretroviral therapy was 6.9 μg/ml (therapeutic range 4.0–12.0 μg/ml).

Physical examination at the time of admission was notable for marked limb and truncal ataxia, resulting in an inability to walk, and stable mild right-sided weakness. A computed tomography scan of the head was normal, and laboratory tests were only notable for hyponatremia (serum sodium 125 meq/l) and a serum carbamazepine concentration of 20.4 μg/ml (see Fig. 1). With the discontinuation of carbamazepine, the ataxia improved. After 9 days, the carbamazepine was re-started at 300 mg per day. However, she developed mild ataxia and the serum carbamazepine concentration was high. Over the following 3 weeks the carbamazepine dose was progressively decreased. At a dose of 100 mg per day the patient was asymptomatic and had a therapeutic serum carbamazepine concentration.

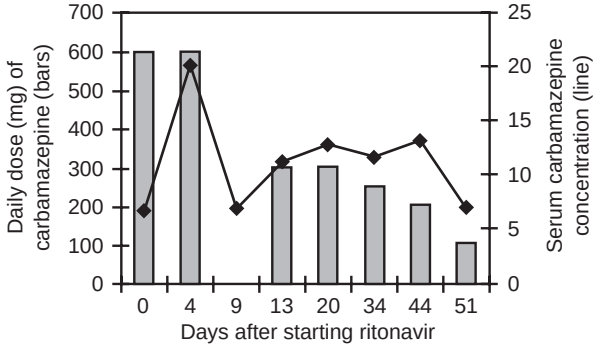

Fig. 1. The relationship between the initiation of a ritonavir-containing antiretroviral treatment regimen and serum concentrations of carbamazepine. The total daily dose of carbamazepine is shown as bars; the serum concentrations of carbamazepine are shown as a line.

The patient remained stable on this dose for approximately 2 weeks, when progressive abdominal pain and nausea led to the discontinuation of the salvage regimen. Her symptoms improved, but a repeat carbamazepine serum concentration was sub-therapeutic (3.4 μg/ml). The dose of carbamazepine was subsequently increased to the original dose of 600 mg per day, and a therapeutic serum concentration was obtained (6.3 μg/ml).

This case documents an important drug–drug interaction between antiretroviral therapy and carbamazepine, a commonly used anticonvulsant agent. The striking temporal relationships between the change in antiretroviral therapy and the onset of carbamazepine toxicity, and later the need to increase the dose of carbamazepine when this antiretroviral regimen was stopped, implicate the ritonavir-containing regimen as the cause of carbamazepine toxicity. Ritonavir is a very potent inhibitor of the cytochrome P450 3A4 isoform (CYP3A4) [1], the enzyme system primarily responsible for the metabolism of carbamazepine [2]. Saquinavir, as a mild CYP3A4 inhibitor [1], is unlikely to change the concentration of carbamazepine substantially, and efavirenz, a relatively potent 3A4-inducer [3], is likely to decrease carbamazepine concentrations. Therefore, the carbamazepine toxicity was almost certainly caused by ritonavir. The magnitude of this interaction was notable; an 83% reduction in carbamazepine dose (from 600 to 100 mg/day) was required to re-establish a therapeutic concentration.

This case makes two important points. The need to check for drug–drug interactions in HIV care has been well publicized [4]. Our pharmacy checks for drug–drug interactions using a computer program, the Drug–Drug Interaction Module (First DataBank, Inc., Indianapolis, IN, USA). The ritonavir–carbamazepine interaction was not included in this program because it had never previously been reported, although the interaction was suspected on the basis of knowledge of the metabolism of carbamazepine by CYP3A4 [1] and previous reports of CYP3A4 inhibitors causing carbamazepine toxicity [5,6]. Therefore, clinicians and pharmacists need to check for predicted, as well as reported, drug–drug interactions when starting or changing antiretroviral regimens, particularly those including ritonavir.

Second, this case is a reminder of the remarkable potency of ritonavir as a CYP3A4 inhibitor. We did not expect a marked increase in carbamazepine concentrations because the change in antiretroviral regimen involved changing from one CYP3A4 inhibitor (indinavir) to another (ritonavir) at the same time as a relatively potent CYP3A4 inducer (efavirenz) was added. Nevertheless, there was a fourfold increase in carbamazepine concentrations, confirming that ritonavir

is a much more potent CYP3A4 inhibitor of the metabolism of carbamazepine than indinavir [1], and that the CYP3A4 inhibition of ritonavir overcomes the effect of CYP3A4 inducers. It is also possible that the activity of ritonavir as an inhibitor of CYP2C8, an alternative metabolic pathway for carbamazepine [2], played a role in this interaction. Recent drug interaction studies have shown that ritonavir reverses the effects of CYP3A4 inducers such as efavirenz, rifabutin, and rifampin (the most potent CYP3A4 inducer in clinical use), on serum concentrations of other drugs [7–9].

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No drug–drug interaction between nelfinavir or indinavir and mefloquine in HIV-1-infected patients

Indinavir and nelfinavir are potent HIV protease inhibitors (PI). Both drugs are used in highly active antiretroviral combination therapy (HAART). Indinavir and nelfinavir are metabolized by the cytochrome P450 3A4 isoenzyme (CYP3A4). Furthermore, indinavir and nelfinavir inhibit CYP3A4. Mefloquine is a quinolinemethanol antimalarial drug used in chemoprophylaxis. Mefloquine has a long elimination half-life, approximately 3 weeks [1]. The major metabolite is carboxymefloquine. In-vitro data have shown that the formation of this metabolite can be inhibited by ketoconazol, a potent inhibitor of cytochrome P450 enzymes [2]. This finding suggests that cytochrome P450 plays an important role in mefloquine elimination. In theory, the concurrent use of mefloquine and PI might cause drug–drug interactions. In healthy volunteers, ritonavir, the most potent CYP3A4 inhibitor of all currently available PI, did not appear to inhibit mefloquine metabolism [3]. However, in HIV-1-infected patients data on this interaction are not available. To evaluate the effect of mefloquine on indinavir or nelfinavir or vice versa, we measured plasma concentrations of both drugs in HIV-1-infected patients who used the drugs concurrently.

Two HIV-1-infected patients using HAART for at least 6 months, containing indinavir 800 mg three times a day (patient 1) or nelfinavir 1250 mg twice a day (patient 2), took mefloquine (250 mg a week) as malaria prophylaxis when travelling to Africa. We measured the indinavir and nelfinavir plasma concentrations shortly before and indinavir, nelfinavir and mefloquine plasma concentrations during and after the use of mefloquine. In patient 1, we obtained random indinavir and trough mefloquine plasma samples (C_{min}), in patient 2 we obtained trough plasma samples (C_{min}) of both drugs (see Fig. 1). All PI plasma concentrations were measured as previously described [4]. To be able to compare the randomly drawn indinavir plasma concentrations, we determined so-called concentration ratios by dividing the actual indinavir concentration measured by the mean concentration found in a reference population at the same time-point after drug ingestion. Although all nelfinavir samples were trough levels, we also determined concentration ratios for these samples, comparing the concentration with a morning trough population level of 2.6 mg/l. Mefloquine elimination half-life was determined from the decay of the concentration in the mefloquine washout phase. In both patients, we found therapeutic plasma concentrations of mefloquine (> 500 μg/l). Toxic accumulation of the drug did not occur. Mefloquine elimination half-lives were approximately 15 and 27 days in the indinavir and nelfinavir patients, respectively, similar to that found in healthy individuals [1]. No side-effects were reported. We did not find a consistent increase or decrease in indinavir and nelfinavir plasma concentrations.

We concluded that the co-administration of mefloquine to HIV-1-infected patients on HAART,

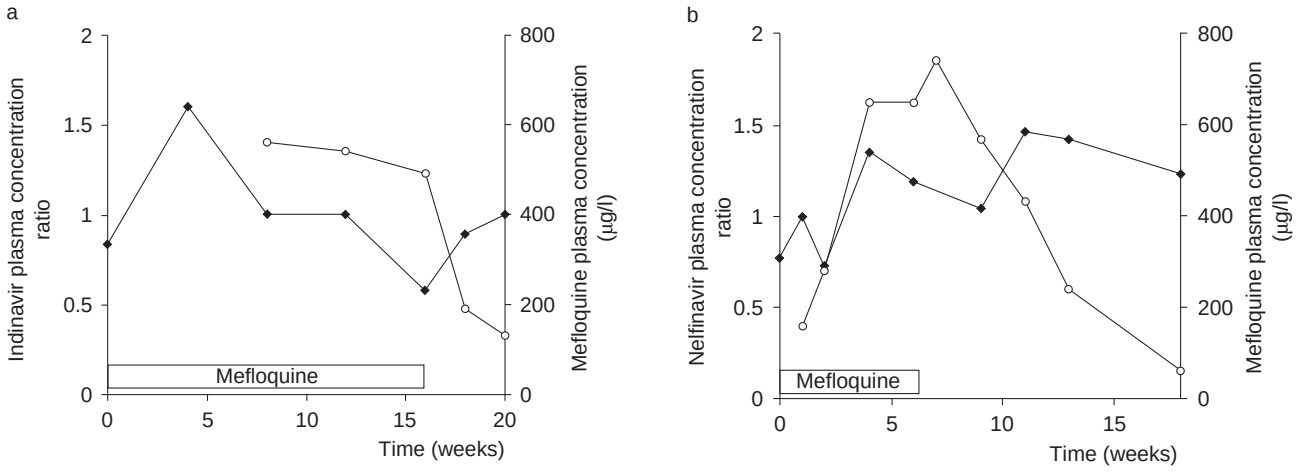

Fig. 1. Pharmacokinetic profiles of the protease inhibitors and mefloquine in two patients treated with a combination of these drugs. (a) Patient 1, indinavir (◆) 800 mg three times a day plus mefloquine (○) 250 mg once a week (weeks 1–16). (b) Patient 2, nelfinavir (◆) 1250 mg twice a day plus mefloquine (○) 250 mg once a week (weeks 1–6).

containing indinavir or nelfinavir, does not result in sub-therapeutic or toxic concentrations of the drugs.

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Switch from indinavir to ritonavir–indinavir regimen in patients treated with highly active antiretroviral therapy co-infected with hepatitis C is not associated with alteration of liver function tests

There is currently great interest in the combination of a small dose of ritonavir with other protease inhibitors, such as indinavir or saquinavir, in order to increase drug potency by improving trough plasma levels [1]. However, the use of ritonavir is associated with a higher risk of hepatotoxicity compared with other protease inhibitors [2,3], in particular in those HIV-1-infected patients co-infected with hepatitis C (HCV) [4–7]. This study includes 19 consecutive HIV-1-infected patients (17 men and two women) co-infected with HCV (n = 18) or hepatitis B (HBV) (n = 1), who have been treated for at least 6 months with indinavir combined with two nucleoside analogues. The protease inhibitor treatment of these patients was next modified through the addition of ritonavir 100 mg twice a day (n = 17) or 200 mg twice a day (n = 2) and a decrease in the indinavir dosage from 800 mg three times a day to 800 mg twice a day. Liver function tests were performed at baseline and after an average follow-up period of 179 days (range 67–313 days) after treatment modification. The median age of the study population was 40 years. HIV infection was acquired through intravenous drug use in 13 patients, homosexual contact in four patients and heterosexual contact in two patients. At the time of the study, 14 patients were included in a methadone substitution programme. The diagnosis of HCV infection was based on the presence of anti-HCV antibodies and that of chronic HBV infection was based on the detection of HBs antigen. Zidovudine plus lamivudine (n = 6) or stavudine plus lamivudine (n = 7) were the most frequent nucleoside analogue combinations. All patients were naive for ritonavir. Before the addition of ritonavir, the baseline median plasma viraemia and CD4 cell counts were

1.84 $\log_{10}$ RNA copies/ml (range 1.0–5.33) and 439 CD4 T cells/mm^3 (range 39–945). After the 179 day follow-up period, viraemia (median 2.53 $\log_{10}$ RNA copies/ml, range 1.0–5.2) and CD4 T cell counts (median 317 cell/mm^3, range 40–976) were in the same range.

The median baseline values for aspartate transaminase (AST) and alanine transaminase (ALT) were 55 U/l (range 15–350) and 51 U/l (range 6–391), respectively (Fig. 1). Liver toxicity was recorded on an individual basis according to the ratio of follow-up values over baseline values for AST and ALT. The median of the ratios was 0.91 for AST (range 0.09–2.11) and 1.40 for ALT (range 0.06–2.74). In one patient, there was a 2.1- and 2.7-fold increase in AST and ALT values, respectively. There was no case of discontinuation of medication.

Long-term treatment with indinavir has been associated with the impairment of renal function, as observed in 18.6% of patients having a 20% increase in creatinine levels, nephrolithiasis and hyperbilirubinemia [8,9]. In our patients, we monitored the renal function and total bilirubin levels from the onset of treatment with indinavir at the dosage of 800 mg three times a day. The average treatment duration with indinavir was 931 days (range 378–1360). Before the initiation of indinavir treatment, median values for creatinine were 76 μmol/l (range 58–103) and reached 83 μmol/l (range 59–143) after 931 days. Two patients had a gradual 1.6- and twofold increase in creatinine levels during the total duration of indinavir treatment from 89 to 143 μmol/l and from 68 to 141 μmmol/l, respectively. During the period of treatment with the indinavir–ritonavir-based regimen, three patients experienced one episode of nephrolithiasis that resolved with increased fluid intake. This was accompanied in one patient by a slight increase in creatinine levels from 112 to 143 μmol/l. There was no change in the bilirubin levels during the whole period of indinavir treatment. The median baseline total bilirubin levels were 24 μmol/l (range 8–94) before the onset of indinavir-based highly active antiretroviral therapy and 23 μmol/l (range 8–112) at the end of the study period.

In HIV-infected patients co-infected with HCV treated with indinavir and nucleoside analogues for a median of 32 months, a switch from indinavir three times a day

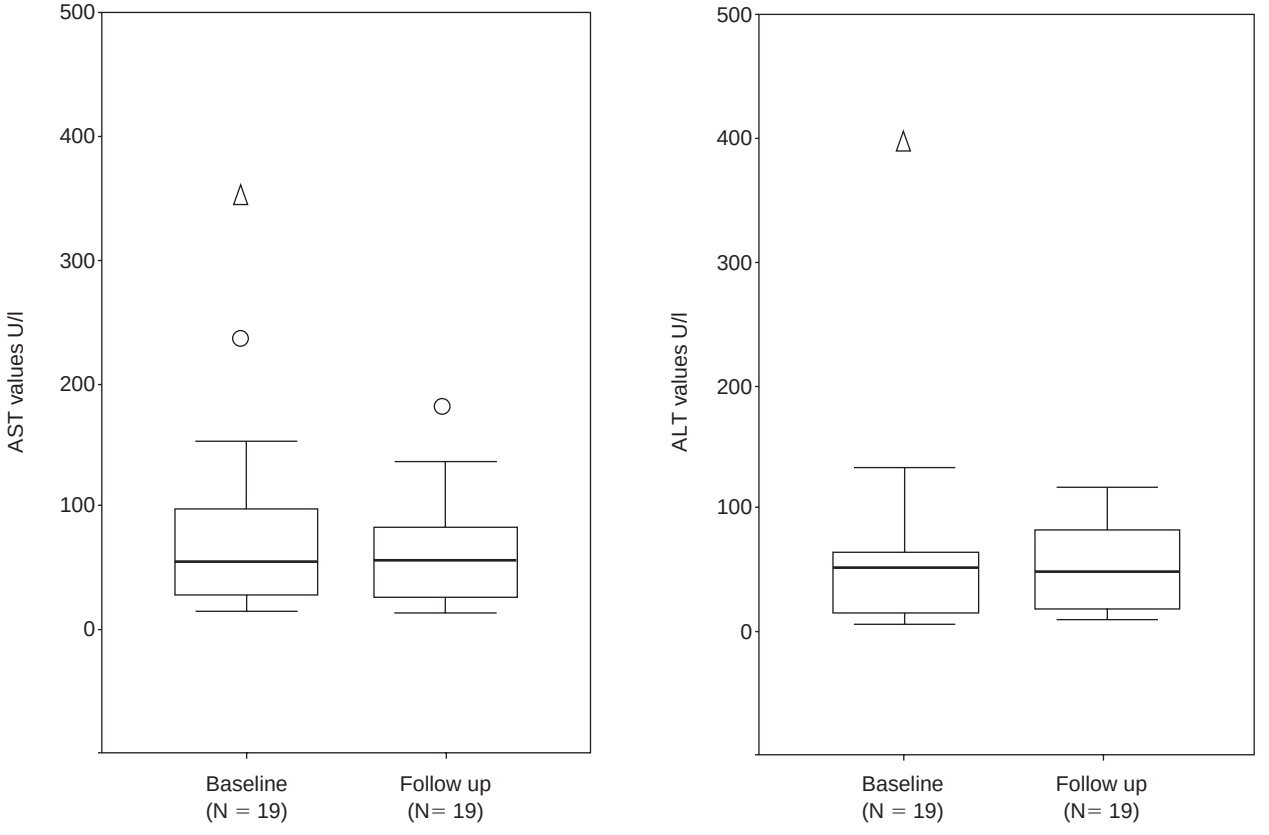

Fig. 1. Liver function tests at baseline and a median of 179 days after switch from a triple-drug regimen containing indinavir three times a day to indinavir twice a day with low dosage ritonavir. Normal values for aspartate transaminase (AST) and alanine transaminase (ALT) were 10–34 and 10–44 U/l, respectively.

to indinavir twice a day in association with a low dose of ritonavir does not result in the impairment of liver function tests during a follow-up of approximately 6 months.

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Selective expansion of a minor proportion of drug-resistant HIV-1 by antiretroviral pressure *in vitro*

The detection of drug-resistant HIV-1 strains by currently available genotypic and phenotypic methods is limited to those that comprise 10–20% of the viral swarm [1]. Drug-resistance mutations generally impart a replication fitness disadvantage (compared with wild-type strains), thereby reducing the ability to isolate such species. Drug-resistant HIV-1 may also be compartmentalized *in vivo*, further complicating resistance testing. Here, we report a modified culture system for the selective expansion of drug-resistant HIV-1, and show that this approach can resolve the presence of minor resistant species for both genotypic and phenotypic determinations.

To verify that minor drug-resistant HIV-1 species can emerge in culture when given a selective advantage, we obtained drug-sensitive and -resistant virus stocks via the National Institutes of Health AIDS Research and Reference Reagent Program. Drug-sensitive (wild-type) HIV-1 stock was mixed at varying ratios with virus resistant to either zidovudine or lamivudine and used to infect phytohemaglutinin-stimulated CD4 T cells. These infected cells were then cultured under the presence or absence of the appropriate selective drug pressure during the following 14 days. Cultures initiated with as little as 1% of virions (based on p24 antigen) resistant for either zidovudine or lamivudine showed the emergence of HIV-1 in the presence of appropriate drug treatment (Fig. 1). Importantly, no evidence of virus expansion was observed in cultures of pure wild-type HIV-1, indicating that these culture conditions did not induce the emergence of drug-resistant virus.

To examine the detection of resistant variants before and after culture under antiretroviral drug pressure, we performed genotypic analysis by chip hybridization-based methods (Affymetrix, Inc., Santa Clara, CA, USA) [2] on the viral mixtures from a second experiment involving lamivudine-resistant virus. Although not apparent in the viral mixture before culture, genotypic analysis confirmed the appropriate mutation in the reverse transcriptase region (M184V) of the HIV-1 isolate expanded from the lamivudine-treated culture, even when initiated with only 0.2% (v/v) of drug-resistant virus.

Therefore, the use of antiretroviral pressure during in-vitro culture may overcome the limitations of conventional genotypic and phenotypic assays to detect minor resistant variants. This approach may be of clinical value in determining a proper therapeutic regimen, especially when considering effective salvage therapy. This relatively simple approach is not limited to any particular group of compounds, and can be used in conjunction with the culturing of patient cells to derive a viral isolate for subsequent testing. Furthermore, the approach could be linked with current recombinant

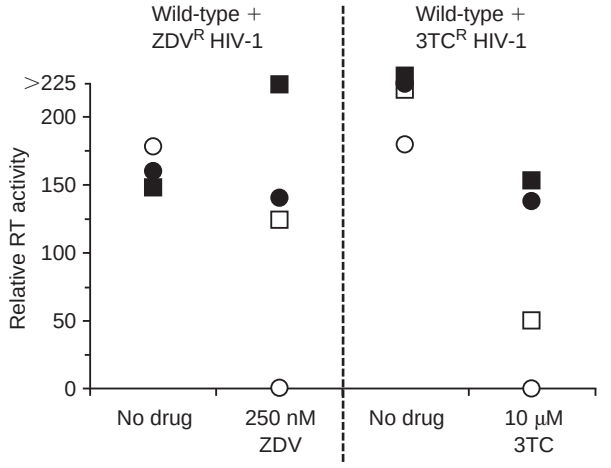

Fig. 1. Proof of concept experiment using known drug-resistant HIV-1 isolates mixed at various ratios with wild-type HIV-1. Zidovudine-resistant (ZDV^R) or lamivudine-resistant (3TC^R) virus stocks were mixed at 1% (□), 10% (■), and 50% (●) with wild-type HIV-1 stock (○), and the mixtures were used to infect CD4 T lymphocytes. Data are reported as relative reverse transcriptase (RT) activity using a standard ^{32}P-incorporation assay (quantitated by phosphoimaging).

phenotypic assays during the expansion of recombinant viruses.

To document the transmission of drug-resistant HIV-1 strains and accurately estimate the prevalence of individuals who harbor such strains, the presence of minor viral species must be considered. Therefore, we have used this approach to characterize archival or compartmentalized drug-resistant virus in drug-naive individuals. Peripheral blood mononuclear cells were obtained from 17 drug-naive, newly infected individuals for whom no conclusive genotypic evidence of resistance was obtained [3]. During a 21 day period, patients' cells were CD8-depleted, stimulated, and co-cultured with phytohemaglutinin-stimulated CD4 T cells (added every 7 days) in the presence and absence of zidovudine or lamivudine.

Among the 17 newly infected individuals tested, viral isolates from 11 were derived under standard isolation and expansion conditions. Interestingly, when drug pressure was used during isolation, the emergence of zidovudine-resistant HIV-1 was observed from the mononuclear cell cultures of five individuals. Furthermore, in three individuals zidovudine-resistant HIV-1 was compartmentalized to either the T cell or monocyte subset when these cells were cultured independently. No lamivudine-resistant isolates were observed.

Phenotypic analysis of HIV-1 isolated from cellular compartments may not reflect the viral population of plasma and may represent archived provirus from the original transmission event. Therefore, identifying differing drug-resistant viral strains among peripheral blood mononuclear cell subsets may lead to a better understanding of the transmission and pathogenesis of drug-resistant HIV-1 mutants.

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Recent increase in diagnoses of HIV infections based on surveillance system data in Belgium

Since 1997, and for the first time in 8 years, an increase in the number of newly diagnosed HIV infections was observed in Belgium. In reference to the 1997 baseline, there was a 9 and 15% increase in the number of new diagnosed cases for 1998 and 1999, respectively (chi square for linear trend, $P < 0.01$). As shown in Fig. 1, this rising trend followed a steady and significant decline in new cases (−29%) observed between 1992 and 1997 ($P < 0.001$). On the other hand, the number of tests performed nation-wide decreased significantly by 17% between 1996 and 1999 ($P < 0.001$). Fifty-one screening tests per 1000 individuals were performed in 1999 versus 61 in 1996.

Nationally, all serums with a positive screening test result are submitted for confirmation to one of the eight AIDS Reference Laboratories [1,2]. Data from all confirmed HIV infections have been included in an

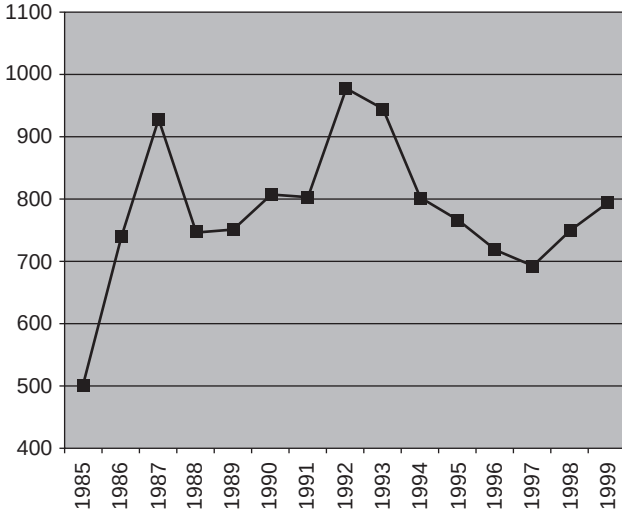

Fig. 1. Number of new HIV diagnoses per year in Belgium, 1985–1999.

HIV database since 1985. HIV and AIDS databases were linked in 1990. HIV and AIDS data are validated for duplicate recording.

The HIV testing volume corresponds to the number of tests reimbursed by the social security system, Institut National d'Assurance Maladie-Invalidité (INAMI). It excludes blood donation-related tests. Free anonymous tests, which correspond to approximately 0.6% of the screening tests performed, were not included in this analysis.

The major part of the recent increase in HIV diagnoses was attributable to female cases. The proportion of female patients has been rising significantly since 1997 (41.1, 44.7 and 48.0% in 1997, 1998, and 1999, respectively; chi square for linear trend, $P < 0.01$).

In 1999, heterosexual transmission remained the first reported mode of infection (62.6%), followed by homo/bisexual transmission (25.8%); only 3.4% of patients were infected by intravenous drug use.

A shift towards older age groups was observed over time. In 1999, the age group 30–34 years was most affected, followed by the groups 35–39 and 25–29 years. In the late 1980s, the age group 25–29 years was most affected, followed by the groups 30–34 and 35–39 years.

Migration may have contributed to the recent increase in HIV infections. The proportion of patients of other nationalities increased significantly during the past 5 years (63.9% in 1998–1999 versus 58.1% in 1995–1996; chi square for linear trend, $P < 0.05$).

No modifications had occurred in the HIV/AIDS surveillance system, which remained remarkably stable over time, to explain the recent trend reversal.

Although the 1997–1999 15% increase was moderate in absolute value (plus 103 new cases), it may initiate a second increasing phase in the evolution of the epidemic. Actually, the 1987 peak (see Fig. 1) may be considered as an artefact related to a catching up phase caused by the commercialization of the HIV antibody test in 1985. As for the 1992–1993 peak, it was characterized by a significantly high proportion of patients from migrant populations (68.3% in 1992–1993 versus 53.9% in 1990–1991; chi square for linear trend, $P < 0.001$).

Different factors may influence the observed HIV curve, including the intrinsic dynamic of the epidemic, information and prevention campaigns, migration [3], attitudes toward HIV testing. However, the respective impacts of each factor can not be easily measured or predicted, and the HIV curve represents the balance between those changing factors.

In recent years, decreasing political interest has been shown towards HIV/AIDS, expressing itself in fewer large-scale prevention campaigns. This factor, along with the announcement of the arrival of highly active antiretroviral therapies in 1996 [4], may have led to less concern or protective behaviours in the population. The recent drop in testing volume may corroborate this behaviour change.

Our observations confirm the importance of an exhaustive HIV surveillance system to monitor an epidemic that may still present unforseeable changes in the near future. It also underlines the need for continuously renewed and adapted health policies and strategies towards HIV/AIDS prevention.

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