Clinical aspects of toxicity in antiretroviral therapy

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Clinical aspects of toxicity in antiretroviral therapy

Klinische aspecten van toxiciteit bij antiretrovirale therapie

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resistance, increase the risk of cardiovascular disease [13-16]
As atherosclerosis increases with age, HIV-patients on chronic treatment will have the same risks as HIV-uninfected patients. Since HIV itself and ART also increase this risk, the overall rise of cardiovascular diseases is much higher than in the normal population. Dyslipidaemia to levels associated with increased cardiovascular disease occur in even up to 70% of HIV-infected patients receiving ART. The prevalence of lipodystrophy varies widely and has been estimated at 7-84% among adult patients and depends on the criteria to define this syndrome. The HIV Lipodystrophy Case Definition Study Group evaluated a model to diagnose lipodystrophy; including a score system for age, sex, duration of HIV-infection, HIV-disease stage, waist:hip ratio, anion gap, serum HDL-cholesterol concentration, trunk:peripheral fat ratio, percentage of leg fat and intra-abdominal:extra-abdominal fat ratio. Application of this score resulted in a sensitivity of 79% and a specificity of 80% [17].
Toxicity is a major problem since it does affect compliance and resistance. Patients experiencing side effects find it difficult being motivated to continue their medication. They might interrupt their treatment which might induce resistance of the virus. In case of serious side effects their physicians will decide to stop ART and change to a better tolerated regimen if possible. In some cases the addition of another drug can solve the problem, for instance lipid lowering drugs in hyperlipidaemia of loperamide in case of diarrhoea.

The following factors play a role in the development of toxicity: pharmaco-dynamic and kinetic parameters of the drugs and genetic parameters of the patients.

Mitochondrial impairment seen in NRTI use is due to a specific pharmaco-dynamic effect of this class of drugs. Selected side effects for NRTIs are neuropathy, myopathy, pancreatitis, anaemia, hepatitis and lactic acidosis. The origin of these adverse events lies partly in the potential of NRTIs to interfere with DNA polymerase γ, an enzyme essential for mitochondrial DNA (mtDNA) strands, resulting in impaired mitochondrial function. In this way NRTI incorporation leads to chain termination. However, the mechanism for mitochondrial toxicity includes also direct inhibition of mtDNA polymerase γ without chain termination, persistence of the incorporated analogues in mtDNA because of inefficient excision resulting in a defective mtDNA template, or combination of these mechanisms. This so-called mitochondrial toxicity is a specific effect of NRTIs as a class. It should be noted that some side effects, which occur by example in stavudine, do not occur in other NRTIs [18].

Kinetic parameters play a role in side effects when toxicity is related to plasma concentrations of drugs. Especially the PI group has been known for its wide inter-subject and intra-subject variation resulting in a wide variability of plasma concentrations. Therefore, therapeutic drug monitoring is advised in these drugs both for prevention of drug levels below the therapeutic range as for prevention of toxic drug levels [19]. Previous studies have demonstrated a relationship between drug exposure and efficacy and toxicity. Nowadays, measurement of plasma drug concentrations is applied for non nucleoside reverse transcriptase inhibitors (NNRTIs) and PIs. It is frequently stated that plasma NRTI levels do not correlate

Outline of the thesis

Despite the progress in the insight of HIV-infection itself and the development of a series of drugs to suppress replication of the humane immunodeficiency virus (HIV), HIV-infection remains a challenging therapeutic problem.
Highly active antiretroviral therapy (HAART) consists of a combination of at least three drugs from two different classes of antiretroviral drugs [1]. By this approach HIV-replication can be suppressed for long time and HIV-infection has turned into a more chronic disease. However, the other side of the picture is the occurrence of serious adverse effects, which is an important problem for physicians treating HIV-patients.
Antiretroviral therapy (ART) has been associated with considerable adverse events (AEs). Incidences vary, but laboratory adverse events have been reported in up to 25% and clinical AEs in even up to 45% of patients [2-4]. One cohort with a median follow up of 6 years showed that during this period laboratory adverse events were associated with a significant higher mortality [5]. In contrast, for clinical AEs no significant association with increased mortality was found. Physicians seem to change treatments to relieve clinical symptoms, while accepting laboratory AEs. However, in this cohort the burden of laboratory AEs to antiretroviral drugs was associated with a higher mortality. In 2007 the monitoring of HIV-infection in the Netherlands showed that older age and lower weight at the start of ART were associated with a shorter time to develop toxicity [6]. Most frequently reported side effects in this report were nausea (18%), anaemia (12%), rash (10%), diarrhoea (10%) and vomiting (10%).
Adverse events seen shortly after the initiation of ART are gastrointestinal problems, such as nausea, vomiting and diarrhoea, which can be seen in all ART drug classes, although diarrhoea has been mainly associated with protease inhibitor (PI) use. Furthermore, hypersensitivity reactions with rash and fever are seen. This is especially known from amprenavir, abacavir and nevirapine, with incidence rates of 28%, 7-16% and 5% respectively [3;7]. It is an important side effect since fatal events have been reported with rechallenge of abacavir. If rash occurs medication has to be discontinued.
There are several important long term side effects which might hamper continuation of ART. The first is mitochondrial impairment seen in nucleoside reverse transcriptase inhibitor (NRTI) use. The second problem is the risk of cardiovascular disease due to metabolic complications of protease inhibitors [8;9]. These metabolic changes with lipid alterations and impaired glucose tolerance with insulin resistance can be seen with or without the so-called lipodystrophy syndrome [10;11]. Hyperglycaemia due to frank diabetes is only seen in a small proportion of patients (1-6%) [2]. This lipodystrophy syndrome or best described as lipohypertrophy/ lipoatrophy syndrome is a demanding problem in HIV-treatment. The lipohypertrophy is mainly attributed to PI use, while the lipoatrophy is mainly attributed to NRTI use; especially stavudine [12]. This syndrome consists of body shape changes with peripheral fat wasting and central fat accumulation. This syndrome is well known among patients and feared for its stigmatising appearance. Dyslipidaemia with hypertriglyceridaemia, decreased high density lipoprotein (HDL) and increased low density lipoprotein (LDL) and total cholesterol together with insulin

with the intracellular active NRTI-triphosphate metabolite and should therefore not be used.
Finally, genetic factors are of great significance in the occurrence of side effects. Genetic constitution is known to make patients more prone for adverse events. One example is the hypersensitivity reaction with (severe) rash in nevirapine and abacavir. For abacavir it has been revealed that there is a relationship between presence of the HLA B5701 allele and hypersensitivity reactions. Currently it is even recommended to test for this HLA type before initiating abacavir therapy. Furthermore, the HLA-DRB101 allele plays an important role in susceptibility to cutaneous reactions associated with nevirapine and efavirenz [20-22].

Diagnosis and early recognition of adverse events is of great importance to protect patients from additional medical problems and avoid serious or even fatal complications.
The aim of this thesis is to consider clinical aspects of toxicity in ART by investigating correlations between patient's blood chemistry (laboratory values), plasma drug concentrations and side effects. The thesis focuses on adverse events related to NRTI-induced mitochondrial toxicity, the stavudine-related lipoatrophy syndrome, and the PI-associated metabolic syndrome with lipid alterations and impaired glucose tolerance.

Section 1

In **chapter 1** we present an overview of adverse events in antiretroviral therapy focusing on metabolic complications and mitochondrial toxicity, its underlying mechanisms and possible therapeutic interventions.
Dyslipidaemia in HIV is revealed to be multifactorial. Factors that play a role in affecting lipid levels include HIV-infection itself, treatment with antiretrovirals (especially PIs and stavudine), the effect of changes in body composition and possible genetic predisposition to dyslipidaemia. These atherogenic effects have their basis on molecular level, were NRTIs interfere with mitochondrial RNA transcription leading to mitochondrial DNA depletion and PIs interfere with cellular transcription factors such as SREBP-1c (sterol regulatory element binding protein 1c) essential in adipose tissue function.

Section 2

In this section laboratory parameters to detect mitochondrial toxicity are studied. The initial interest in the subject of this thesis developed after experience with a fatal case of lactic acidosis in our own hospital, which was due to a serious side effect of stavudine. Our curiosity for the incidence and mechanism further increased after collecting more of these cases, which are presented in **chapter 2**. The use of the NRTI stavudine was recognised as risk factor for this fatal lactic acidosis. We addressed the question to what extent hyperlactataemia plays a role in the development of adverse events. Lactate levels were collected in a series of patients of our outpatient department. Serum lactate and pyruvate levels were compared between different groups consisting of HIV-patients with mitochondrial toxicity, HIV-patients on antiretroviral therapy without toxic effects, HIV-patients without antiretroviral treatment and in healthy volunteers.

The analysis is outlined in **chapter 3**.
In paediatric medicine application of an oral glucose loading test (OGTT) had proven to be useful to diagnose patients with occult hyperlactataemia due to mitochondrial disorders. We wondered whether this method would be useful in daily clinical practice for detection of toxicity in HIV-treated patients. Therefore, an OGTT with measurement of serum lactates was performed in patients on NRTI therapy with and without NRTI-related mitochondrial toxicity. The predictive value of an OGTT for detection of NRTI-related mitochondrial toxicity is evaluated in **chapter 4**.

Section 3

In this section special attention is paid to serum lipids and glucose alterations after initiation of ART with a PI. PIs are known to interfere with lipid and glucose metabolism resulting in insulin resistance with hyperglycaemia, hypertriglyceridaemia, elevated total and LDL-cholesterol and often reduced HDL-cholesterol concentrations. Even after short-term therapy with antiretroviral drugs, these metabolic changes have been observed.
In **chapter 5**, we explore whether antiretroviral drugs influence glucose- and lipid- metabolism in therapy-naive HIV-infected patients. We investigated serum lipid parameters, glucose, insulin, highly sensitive C-reactive protein and leptin concentrations during 72 weeks of antiretroviral therapy. Data were collected within the so called FREE study: a randomised, open label, multicentre strategic study to evaluate the efficacy and toxicity of an early switch from a PI-containing regimen (ritonavir boosted lopinavir) to trizivir® (combination drug of zidovudine, lamivudine and abacavir) on guidance of viral load in HIV-1 infected, antiretroviral therapy-naive adults.

Section 4

This section looks at pharmacokinetics more closely and investigated if adverse events are related to drug exposure and thereby plasma drug concentrations. Since lipoatrophy has been associated with stavudine use, we wanted to test the hypothesis that patients with lipoatrophy are the ones with the highest stavudine plasma levels. In **chapter 6** stavudine plasma concentrations are analysed in patients with and without lipoatrophy on a stavudine containing regimen for at least 12 months.
In literature few data are available describing the influence of PI plasma concentrations on lipid changes. Studies in HIV-patients on antiretroviral therapy have elucidated that there is a wide inter-patient variability when measuring drug exposure to a standard dose of lopinavir. Lopinavir is known to cause hyperlipidaemia. Grade 3 hypercholesterolaemia and hypertriglyceridaemia have even been reported in 10% and up to 40% of antiretroviral therapy-naive and previous PI-experienced patients respectively. In **chapter 7** plasma lipids and lopinavir plasma concentrations were measured in a prospective study (FREE study), treating therapy-naive patients initially with ritonavir boosted lopinavir. By correlating lipid levels with lopinavir plasma concentrations we wanted to investigate if higher plasma drug concentrations are related to higher plasma lipid levels.
In the **general discussion and summary** the results of the above studies and future perspectives are provided.

References

1 Yeni PG, Hammer SM, Hirsch MS, Saag MS, Schechter M, Carpenter CC, Fischl MA, Gatell JM, Gazzard BG, Jacobsen DM, Katzenstein DA, Montaner JS, Richman DD, Schooley RT, Thompson MA, Vella S, Volberding PA. Treatment for adult HIV-infection: 2004 recommendations of the International AIDS Society-USA Panel. **JAMA** 2004; 292:251-65.

2 Aberg JA, Gallant JE, Anderson J, Oleske JM, Libman H, Currier JS, Stone VE, Kaplan JE. Primary care guidelines for the management of persons infected with human immunodeficiency virus: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. **Clin Infect Dis** 2004; 39:609-29.

3 Montessori V, Press N, Harris M, Akagi L, Montaner JS. Adverse effects of antiretroviral therapy for HIV-infection. **CMAJ** 2004; 170:229-38.

4 Carr A, Cooper DA. Adverse effects of antiretroviral therapy. **Lancet** 2000; 356:1423-30.

5 Keiser O, Fellay J, Opravil M, Hirsch HH, Hirschel B, Bernasconi E, Vernazza PL, Rickenbach M, Telenti A, Furrer H. Adverse events to antiretrovirals in the Swiss HIV Cohort Study: effect on mortality and treatment modification. **Antivir Ther** 2007; 12:1157-64.

6 Annual report HIV monitoring foundation Academic Hospital Medical Center University of Amsterdam 2007.

7 Adkins JC, Faulds D. Amprenavir. **Drugs** 1998; 55:837-42.

8 d'Amati G, di Gioia CR, Gallo P. Pathological findings of HIV-associated cardiovascular disease. **Ann N Y Acad Sci** 2001; 946:23-45.

9 Periard D, Cavassini M, Taffe P, Chevalley M, Senn L, Chapuis-Taillard C, de VS, Hayoz D, Tarr PE. High prevalence of peripheral arterial disease in HIV-infected persons. **Clin Infect Dis** 2008; 46:761-67.

10 Wohl DA, McComsey G, Tebas P, Brown TT, Glesby MJ, Reeds D, Shikuma C, Mulligan K, Dube M, Wininger D, Huang J, Revuelta M, Currier J, Swindells S, Fichtenbaum C, Basar M, Tungsiripat M, Meyer W, Weihe J, Wanke C. Current concepts in the diagnosis and management of metabolic complications of HIV-infection and its therapy. **Clin Infect Dis** 2006; 43:645-53.

11 Mallon PW. Pathogenesis of lipodystrophy and lipid abnormalities in patients taking antiretroviral therapy. **AIDS Rev** 2007; 9:3-15.

12 Milinkovic A, Martinez E. Current perspectives on HIV-associated lipodystrophy syndrome. **J Antimicrob Chemother** 2005; 56:6-9.

13 Calza L, Manfredi R, Pocaterra D, Chiodo F. Risk of premature atherosclerosis and ischemic heart disease associated with HIV-infection and antiretroviral therapy. **J Infect** 2008; 57:16-32.

14 Oh J, Hegele RA. HIV-associated dyslipidaemia: pathogenesis and treatment. **Lancet Infect Dis** 2007; 7:787-96.

15 Samaras K. Metabolic consequences and therapeutic options in highly active antiretroviral therapy in human immunodeficiency virus-1 infection. **J Antimicrob Chemother** 2008; 61:238-45.

16 Carpentier A, Patterson BW, Uffelman KD, Salit I, Lewis GF. Mechanism of highly active antiretroviral therapy-induced hyperlipidemia in HIV-infected individuals. **Atherosclerosis** 2005; 178:165-72.

17 Carr A, Emery S, Law M, Puls R, Lundgren JD, Powderly WG. An objective case definition of lipodystrophy in HIV-infected adults: a case-control study. **Lancet** 2003; 361:726-35.

18 Kohler JJ, Lewis W. A brief overview of mechanisms of mitochondrial toxicity from NRTIs. **Environ Mol Mutagen** 2007; 48:166-72.
19 Burger DM, Aarnoutse RE, Hugen PW. Pros and cons of therapeutic drug monitoring of antiretroviral agents. **Curr Opin Infect Dis** 2002; 15:17-22.
20 Vitezica ZG, Milpied B, Lonjou C, Borot N, Ledger TN, Lefebvre A, Hovnanian A. HLA-DRB1*01 associated with cutaneous hypersensitivity induced by nevirapine and efavirenz. **AIDS** 2008; 22:540-541.
21 Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, Jagel-Guedes E, Rugina S, Kozyrev O, Cid JF, Hay P, Nolan D, Hughes S, Hughes A, Ryan S, Fitch N, Thorborn D, Benbow A. HLA-B*5701 screening for hypersensitivity to abacavir. **N Engl J Med** 2008; 358:568-79.
22 Stekler J, Maenza J, Stevens C, Holte S, Malhotra U, McElrath MJ, Corey L, Collier AC. Abacavir hypersensitivity reaction in primary HIV-infection. **AIDS** 2006; 20:1269-74.

Section

General introduction

Chapter 1

Antiretroviral therapy in HIV-patients: Aspects of metabolic and mitochondrial toxicity

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Peter P. Koopmans

The Netherlands Journal of Medicine 2003; 61:393-403.

Abstract

In the last decade the use of antiretroviral therapy has lead to dramatic improvement in the prognosis of HIV-infection. However, in the long term the use of combination antiretroviral therapy is frequently associated with side effects. Two major problems concerning adverse events are redistribution of body fat (the syndrome of lipoatrophy and/or lipodystrophy) and toxicity related to mitochondrial damage. In a large proportion of patients the fat redistribution is accompanied by development of insulin resistance and elevated plasma lipid concentrations. This review will focus on the possible pathogenesis and the clinical consequences of these side effects.

Introduction

Since the introduction of highly active antiretroviral therapy (HAART) in HIV-infected patients, morbidity and mortality due to HIV-infection have been dramatically decreased [1]. Although resistance to antiretroviral therapy is an important issue, toxicity is becoming a large problem. In the ATHENA cohort the major reason to switch antiretroviral therapy is toxicity, this is 44-58% in patients on their first regimen and 56% on subsequent regimens [2]. Since HIV cannot be cured, chronic therapy is needed to suppress HIV-replication, which increases the risk of adverse events. The benefits of HAART have led to a great number of HIV-patients receiving antiretroviral therapy [3-5]. One of the major problems at the moment is change in body composition due to fat redistribution; the syndrome of lipoatrophy and/or lipodystrophy [6;7].

In this review we will discuss two major groups of antiretroviral therapy-related toxicity: the lipodystrophy-lipoatrophy syndrome with metabolic changes and mitochondrial toxicity. Another important group of antiretroviral therapy-related toxicity is (severe) hypersensitivity with systemic reactions; this will not be discussed here.

Pathogenesis and predisposing factors

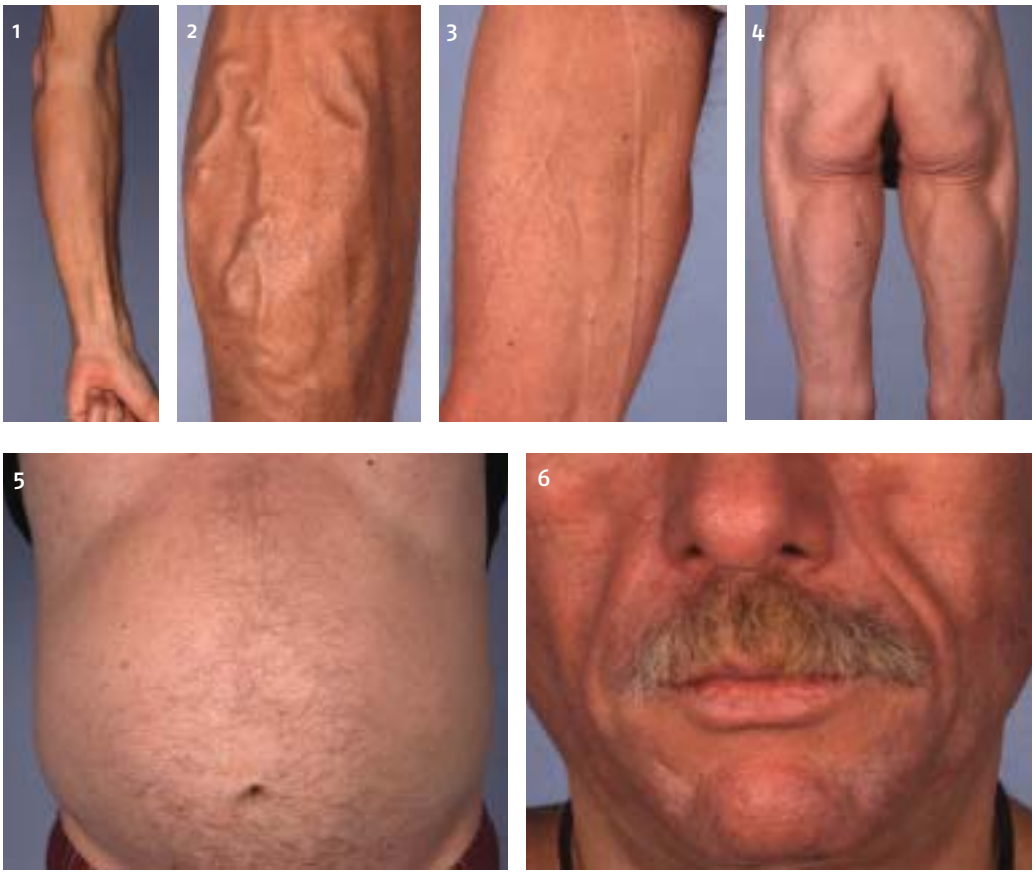
Lipodystrophy, lipoatrophy and metabolic disturbances

One of the most expanding problems, which have been underestimated in the beginning of HAART introduction, is discussed here. Exposure to antiretroviral drugs has been associated with the development of significant metabolic adverse effects such as hyperlipidaemia, hyperglycaemia and insulin resistance with diabetes mellitus, peripheral fat wasting (lipoatrophy) and central adiposity (lipohypertrophy). Fat loss on the extremities, buttocks and in the face together with localised deposits of fat, particularly in the abdomen, breasts or neck region (Buffalo hump), is very stigmatising for patients on HAART *(picture 1-6)* [8-13].

The prevalence of lipodystrophy varies widely and has been estimated at 7-84% among adult patients. A prospective cohort study from 1996 till 1999 found the following incidences: any lipodystrophy 11.7, lipodystrophy with subcutaneous lipoatrophy 9.2 and lipodystrophy with central obesity 7.7 per 100 patient years. Data from the Dutch ATHENA cohort including 1952 patients, demonstrated 261 patients who developed lipodystrophy/lipoatrophy. The incidence rate was 6.2 per 100 person years with a four-year cumulative incidence of 25% [14;15]. However, most lipodystrophy studies included HIV-positive Caucasian men. The incidence of lipodystrophy in other than Caucasian subjects is not well studied. One study showed that lipodystrophy was only seen in 3.5% of a cohort of Koreans [16].

The authors of the LIPOCO study, evaluating fat distribution by computed tomography and metabolic changes in patients on antiretroviral therapy, suggested that there are three major types of fat distribution. First of all the lipoatrophy syndrome (fat depletion), which may be related to stavudine use, second a mixed or fat redistribution syndrome related to an unusual side-product of effective virus-control, and finally a subcutaneous adiposity syndrome reflecting increase in

Picture 1–6 Patients with lipodystrophy.



1 Peripheral fat loss arm (lipoatrophy)
2–3 Peripheral fat loss legs (lipoatrophy)
4 Peripheral fat loss buttocks (lipoatrophy)
5 Central fat accumulation (lipohypertrophy)
6 Facial fat wasting (lipoatrophy)

calorie intake (often due to diet prescriptions when using some types of PIs) [10]. Recent reports highlight the fact that lipodystrophy, mainly the lipoatrophy component, is primarily linked to NRTI therapy, while dyslipidaemia and insulin resistance are more readily associated with PI therapy. Although risk factors for this syndrome are not exactly known, the following factors have been suggested to play a role: low body weight before start of HAART, elevation of C-peptide and fasting triglyceride concentrations early in therapy, female gender, age >40 years, baseline viral load >100.000 copies/ml, white ethnicity, duration of HAART and the use of a HAART regimen containing stavudine and combinations of PIs (especially saquinavir and ritonavir) [10;14;17;18]. Lipodystrophy occurs frequently in regimens including NRTIs and is rare in NRTI sparing regimens. Several studies confirmed the observation that not only PIs but also the use of NRTIs contribute to

the development of lipoatrophy/lipodystrophy, especially when hyperlactataemia occurs, which is often seen in patients on stavudine [6;9;10;19;20]. The risk to develop lipodystrophy was assessed in a study with 158 HIV-infected patients of whom 113 received a PI containing regimen and 45 were never treated with a PI. Predictors of subsequent lipodystrophy severity included weight before PI therapy, duration of therapy and fasting triglyceride and C-peptide concentrations on therapy. Lipodystrophy was very common and even progressive in most cases, after two years of HAART with a PI [17]. In contrast to a prospective cohort study following almost 500 patients for 18 months, in which the risk factors for developing lipoatrophy/dystrophy were multifactorial and overlapping and could not be ascribed to the duration of exposure to a particular antiretroviral drug [14].

Adipose tissue changes

Different aspects of adipose tissue disturbances have been postulated in the development of lipodystrophy/lipoatrophy. One of the hypotheses of this syndrome is that it occurs due to inhibition of lipid and adipocyte regulatory proteins that have partial homology to the catalytic site of HIV-1 protease, to which PIs all bind. PIs are suggested to inhibit CRABP-1-modified and cytochrome P450-3A-mediated synthesis of cis-9-retinoic acid and peroxisome proliferator-activated receptor type gamma (PPAR-γ) heterodimer [21]. The inhibition increases the rate of apoptosis of adipocytes and reduces the rate at which pre-adipocytes differentiate into adipocytes, reducing triglyceride storage and increasing lipid release. PI binding to low density lipoprotein receptor related protein would impair hepatic chylomicron uptake and endothelial triglyceride clearance, resulting in hyperlipidaemia and insulin resistance [6;17;22]. Another factor suggested to play a role in lipodystrophy is the transcription protein sterol regulatory element binding protein 1 (SREBP1). SREBP1 is necessary for adipocyte differentiation. In vivo investigation of fat tissue in HIV-patients with lipoatrophy versus HIV-negative healthy controls, demonstrated a higher proportion of small adipocytes and a reduced expression of SREBP1 in patients with lipoatrophy [22].

Recent investigations show the role of the autonomic nervous system in regulation of adipose tissue. Parasympathetic stimulation of adipose tissue results in glucose and free fatty acid (FFA) uptake, resulting in an increase of adipose tissue. Autonomic neurons in the brainstem or the spine are able to innervate abdominal and subcutaneous fat tissue. A misbalance in the autonomic nervous system could lead to a different distribution of fat tissue intra-abdominal and subcutaneous, leading to central fat accumulation and peripheral fat loss as seen in the lipodystrophy/lipoatrophy syndrome in HIV-infected patients [23].

Endocrine changes

In addition, endocrine changes have been revealed in lipodystrophy. The natural course of HIV-1 infection is associated with an increase in whole body lipolysis and an increase in resting energy expenditure (REE) without an increase in catecholamines (normally stimulators of these processes). However, in patients with lipodystrophy, plasma concentrations of norepinephrine have been found to be increased, indicating increased sympathetic activity. In the same study, lipodystrophy patients had a lower REE compared to HIV-patients without lipo-

dystrophy. HAART-associated lipodystrophy is therefore suggested to have only minor effects on lipolysis induced by HIV-infection itself, as a result of concomitant sympathetic stimulation of adipose tissue [24]. Also an imbalance between peripheral lipolysis and lipogenesis, both regulated by cortisol and dehydro-epiandrosterone, has been hypothesised to play a role in lipodystrophy. Furthermore, subcutaneous adipocyte apoptosis has been found in lipoatrophic areas of HIV-patients treated with PIs [25;26].

Insulin resistance in antiretroviral therapy–associated lipodystrophy has been attributed to impaired glucose transport and phosphorylation. PIs interfere with glucose metabolism in muscle and adipocyte cells, leading to an increase of basal lipolysis. PIs have also been found to directly inhibit the activity of GLUT4, an important cellular glucose transporter, which is generally thought to be the major contributor to insulin-stimulated glucose uptake in adipocytes and skeletal muscle. GLUT4 inhibiton by PIs is thereby leading to a decrease in insulin-stimulated glucose uptake [27-29].

Mitochondrial toxicity

NRTI-associated mitochondrial toxicity has also been postulated to play a role in antiretroviral therapy related lipodystrophy [19;20;30]. Investigation of muscle mitochondria in HIV-patients with lipodystrophy, have shown abnormalities in mitochondrial respiratory chain complexes, mtDNA and mitochondrial morphology suggestive for mitochondrial dysfunction [31]. Adipocyte mitochondria have been studied in HIV-infected patients on NRTIs, therapy-naive patients and healthy controls, revealing mtDNA depletion and mitochondrial proliferation in adipocytes in the first group [32;33].

Cardiovascular risk of antiretroviral therapy

Lipodystrophy with peripheral lipoatrophy is mostly seen in HIV-infected patients receiving HAART and therefore not likely associated with HIV-infection itself. Furthermore, the occurrence and severity of the syndrome is independent of HIV load. Even after short-term therapy with antiretroviral drugs, metabolic changes have been observed. In a recent study in which healthy volunteers were treated with indinavir, insulin resistance was found already after four weeks of drug use [34]. Prior to the availability of protease inhibitor therapy, endothelial dysfunction, hypercoagulability, hypertriglyceridaemia and abnormal coronary artery pathology were associated with HIV-infection. This was even seen in children and young adults. Autopsy reports showed major atherosclerotic lesions in HIV subjects in the absence of traditionally cardiovascular risk factors. A cohort of over 5000 HIV-patients in the US followed between 1993 and 2002, showed an increase in myocardial infarctions after the introduction of protease inhibitors in 1996 [35]. In contrast, a retrospective analysis of a cohort of 36.000 HIV-infected patients between 1993 and 2001, demonstrated a decline in the rate of admissions for cardiovascular or cerebrovascular disease from 1.7 to 0.9 per 100 patient-years. The authors did not find a relation between the use of NRTIs, NNRTIs or PIs and the cardiovascular and cerebrovascular events. Antiretroviral therapy was associated with an overall decrease in death from any cause [36]. The DAD study assessed the risk of cardiovascular disease in HAART-treated patients in a prospective

table 1 Risk factors for atherosclerosis in HIV-patients on HAART.

Risk factors for atherosclerosis in HAART
Increased triglycerides
Increased total cholesterol
Increased LDL-cholesterol
Decreased HDL-cholesterol
Insulin resistance
Increased visceral fat
Increased plasminogen-activator inhibitor type I
Increased apolipoprotein B

multinational cohort study including around 17.000 subjects. Data from this study indicate that NNRTIs and PIs (and especially in combination) are associated with a lipid profile known to increase the risk of coronary heart disease. This was particularly seen in older patients with normalised CD_4 counts and suppressed HIV-replication [37].

Plasma markers that play a role in endothelial function have been shown to be significantly elevated in HIV-patients (von Willebrand factor, tissue plasminogen activator, β2-microglobulin and soluble thrombomodulin). Levels of these markers have been found to be related to the stage of HIV-disease and may be proportional to the viral load. The course of vascular disease may be accelerated in HIV-infected patients because of atherogenesis stimulated by HIV-infected monocyte-macrophages, possibly via altered leukocyte adhesion or arteritis. In recent literature, a relation between chronic inflammation and atherosclerosis has been postulated. In this light, chronic HIV-infection with the occurrence of co-infections (opportunistic or not) makes subjects prone for atherogenic disturbances. In addition, insulin resistance, hypercholesterolaemia and the fat redistribution syndrome (increase of visceral fat) associated with HIV therapy, may exacerbate these HIV-associated atherosclerotic risk factors [22;38-40]. Especially protease inhibitors are known to interfere with lipid and glucose metabolism resulting in insulin resistance with hyperglycaemia, hypertriglyceridaemia and elevated total cholesterol (HDL-cholesterol is often reduced) [35;41]. However, lipid changes have also been seen in patients on efavirenz. Moreover, NRTIs may play a significant role in a synergistic effect on these metabolic changes.

Overall, there are conflicting data on cardiovascular effects of HAART. The growing concern that the metabolic complications associated with HIV and antiretroviral therapy may lead to accelerated coronary artery disease has to be evaluated in large prospective trials with long-term follow up. Till then, monitoring traditional cardiovascular risk factors and risk factors associated with HAART such as dys-lipidaemia, glucose levels and visceral fat accumulation in HIV-patients is justified *(table 1)*. Before the introduction of HAART, AIDS was still a disease resulting in death often within two years, making treatment of opportunistic infections the most important goal in therapy. With increase in life expectancy, the concern of other risk factors for disease in the near future such as cardiovascular diseases, is rising [22;35;36;42-44].

Toxicity associated with mitochondrial damage

Clinical symptoms that might occur during NRTI therapy are: (cardio)myopathy, neuropathy, pancreatitis, hepatic steatosis and lactic acidosis *(table 2)*. These adverse events mostly occur after at least three to four months of NRTI treatment. The clinical symptoms resemble symptoms that occur in paediatric patients with congenital mitochondrial diseases.

Incidences are not well known and vary dependent on the analysis: such as neuropathy 12-46%, myopathy 17% and pancreatitis 0.5-7%. The most life-threatening NRTI related toxic event reported, is lactic acidosis with hepatic failure. It has been described for zidovudine, didanosine and stavudine. The incidence is estimated around 1.3 per 1000 person-years, retrospectively found in a cohort of antiretroviral drug users [5;20;45-54]. This number fits reasonably well with our own experience, when we found four fatal cases within one year in the Netherlands, where around 3000 patients are treated with antiretroviral combination therapy [55]. Imminent lactic acidosis should be suspected if a patient complains of malaise, nausea and vomiting, often accompanied by abdominal pain and hyperventilation, followed by rapid liver failure and uncontrollable arrhythmias.

The common pathway of this NRTI toxicity is induced mitochondrial dysfunction. NRTIs are supposed to interfere with an enzyme (polymerase γ), essential for the synthesis of mitochondrial DNA strands. The triphosphate compounds of the nucleoside analogues inhibit polymerase γ in two ways; direct inhibition of mtDNA polymerase γ without NRTI incorporation or chain termination by incorporation of NRTI triphosphate into mtDNA. Additionally, the incorporated analogues in mtDNA persist because of inefficient excision resulting in a defective DNA strand. Combinations of these three mechanisms are possible as well [56]. Direct proof for NRTI induced mitochondrial toxicity has been demonstrated in zidovudine related myopathy in humans, showing ragged-red fibres and moderate lipid droplet accumulation and myofilamentous loss without inflammatory infiltration [46;47;51;54].

In case of toxicity and discontinuation of medication, some tissues seem to recover only very slowly. Factors that determine this recuperation capacity are unknown, but tissues with a low cell turnover like neurones, seem to recover very slowly and appear to be more vulnerable for this kind of toxicity. An example is NRTI induced neuropathy that can last for months after NRTIs have been stopped.

Another striking feature of the NRTI toxicity is its apparent tissue specificity: myopathy can be caused by zidovudine, but hardly by any of the other NRTIs and conversely, neuropathy and pancreatitis are common features in treatment with zalcitabine, didanosine and stavudine, but not with the other NRTIs *(table 2)*.

A possible explanation for this phenomenon of apparent tissue specificity is the so-called polymerase γ hypothesis, which states that four factors contribute to an effective inhibition of DNA polymerase γ by a certain NRTI at a special tissue level. These factors are: pharmacodynamic capability to enter the target cells, the right cellular nucleoside kinases to triphosphorylate the NRTI, inhibition of DNA polymerase γ by the triphosphorylated NRTI either by serving as a competitive (ineffective) alternate substrate or by chain termination of the nascent mtDNA strand (non-competitive) and finally metabolic reliance on oxidative phosphorylation by the target tissues [5].

table 2 Adverse events in NRTIs/NtRTI associated with mitochondrial toxicity.

	AZT	3TC	d4T	ddI	ddC	ABV	TFV*
Anaemia	+	–	–	–	–	–	–
(Cardio)myopathy	+	–	–	–	–	–	–
Neuropathy	–	–	+	+	+	–	–
Pancreatitis	–	+/–	–	+	–	–	–
Hepatic steatosis	+	–	+	+	+	–	–
Lactic acidosis	+	–	+	+	+	–	–

* = NtRTI (nucleotide reverse transcriptase inhibitor), AZT = zidovudine, 3TC = lamivudine, d4T = stavudine, ddI = didanosine, ddC = zalcitabine, ABV = abacavir and TFV = tenofovir.

At present, the reason why some individuals suffer from mitochondrial toxicity is not clearly understood, although several factors have been identified to play a role in this mechanism of toxicity. As in congenital mitochondrial diseases this mitochondrial impairment might have a genetic basis. Patients with suboptimal mitochondrial function due to inherited mitochondrial DNA (mtDNA) mutations or deletions, with no symptoms yet, might be more susceptible to develop severe dysfunction of mitochondria on NRTI therapy. In a comparable fashion, ageing will result in increasing mitochondrial dysfunction, since mutations in mtDNA accumulate in time and repair mechanisms in mitochondrial DNA replication are limited compared to nuclear DNA replication. Elder people might therefore be more susceptible than younger. A certain time span appears to be necessary for the accumulation of toxic effects. Consequently, long-term exposure to antiretroviral nucleoside analogues is likely to result in symptomatic mitochondrial dysfunction. Accumulative toxicity with mitochondrial toxicity due to prior NRTI use is also seen. Another predisposing factor might be the patient's nutritional state. Biochemical reactions in mitochondria depend on a large scale of vitamins, co-factors and substrates. In malnourished patients there might be a deficiency for these co-factors, in this way contributing to impaired mitochondrial function [5;55].

Diagnosis

Lipodystrophy, lipoatrophy and metabolic disturbances

The diagnosis of this syndrome is difficult and is based on objective and subjective parameters such as physical examination with measurement of hip waist ratio, presence of fat disposition/loss and visible veins on the extremities together with patient's report of a changed body composition. The HIV Lipodystrophy Case Definition Study Group evaluated a model to diagnose lipodystrophy. This model included a score system for age, sex, duration of HIV-infection, HIV-disease stage, waist: hip ratio, anion gap, serum HDL-cholesterol concentration, trunk: peripheral fat ratio, percentage of leg fat and intra-abdominal: extra-abdominal fat ratio. By using this score the diagnosis lipodystrophy had a sensitivity of 79% and a specificity of 80%, which is far more than in scores

using only clinical or metabolic variables [57].
Lipoatrophy/dystrophy with lipid and glucose disturbances have been studied by means of blood measurements, CT (computed tomography) scans, DEXA scans and Body Impedance Amplitude (BIA) in combination with weight and measurement of subcutaneous fat. A problem with this syndrome is the subjective part of it; changes in body composition experienced by the patients cannot always be evaluated by standard tests [58-60]. Changes in fat distribution have been objectively confirmed by dual energy X-ray absorption (DEXA) and computed tomography. However, DEXA and CT scanning cannot demonstrate a change in all aspects concerning the lipoatrophy/lipodystrophy syndrome. Facial lipoatrophy, one of the most distressing features of the syndrome, cannot be measured in this way [61]. In addition, sonography can be considered as an additional tool to quantify regional fat distribution. The measurement of subcutaneous facial and arm fat seems to be simpler, less variable and more discriminative to diagnose abnormal regional fat distribution than that of intra-abdominal fat [62].

Toxicity associated with mitochondrial damage

Mitochondrial disease are clinically heterogeneous ranging from mild myopathy to early fatal multi-system disease. Clinical chemical and metabolic results of body fluids like increased lactic acid and alanine concentrations point to a mitochondrial disturbance. If so, evaluating those organs and tissues with a high-energy demand, like brain, the eyes, the heart and skeletal muscle is warranted. For this a variety of imaging and electrophysiological studies can be performed. Final diagnosis can be reached by biochemical, histological, histochemical and molecular studies in muscle biopsy samples [63].
Histology is the only method to demonstrate mitochondrial disorders definitely. It is an invasive examination and the biopsy site has to be targeted to the tissue affected. For instance, in muscle biopsies the difference between HIV myopathy and AZT induced myopathy can be established. The presence of ragged-red fibres and myofilamentous, is characteristic for an acquired mitochondrial related myopathy. Damaged mitochondria can be seen with electron microscopy, showing swollen mitochondria with disruption and fragmentation of cristae, cristallic inclusions and lipid droplets. However, tissue biopsies are not useful as screening tests and not every tissue can be sampled [5;64].
For more then a decade specific knowledge of mitochondrial dysfunction has been obtained during zidovudine induced myopathy by evaluating cytochrome c oxidase histochemical reaction in muscle biopsies [51;65;66]. Cytochrome c oxidase (COX), is an enzyme essential in complex IV of the mitochondrial respiratory chain. Deficiency of this enzyme was found in all patients with AZT-related myopathy and in the majority of AZT treated patients, however, without myopathy. In contrast, no deficiency was detected in patients with HIV-related myopathy. COX deficiency was found in patients with full-blown AZT myopathy as well as in myopathic tissue from asymptomatic AZT recipients with only histological changes characteristic of AZT myopathy [46;67-69]. Histochemical reaction for COX could therefore be a marker of AZT induced mitochondrial toxicity in HIV-infected patients. Disadvantages of mitochondrial function tests are the invasive character of these

tests and the results do not always correlate with the clinical picture.
NRTI induced hepatic steatosis and neuropathy are diagnoses often detected by exclusion of other possibilities. Histological examination of the liver reveals macrovesicular steatosis without necrosis, in contrast to, steatosis induced by other toxic agents and diseases, which show a microvesicular pattern with necrosis. Additional histochemistry has to be performed to exclude infectious causes. The diagnosis neuropathy can be achieved by electromyography (EMG); however this does not discriminate between HIV and other causes.
Less invasive tests, such as blood tests, are easier to perform and therefore, might be more useful. As a result of mitochondrial dysfunction, pyruvate can only be metabolised into lactate, which leads to an increased lactate and lactate/pyruvate ratio. Determination of blood lactate/pyruvate ratio (L/P) is used as diagnostic screening method in patients with mitochondrial diseases. Chariot et al performed a small pilot study in 20 HIV-infected patients with AZT induced myopathy [70;71]. Although elevated lactate levels have been described in patients suspected from mitochondrial toxicity, not all patients with mitochondrial toxicity develop hyperlactataemia and lactataemia does not always result in lactic acidosis. Zidovudine, didanosine and stavudine have all been described to cause lactic acidosis [72-74]. In addition, several studies found elevated lactate levels more frequently in stavudine containing regimens. Hyperlactataemia in patients on stavudine is noticeable and not always directly related to adverse events [75-82]. Interestingly, serum lactate analysis in a group of our own patients revealed the highest lactate values in patients with presumed NRTI-related neuropathy. This is consistent with a recent study using serum lactate levels in distinguishing between HIV- and NRTI-associated neuropathy [83]. Overall, asymptomatic mild hyperlactataemia is a rather common feature of antiretroviral therapy. In recent publications the use of lactate to monitor complications of antiretroviral therapy has been discussed [75;78;79;84-86]. Routine lactate measurement is not recommended; a difference has to be made between symptomatic and asymptomatic hyperlactataemia and lactic acidosis [82]. Mild asymptomatic hyperlactataemia (lactate levels of ≥2500 µmol/L) requires careful monitoring but no immediate action. Symptomatic hyperlactataemia and lactate levels above 5000 µmol/L are clinically relevant and need intervention. It is advisable to check lactates in patients with NRTI-related neuropathy, hepatic steatosis or elevated transaminases, myopathy and in case of extreme fatigue, unexplained nausea, vomiting, dyspnoea or abdominal pain. Patients with hyperlactataemia should be closely monitored and in case of increasing lactates or lactic acidosis, nucleoside analogues should be discontinued [64;76;87;88].

Another option to study mitochondrial toxicity is to measure mitochondrial DNA (mtDNA) contents. In vitro studies have been performed in different cell lines showing time- and dose dependent mtDNA depletion in cells incubated with zalcitabine, didanosine and stavudine in combination with an increase in lactate production. This effect is also seen in zidovudine treated cells however to a lesser extent. Lamivudine and efavirenz did not affect any of these measurements. In conclusion, NRTIs (except lamivudine) can inhibit mtDNA polymerase γ and cause termination of synthesis of growing mtDNA strands and mtDNA depletion, however the propensity to injure particular target tissues is unexplained [5].

In patients with symptomatic hyperlactataemia changes in mtDNA relative to nuclear DNA in peripheral blood cells were studied. The ratios of mitochondrial to nuclear DNA in HIV-patients with symptomatic hyperlactataemia were 68% lower compared to non-HIV-infected subjects and 48% lower than the ratios in asymptomatic HIV-patients. The decline in mtDNA resolved partially after discontinuation of antiretroviral therapy [89]. Depletion of mtDNA seems at least to be partially reversible. Patients followed longitudinally, showed a decline in mtDNA that preceded the increase in lactate levels. In patients on HAART (including NRTIs) with peripheral fat wasting (lipoatrophy) mtDNA contents of subcutaneous fat tissue from the neck, abdomen and thigh were measured. The results showed a decline in mtDNA content compared to HIV-patients on HAART without lipodystrophy/atrophy, HIV therapy-naive patients and HIV negative controls. Unfortunately not all studies show consistency. One study showed higher mtDNA levels in patients on antiretroviral therapy compared to HIV-infected patients without HAART. This suggests mitochondrial recovery during HAART. Apparently more mechanisms play a role in the mechanism of toxicity [65;87;90-96].

TREATMENT

Lipodystrophy, lipoatrophy and metabolic disturbances

HAART receiving patients with hyperlipidaemia and diabetes are currently treated with lipid lowering agents and oral anti-diabetics or even subcutaneous insulin. Thiazolidinediones such as troglitazone and rosiglitazone have shown to improve insulin sensitivity and promote adipocyte differentiation in vitro. Small studies with metformin and thiazolidinediones in HIV-patients with impaired insulin sensitivity and diabetes during HAART, showed amelioration of antiretroviral therapy-associated insulin resistance, improvement of body fat distribution (increase in lean body mass), a decrease in total body fat and decline of triglycerides and VLDL-cholesterol [99-101].

Leptin therapy is suggested to be of use in lipodystrophy. A study in mice demonstrated a positive effect of leptin replacement with reduction of ritonavir-induced hypercholesterolaemia, interscapular fat mass and improvement of liver steatosis. In contrast to the administration of a polyunsaturated fatty acid diet, which did not alleviate PI-induced metabolic abnormalities [102]. Patients with congenital lipodystrophy have been studied while on chronic leptin therapy. An improvement of insulin-stimulated hepatic and peripheral glucose metabolism in severe insulin-resistance was demonstrated with marked reduction of hepatic and muscle triglyceride content [103;104]. However, the use in HIV-lipodystrophy has to be proven.

Lipid disorders are treated with statins in case of elevated cholesterol and fibrates are used in patients with elevated triglycerides. Till now, pravastatine is the statin of choice since it has no potential to cause rhabdomyolysis or to interact with the other medication often used in HAART regimens (especially the PIs) [105;106]. Furthermore, traditional risk factors such as age greater than 40 years, positive family history, male gender and smoking, should be taken into account when

treating patients with abnormal lipid values and insulin resistance. Also dietary modifications and exercise are general health measures that have been proven to be beneficial in HIV-infected patients with lipodystrophy and for lipid and glucose disturbances [8;22;44].

There is conflicting information on whether switching to a PI depleted regimen reverses the lipoatrophy/dystrophy syndrome and if this leads to lower lipid levels and improvement of insulin resistance. There have been a few studies switching PIs to abacavir, efavirenz or nevirapine showing improvement of insulin resistance. Overall there seems to be a favourable change in lipid concentrations, particularly after switching to nevirapine and abacavir [42;107]. Metabolic disturbances due to HAART appear to be at least partially reversible. However, improvement of the fat redistribution has not been clearly demonstrated. The reason for these conflicting data might perhaps be due to limitations in study design, patient numbers and duration of follow up. One study with PI-treated patients, who presented with lipoatrophy, hyperlipidaemia and insulin resistance, analysed the switch from a PI containing regimen to one containing efavirenz [107-109]. Even one year after substitution of efavirenz for PIs the lipid profile did not improve nor did it resolve the insulin resistance or lipoatrophy. Another study, replacing ritonavir by nelfinavir or nelfinavir/saquinavir in HAART combinations, led to improvement of triglyceride levels [110]. Decrease of lipids was also seen in a randomised study in which patients were switched from a PI containing regimen to a combination of zidovudine, lamivudine and abacavir [111-113].

Peripheral fat loss can sometimes be treated with implantation of subcutaneous fat of the patient or synthetic material (bio-alcamid, New Fill® and botulin toxin); however the results are not always satisfactory. Cosmetic surgery with liposuction can be performed in patients with abdominal fat disposition and buffalo hump, however it is not clear if the fat accumulation will re-occur [6;114].

Toxicity associated with mitochondrial damage

There is no real treatment for mitochondrial toxicity. Case reports have been described treating lactic acidosis due to severe HIV-induced mitochondrial damage with co-enzyme Q, thiamine, L-carnitine and riboflavin. These substrates play an important role in mitochondrial biochemical reactions, however it is not clear what effect they had in these case reports, since antiretroviral therapy was always discontinued in these cases [82;115-117].

CONCLUSION

Awareness of adverse effects of antiretroviral therapy will help to improve strategies to reduce toxicity. Additional assays and tests are needed in order to predict and prove toxicity. Furthermore, new generations of antiretroviral agents will have to be discovered to improve adherence by reducing toxicity in patients on antiretroviral therapy. Finally, still more studies are necessary to get more insight in the mechanisms of toxicity, since treatment options for all these adverse events have been postulated, however none resolved the original problem.

References

1 Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. **N Engl J Med** 1998; 338(13):853-860.

2 Dieleman JP, Jambroes M, Gyssens IC, Sturkenboom MC, Stricker BH, Mulder WM, et al. Determinants of recurrent toxicity-driven switches of highly active antiretroviral therapy. The ATHENA cohort. **AIDS** 2002; 16(5):737-745.

3 Struble KA, Pratt RD, Gitterman SR. Toxicity of antiretroviral agents. **Am J Med** 1997; 102(5B):65-67.

4 Carr A, Cooper DA. Adverse effects of antiretroviral therapy. **Lancet** 2000; 356(9239): 1423-1430.

5 Brinkman K, ter Hofstede HJM, Burger DM, Smeitink JA, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. **AIDS** 1998; 12(14):1735-1744.

6 Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. **AIDS** 1998; 12(7):F51-F58.

7 Carr A. HIV protease inhibitor-induced lipodystrophy syndrome. AIDS Reviews 2008; 1:29-36.

8 Blanco F, Carr A. Lipodystrophy syndrome: Diagnostic, clinic and therapeutic aspects. **AIDS** rev 2004; 3:98-105.

9 Saint-Marc T, Partisani M, Poizot-Martin I, Bruno F, Rouviere O, Lang JM, et al. A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy. **AIDS** 1999; 13(13):1659-1667.

10 Saint-Marc T, Partisani M, Poizot-Martin I, Rouviere O, Bruno F, Avellaneda R, et al. Fat distribution evaluated by computed tomography and metabolic abnormalities in patients undergoing antiretroviral therapy: preliminary results of the LIPOCO study. **AIDS** 2000; 14(1):37-49.

11 Carr A, Miller J, Law M, Cooper DA. A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. **AIDS** 2000; 14(3):F25-F32.

12 Carr A. HIV protease inhibitor-related lipodystrophy syndrome. **Clin Infect Dis** 2000; 30 Suppl 2:S135-S142.

13 Carr A, Cooper DA. Images in clinical medicine. Lipodystrophy associated with an HIV-protease inhibitor. **N Engl J Med** 1998; 339(18):1296.

14 Martinez E, Mocroft A, Garcia-Viejo MA, Perez-Cuevas JB, Blanco JL, Mallolas J, et al. Risk of lipodystrophy in HIV-1-infected patients treated with protease inhibitors: a prospective cohort study. **Lancet** 2001; 357(9256):592-598.

15 Dieleman JP. Risk of lipodystrophy is highest after prolonged exposure to stavudine containing antiretroviral treatment. **The ATHENA cohort.** In: 2003. pp. 108-122.

16 Chang KH, Kim JM, Song YG, Hong SK, Lee HC, Lim SK. Does race protect an oriental population from developing lipodystrophy in HIV-infected individuals on HAART? **J Infect** 2002; 44(1):33-38.

17 Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. **Lancet** 1999; 353(9170):2093-2099.

18 Bogner JR, Vielhauer V, Beckmann RA, Michl G, Wille L, Salzberger B, et al. Stavudine versus zidovudine and the development of lipodystrophy. **J Acquir Immune Defic Syndr** 2001; 27(3):237-244.
19 McComsey G. Update on mitochondrial toxicity of antiretrovirals and its link to lipodystrophy. **AIDS** Rev 2002; 4(3):140-147.
20 van de Valk M, Gisolf EH, Reiss P, Wit FW, Japour A, Weverling GJ, et al. Increased risk of lipodystrophy when nucleoside analogue reverse transcriptase inhibitors are included with protease inhibitors in the treatment of HIV-1 infection. **AIDS** 2001; 15(7):847-855.
21 Carr A, Samaras K, Chisholm DJ, Cooper DA. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. **Lancet** 1998; 351(9119):1881-1883.
22 Barbaro G. HIV-infection, antiretroviral therapy and cardiovascular risk. **J Cardiovasc Risk** 2002; 9(5):295-300.
23 Kreier F, Fliers E, Voshol PJ, Van Eden CG, Havekes LM, Kalsbeek A, et al. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat—functional implications. **J Clin Invest** 2002; 110(9):1243-1250.
24 van de Valk M, Reiss P, van Leth FC, Ackermans MT, Endert E, Romijn JA, et al. Highly active antiretroviral therapy-induced lipodystrophy has minor effects on human immunodeficiency virus-induced changes in lipolysis, but normalizes resting energy expenditure. **J Clin Endocrinol Metab** 2002; 87(11):5066-5071.
25 Christeff N, Melchior JC, de TP, Perronne C, Nunez EA, Gougeon ML. Lipodystrophy defined by a clinical score in HIV-infected men on highly active antiretroviral therapy: correlation between dyslipidaemia and steroid hormone alterations. **AIDS** 1999; 13(16):2251-2260.
26 Domingo P, Matias-Guiu X, Pujol RM, Francia E, Lagarda E, Sambeat MA, et al. Subcutaneous adipocyte apoptosis in HIV-1 protease inhibitor-associated lipodystrophy. **AIDS** 1999; 13(16):2261-2267.
27 Behrens G, Dejam A, Schmidt H, Balks HJ, Brabant G, Korner T, et al. Impaired glucose tolerance, beta cell function and lipid metabolism in HIV-patients under treatment with protease inhibitors. **AIDS** 1999; 13(10):F63-F70.
28 Murata H, Hruz PW, Mueckler M. Indinavir inhibits the glucose transporter isoform Glut4 at physiologic concentrations. **AIDS** 2002; 16(6):859-863.
29 Ben-Romano R, Rudich A, Torok D, Vanounou S, Riesenberg K, Schlaeffer F, et al. Agent and cell-type specificity in the induction of insulin resistance by HIV protease inhibitors. **AIDS** 2003; 17(1):23-32.
30. Brinkman K, Smeitink JA, Romijn JA, Reiss P. Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. **Lancet** 1999; 354(9184):1112-1115.
31 Zaera MG, Miro O, Pedrol E, Soler A, Picon M, Cardellach F, et al. Mitochondrial involvement in antiretroviral therapy-related lipodystrophy. **AIDS** 2001; 15(13):1643-1651.
32 Nolan D, Hammond E, Martin A, Taylor L, Herrmann S, McKinnon E, et al. Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. **AIDS** 2003; 17(9):1329-1338.
33 Walker UA, Bickel M, Lutke Volksbeck SI, Ketelsen UP, Schofer H, Setzer B, et al. Evidence of nucleoside analogue reverse transcriptase inhibitor—associated genetic and structural defects of mitochondria in adipose tissue of HIV-infected patients. **J Acquir Immune Defic Syndr** 2002; 29(2):117-121.

34 Noor MA, Lo JC, Mulligan K, Schwarz JM, Halvorsen RA, Schambelan M, et al. Metabolic effects of indinavir in healthy HIV-seronegative men. **AIDS** 2001; 15(7):F11-F18.

35 Holmberg SD, Moorman AC, Williamson JM, Tong TC, Ward DJ, Wood KC, et al. Protease inhibitors and cardiovascular outcomes in patients with HIV-1. **Lancet** 2002; 360(9347):1747-1748.

36 Bozzette SA, Ake CF, Tam HK, Chang SW, Louis TA. Cardiovascular and cerebrovascular events in patients treated for human immunodeficiency virus infection. **N Engl J Med** 2003; 348(8):702-710.

37 Friis-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio MA, et al. Cardiovascular disease risk factors in HIV-patients—association with antiretroviral therapy. Results from the DAD study. **AIDS** 2003; 17(8):1179-1193.

38 Stein JH, Klein MA, Bellehumeur JL, McBride PE, Wiebe DA, Otvos JD, et al. Use of human immunodeficiency virus-1 protease inhibitors is associated with atherogenic lipoprotein changes and endothelial dysfunction. **Circulation** 2001; 104(3):257-262.

39 Passalaris JD, Sepkowitz KA, Glesby MJ. Coronary artery disease and human immunodeficiency virus infection. **Clin Infect Dis** 2000; 31(3):787-797.

40 Acevedo M, Sprecher DL, Calabrese L, Pearce GL, Coyner DL, Halliburton SS, et al. Pilot study of coronary atherosclerotic risk and plaque burden in HIV-patients: 'a call for cardiovascular prevention'. **Atherosclerosis** 2002; 163(2):349-354.

41 Tashima KT, Bausserman L, Alt EN, Aznar E, Flanigan TP. Lipid changes in patients initiating efavirenz- and indinavir-based antiretroviral regimens. **HIV Clin Trials** 2003; 4(1):29-36.

42 van de Valk M, Kastelein JJ, Murphy RL, van Leth F, Katlama C, Horban A, et al. Nevirapine-containing antiretroviral therapy in HIV-1 infected patients results in an anti-atherogenic lipid profile. **AIDS** 2001; 15(18):2407-2414.

43 Safrin S, Grunfeld C. Fat distribution and metabolic changes in patients with HIV infection. AIDS 1999; 13(18):2493-2505.

44 Tsiodras S, Mantzoros C, Hammer S, Samore M. Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study. **Arch Intern Med** 2000; 160(13):2050-2056.

45 Moyle G. Toxicity of antiretroviral nucleoside and nucleotide analogues: is mitochondrial toxicity the only mechanism? **Drug Saf** 2000; 23(6):467-481.

46 Dalakas MC, Illa I, Pezeshkpour GH, Laukaitis JP, Cohen B, Griffin JL. Mitochondrial myopathy caused by long-term zidovudine therapy. **N Engl J Med** 1990; 322(16): 1098-1105.

47 Swartz MN. Mitochondrial toxicity—new adverse drug effects. **N Engl J Med** 1995; 333(17):1146-1148.

48 Lea AP, Faulds D. Stavudine: a review of its pharmacodynamic and pharmacokinetic properties and clinical potential in HIV-infection. **Drugs** 1996; 51(5):846-864.

49 Perry CM, Balfour JA. Didanosine. An update on its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV-disease. **Drugs** 1996; 52(6):928-962.

50 Adkins JC, Peters DH, Faulds D. Zalcitabine. An update of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in the management of HIV-infection. **Drugs** 1997; 53(6):1054-1080.

51 Peters BS, Winer J, Landon DN, Stotter A, Pinching AJ. Mitochondrial myopathy associated with chronic zidovudine therapy in AIDS. **Q J Med** 1993; 86(1):5-15.

52 Hoetelmans RM, Burger DM, Meenhorst PL, Beijnen JH. Pharmacokinetic individualisation of zidovudine therapy. Current state of pharmacokinetic-pharmacodynamic relationships. **Clin Pharmacokinet** 1996; 30(4):314-327.
53 Wilde MI, Langtry HD. Zidovudine. An update of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. **Drugs** 1993; 46(3):515-578.
54 Fortgang IS, Belitsos PC, Chaisson RE, Moore RD. Hepatomegaly and steatosis in HIV-infected patients receiving nucleoside analog antiretroviral therapy. **Am J Gastroenterol** 1995; 90(9):1433-1436.
55 ter Hofstede HJM, de Marie S, Foudraine NA, Danner SA, Brinkman K. Clinical features and risk factors of lactic acidosis following long-term antiretroviral therapy: 4 fatal cases. **Int J STD AIDS** 2000; 11(9):611-616.
56 Kohler JJ, Lewis W. A brief overview of mechanisms of mitochondrial toxicity from NRTIs. **Environ Mol Mutagen** 2007; 48(3-4):166-172.
57 Carr A, Emery S, Law M, Puls R, Lundgren JD, Powderly WG. An objective case definition of lipodystrophy in HIV-infected adults: a case-control study. **Lancet** 2003; 61(9359):726-735.
58 Kaul DR, Cinti SK, Carver PL, Kazanjian PH. HIV protease inhibitors: advances in therapy and adverse reactions, including metabolic complications. **Pharmacotherapy** 1999; 19(3):281-298.
59 Sluys TE, van der Ende ME, Swart GR, van den Berg JW, Wilson JH. Body composition in patients with acquired immunodeficiency syndrome: a validation study of bioelectric impedance analysis. **JPEN J Parenter Enteral Nutr** 1993; 17(5):404-406.
60 Hadigan C, Rabe J, Meininger G, Aliabadi N, Breu J, Grinspoon S. Inhibition of lipolysis improves insulin sensitivity in protease inhibitor-treated HIV-infected men with fat redistribution. **Am J Clin Nutr** 2003; 77(2):490-494.
61 Carr A, Law M. An objective lipodystrophy severity grading scale derived from the lipodystrophy case definition score. **J Acquir Immune Defic Syndr** 2003; 33(5):571-576.
62 Martinez E, Bianchi L, Garcia-Viejo MA, Bru C, Gatell JM. Sonographic assessment of regional fat in HIV-1-infected people. **Lancet** 2000; 356(9239):1412-1413.
62 Martinez E, Bianchi L, Garcia-Viejo MA, Bru C, Gatell JM. Sonographic assessment of regional fat in HIV-1-infected people. **Lancet** 2000; 356(9239):1412-1413.
63 Smeitink J, van den HL, DiMauro S. The genetics and pathology of oxidative phosphorylation. **Nat Rev Genet** 2001; 2(5):342-352.
64 ter Hofstede HJM, Brinkman K. Mitochondrial toxicity owing to nucleoside reverse transcriptase inhibitors: importance of clinical features and early diagnosis. **Int Antiviral News** 1999; 7:148-151.
65 Arnaudo E, Dalakas M, Shanske S, Moraes CT, DiMauro S, Schon EA. Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy. **Lancet** 1991; 337(8740):508-510.
66 Chariot P, Gherardi R. Myopathy and HIV-infection. **Curr Opin Rheumatol** 1995; 7(6):497-502.
67 Lai KK, Gang DL, Zawacki JK, Cooley TP. Fulminant hepatic failure associated with 2',3'-dideoxyinosine (ddI). **Ann Intern Med** 1991; 115(4):283-284.
68 Chariot P, Gherardi R. Partial cytochrome c oxidase deficiency and cytoplasmic bodies in patients with zidovudine myopathy. **Neuromuscul Disord** 1991; 1(5):357-363.
69 Chariot P, Monnet I, Gherardi R. Cytochrome c oxidase reaction improves histopathological assessment of zidovudine myopathy. **Ann Neurol** 1993; 34(4):561-565.
70 Munnich A, Rustin P, Rotig A, Chretien D, Bonnefont JP, Nuttin C, et al. Clinical aspects of mitochondrial disorders. **J Inherit Metab Dis** 1992; 15(4):448-455.

71 Chariot P, Monnet I, Mouchet M, Rohr M, Lefaucheur JP, Dubreuil-Lemaire ML, et al. Determination of the blood lactate:pyruvate ratio as a noninvasive test for the diagnosis of zidovudine myopathy. **Arthritis Rheum** 1994; 37(4):583-586.
72 Sundar K, Suarez M, Banogon PE, Shapiro JM. Zidovudine-induced fatal lactic acidosis and hepatic failure in patients with acquired immunodeficiency syndrome: report of two patients and review of the literature. **Crit Care Med** 1997; 25(8): 1425-1430.
73 Falco V, Rodriguez D, Ribera E, Martinez E, Miro JM, Domingo P, et al. Severe nucleoside-associated lactic acidosis in human immunodeficiency virus-infected patients: report of 12 cases and review of the literature. **Clin Infect Dis** 2002; 34(6): 838-846.
74 Lenzo NP, Garas BA, French MA. Hepatic steatosis and lactic acidosis associated with stavudine treatment in an HIV patient: a case report. **AIDS** 1997; 11(10):1294-1296.
75 Lonergan JT, Behling C, Pfander H, Hassanein TI, Mathews WC. Hyperlactatemia and hepatic abnormalities in 10 human immunodeficiency virus-infected patients receiving nucleoside analogue combination regimens. **Clin Infect Dis** 2000; 31(1):162-166.
76 Mokrzycki MH, Harris C, May H, Laut J, Palmisano J. Lactic acidosis associated with stavudine administration: a report of five cases. **Clin Infect Dis** 2000; 30(1):198-200.
77 Miller KD, Cameron M, Wood LV, Dalakas MC, Kovacs JA. Lactic acidosis and hepatic steatosis associated with use of stavudine: report of four cases. **Ann Intern Med** 2000; 133(3):192-196.
78 Boubaker K, Flepp M, Sudre P, Furrer H, Haensel A, Hirschel B, et al. Hyperlactatemia and antiretroviral therapy: the Swiss HIV Cohort Study. **Clin Infect Dis** 2001; 33(11):1931-1937.
79 Gerard Y, Maulin L, Yazdanpanah Y, De LT, X, Amiel C, Maurage CA, et al. Symptomatic hyperlactataemia: an emerging complication of antiretroviral therapy. **AIDS** 2000; 14(17):2723-2730.
80 Delgado J, Harris M, Tesiorowski A, Montaner JS. Symptomatic elevations of lactic acid and their response to treatment manipulation in human immunodeficiency virus-infected persons: a case series. **Clin Infect Dis** 2001; 33(12):2072-2074.
81 Vrouenraets SM, Treskes M, Regez RM, Troost N, Smulders YM, Weigel HM, et al. Hyperlactataemia in HIV-infected patients: the role of NRTI treatment. **Antivir Ther** 2002; 7(4):239-244.
82 John M, Moore CB, James IR, Nolan D, Upton RP, McKinnon EJ, et al. Chronic hyperlactatemia in HIV-infected patients taking antiretroviral therapy. **AIDS** 2001; 15(6):717-723.
83 Brew BJ, Tisch S, Law M. Lactate concentrations distinguish between nucleoside neuropathy and HIV neuropathy. **AIDS** 2003; 17(7):1094-1096.
84 Brinkman K. Editorial response: hyperlactatemia and hepatic steatosis as features of mitochondrial toxicity of nucleoside analogue reverse transcriptase inhibitors. **Clin Infect Dis** 2000; 31(1):167-169.
85 Brinkman K. Management of hyperlactatemia: no need for routine lactate measurements. **AIDS** 2001; 15(6):795-797.
86 Boffito M, Marietti G, Audagnotto S, Raiter R, Di PG. Lactacidemia in asymptomatic HIV-infected subjects receiving nucleoside reverse-transcriptase inhibitors. **Clin Infect Dis** 2002; 34(4):558-559.
87 Benbrik E, Chariot P, Bonavaud S, mmi-Said M, Frisdal E, Rey C, et al. Cellular and mitochondrial toxicity of zidovudine (AZT), didanosine (ddI) and zalcitabine (ddC) on cultured human muscle cells. **J Neurol Sci** 1997; 149(1):19-25.

88 Bartley PB, Westacott L, Boots RJ, Lawson M, Potter JM, Hyland VJ, et al. Large hepatic mitochondrial DNA deletions associated with L-lactic acidosis and highly active antiretroviral therapy. **AIDS** 2001; 15(3):419-420.

89 Cote HC, Brumme ZL, Craib KJ, Alexander CS, Wynhoven B, Ting L, et al. Changes in mitochondrial DNA as a marker of nucleoside toxicity in HIV-infected patients. **N Engl J Med** 2002; 346(11):811-820.

90 Walker UA, Setzer B, Venhoff N. Increased long-term mitochondrial toxicity in combinations of nucleoside analogue reverse-transcriptase inhibitors. **AIDS** 2002; 16(16):2165-2173.

91 Vittecoq D, Jardel C, Barthelemy C, Escaut L, Cheminot N, Chapin S, et al. Mitochondrial damage associated with long-term antiretroviral treatment: associated alteration or causal disorder? **J Acquir Immune Defic Syndr** 2002; 31(3):299-308.

92 Cossarizza A, Troiano L, Mussini C. Mitochondria and HIV-infection: the first decade. **J Biol Regul Homeost Agents** 2002; 16(1):18-24.

93 Dalakas MC, Semino-Mora C, Leon-Monzon M. Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 2'3'-dideoxycytidine (ddC). **Lab Invest** 2001; 81(11):1537-1544.

94 Shikuma CM, Hu N, Milne C, Yost F, Waslien C, Shimizu S, et al. Mitochondrial DNA decrease in subcutaneous adipose tissue of HIV-infected individuals with peripheral lipoatrophy. **AIDS** 2001; 15(14):1801-1809.

95 Casademont J, Barrientos A, Grau JM, Pedrol E, Estivill X, Urbano-Marquez A, et al. The effect of zidovudine on skeletal muscle mtDNA in HIV-1 infected patients with mild or no muscle dysfunction. **Brain** 1996; 119 (Pt 4):1357-1364.

96 Tsai CH, Doong SL, Johns DG, Driscoll JS, Cheng YC. Effect of anti-HIV 2'-beta-fluoro-2',3'-dideoxynucleoside analogs on the cellular content of mitochondrial DNA and on lactate production. **Biochem Pharmacol** 1994; 48(7):1477-1481.

97 Hadigan C, Corcoran C, Basgoz N, Davis B, Sax P, Grinspoon S. Metformin in the treatment of HIV lipodystrophy syndrome: A randomized controlled trial. **JAMA** 2000; 284(4):472-477.

98 Gelato MC, Mynarcik DC, Quick JL, Steigbigel RT, Fuhrer J, Brathwaite CE, et al. Improved insulin sensitivity and body fat distribution in HIV-infected patients treated with rosiglitazone: a pilot study. **J Acquir Immune Defic Syndr** 2002; 31(2): 163-170.

99 Walli R, Michl GM, Muhlbayer D, Brinkmann L, Goebel FD. Effects of troglitazone on insulin sensitivity in HIV-infected patients with protease inhibitor-associated diabetes mellitus. **Res Exp Med (Berl)** 2000; 199(5):253-262.

100 Riddle TM, Fichtenbaum CJ, Hui DY. Leptin replacement therapy but not dietary polyunsaturated fatty acid alleviates HIV protease inhibitor-induced dyslipidemia and lipodystrophy in mice. **J Acquir Immune Defic Syndr** 2003; 33(5):564-570.

101 Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, et al. Leptin-replacement therapy for lipodystrophy. **N Engl J Med** 2002; 346(8):570-578.

102 Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. **J Clin Invest** 2002; 109(10):1345-1350.

103 Manfredi R, Chiodo F. Disorders of lipid metabolism in patients with HIV-disease treated with antiretroviral agents: frequency, relationship with administered drugs, and role of hypolipidaemic therapy with bezafibrate. **J Infect** 2001; 42(3):181-188.

104 Moyle GJ, Lloyd M, Reynolds B, Baldwin C, Mandalia S, Gazzard BG. Dietary advice with or without pravastatin for the management of hypercholesterolaemia associated with protease inhibitor therapy. **AIDS** 2001; 15(12):1503–1508.
105 Drechsler H, Powderly WG. Switching effective antiretroviral therapy: a review. **Clin Infect Dis** 2002; 35(10):1219–1230.
106 Carr A, Workman C, Smith DE, Hoy J, Hudson J, Doong N, et al. Abacavir substitution for nucleoside analogs in patients with HIV lipoatrophy: a randomized trial. **JAMA** 2002; 288(2):207–215.
107 Estrada V, De Villar NG, Larrad MT, Lopez AG, Fernandez C, Serrano-Rios M. Long-term metabolic consequences of switching from protease inhibitors to efavirenz in therapy for human immunodeficiency virus-infected patients with lipoatrophy. **Clin Infect Dis** 2002; 35(1):69–76.
108 Wensing AM, Reedijk M, Richter C, Boucher CA, Borleffs JC. Replacing ritonavir by nelfinavir or nelfinavir/saquinavir as part of highly active antiretroviral therapy leads to an improvement of triglyceride levels. **AIDS** 2001; 15(16):2191–2193.
109 Murphy RL, Smith WJ. Switch studies: a review. **HIV Med** 2002; 3(2):146–155.
110 Opravil M, Hirschel B, Lazzarin A, Furrer H, Chave JP, Yerly S, et al. A randomized trial of simplified maintenance therapy with abacavir, lamivudine, and zidovudine in human immunodeficiency virus infection. **J Infect Dis** 2002; 185(9):1251–1260.
111 Negredo E, Cruz L, Paredes R, Ruiz L, Fumaz CR, Bonjoch A, et al. Virological, immunological, and clinical impact of switching from protease inhibitors to nevirapine or to efavirenz in patients with human immunodeficiency virus infection and long-lasting viral suppression. **Clin Infect Dis** 2002; 34(4):504–510.
112 Cheonis N. New-Fill to treat facial wasting. **BETA** 2002; 15(2):10–15.
113 Fouty B, Frerman F, Reves R. Riboflavin to treat nucleoside analogue-induced lactic acidosis. **Lancet** 1998; 352(9124):291–292.
114 Luzzati R, Del BP, Di PG, Luzzani A, Concia E. Riboflavine and severe lactic acidosis. **Lancet** 1999; 353(9156):901–902.
115 Przyrembel H. Therapy of mitochondrial disorders. **J Inherit Metab Dis** 1987; 10 Suppl 1:129–146.
116 Walker UA, Byrne E. The therapy of respiratory chain encephalomyopathy: a critical review of the past and current perspective. **Acta Neurol Scand** 1995; 92(4):273–280.
117 Semino-Mora MC, Leon-Monzon ME, Dalakas MC. Effect of L-carnitine on the zidovudine-induced destruction of human myotubes. Part I: L-carnitine prevents the myotoxicity of AZT in vitro. **Lab Invest** 1994; 71(1):102–112.

Section

Mitochondrial toxicity

Chapter 2

Clinical features and risk factors of lactic acidosis following long-term antiretroviral therapy: 4 fatal cases

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Abstract

To describe clinical features and predisposing factors attributed to lactic acidosis in four HIV-infected patients on long-term NRTI therapy.
All patients had received at least 6–20 months of NRTI containing antiretroviral therapy: all used d4T, in one combined with 3TC, in the other three with ddI; in one hydroxyurea was added. In all, the initial symptoms were gastrointestinal (nausea and vomiting), followed by tachypnoea preceding the lactic acidosis; death followed 6–22 days after admission (liver failure and uncontrollable arrhythmias). Treatment with riboflavin was unsuccessful in one patient. The only definite risk factor in all cases was NRTI induced mitochondrial toxicity; one patient was concomitantly treated for Kaposi sarcoma (with bleomycin and vinblastine) and one just recovered from pneumococcal sepsis. None of the patients had a history of chronic hepatitis B or hepatitis C infection. In all patients, some sort of toxicity to other previously used NRTIs had occurred earlier. Lactic acidosis occurred after months of NRTI therapy in patients who had already suffered other forms of NRTI toxicity. Concomitant diseases or co-medication might have aggravated the mitochondrial toxicity of the NRTIs. Screening methods to detect mitochondrial toxicity are necessary, since lactic acidosis occurs rather unexpectedly, with a rapid, fatal course.

Introduction

Although the incidence is very low, fatal lactic acidosis with hepatic failure has been attributed to the use of nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine (AZT) [1-5] and didanosine (ddI) [6;7]. Also for stavudine (d4T), lactic acidosis has been reported [8-10]. This adverse event is believed to be caused by mitochondrial toxicity and in fact can occur with any of the antiretroviral nucleoside analogues, except possibly lamivudine (3TC) [11;12].
In this paper we report four additional cases of fatal lactic acidosis associated with the use of antiretroviral nucleoside analogues as part of highly active antiretroviral therapy (HAART). We compare and discuss the clinical presentation of fatal lactic acidosis and analyse possible underlying risk factors which might have led to the course of this event.

Case reports

Patient 1

A 25-year-old Ethiopian woman was found to be HIV positive in October 1996. Because she had a CD_4 count of 0.18 x 10^9/L and a viral load of 60.000 copies/ml, HAART was initiated. The first combination consisted of zidovudine (AZT), lamivudine (3TC) and ritonavir, which was later switched to stavudine (d4T), zalcitabine (ddC) and indinavir. However, after she developed AZT-related anaemia and ddC-related neuropathy, the HAART was finally set to a combination of d4T (40 mg b.i.d.), 3TC (150 mg b.i.d.) and saquinavir (1200 mg t.i.d.). After this triple therapy had started in April 1997, the CD_4 count rose to 0.31 x 10^9/L and the viral load became undetectable (<400 copies/ml) in September 1997. In October 1997 the patient started to complain of nausea and was admitted because of persistent vomiting. No explanation for the persistent vomiting was found after extensive biochemical, neurological, endoscopic and radiological examination. Her medication had already been stopped 4 days before admission. On the 9th day of admission biochemical tests revealed severe lactic acidosis (pH 7.04; normally 7.35-7.45, bicarbonate 4.8 mmol/L; normally 24-28 mmol/L; lactate 9.8 mmol/L; normally <1.8 mmol/L; not measured on the day of admission) without signs of sepsis. Further analysis revealed increased ratios of lactate/pyruvate (50; normal <20) and β-hydroxybutyrate/acetoacetate (3.5; normal <2). Despite mechanical ventilation and bicarbonate dialysis, she developed hepatic failure, inotropic agents dependent hypotension and arrhythmias (recurrent ventricular tachy-cardias) and when the clinical course was further complicated by bacterial sepsis she died on the 22nd day of admission. Autopsy revealed severe hepatomegaly with microscopically marked cholestasis and moderate, pericentral, micro- and macrovesicular steatosis.

Patient 2

A 31-year-old Dutch woman was found to be HIV positive in 1987. In 1992 AZT (200 mg t.i.d.) therapy was started because of a CD_4 count of 0.03 x 10^9/L, but dose reduction was necessary due to AZT-related anaemia (100 mg t.i.d.).

Furthermore, co-trimoxazole was initiated as prophylaxis for Pneumocystis Carinii Pneumonia (PCP). In 1994 ddI (125 mg b.i.d.) was added, but hair loss developed and treatment was switched to ddC (0.75 mg t.i.d.) after 1 month. Since there was no response in CD_4 counts, AZT resistance was presumed and AZT was switched to 3TC (150 mg b.i.d.). She developed paraesthesia mainly in feet and fingers. Saquinavir was added to the 3TC/ddC combination in February 1996, but the CD_4 count remained very low (<0.01 x 10^9/L) and the first measured viral load was 15.000 copies/ml (September 1996). She had recurrent periods of perianal genital herpes and developed a cytomegalovirus retinitis, which was initially treated with ganciclovir, later with foscarnet. In November 1996 the complete antiretroviral therapy was switched to ddI, d4T and ritonavir. Doses of ddI (200 q.d.) and d4T (15 mg b.i.d.) were adjusted to a foscarnet related renal dysfunction. Although there was an initial viral response after 5 months (<500 copies/ml), virological failure necessitated alteration of antiretroviral therapy. Because genotypic viral resistance analysis demonstrated resistance to protease inhibitors and most of the NRTIs (except ddI and d4T), therapy was switched to nevirapine (200 mg b.i.d.), ddI, d4T and hydroxyureum (500 mg b.i.d.) in March 1998. The viral load became undetectable and her clinical condition stabilised. In may 1998 she complained of continuous stomach pain and nausea. Antiretroviral therapy was stopped for 2 days, but within one week she developed a flu-like syndrome, followed by two-sided pneumococcal pneumonia with bacteraemia. Despite effective antibiotic treatment she became hypotensive and metabolic acidosis (pH 7.17, bicarbonate 8 mmol/L) evolved. The lactate level was 19 mmol/L and though the circulation had normalised, these levels remained high (16 to 26 mmol/L) on hemodialysis, with lactate/pyruvate ratios of 64. Complete hepatic failure became apparent and together with recurrent arrhythmias (ventricular tachycardia) she died on the 6th day of admission. Autopsy was denied.

Patient 3

A 42-year old Dutch man was found to be HIV positive in 1984. In 1992 antiretroviral therapy with AZT (300 mg b.i.d.) was started (CD_4 count of 0.18 x 10^9/L). In 1994 ddC (0.75 mg t.i.d.) was added, but since there was no response, ddC was switched to 3TC (150 mg b.i.d.). In 1996 Pneumocystis carinii pneumonia was diagnosed. Leucopenia developed, possibly related to AZT toxicity and AZT was switched to d4T (40 mg b.i.d.), later supplemented with saquinavir. Because of virological failure, the antiretroviral therapy was changed to d4T, ddI (400 mg q.d.) and indinavir (800 mg t.i.d.) in April 1997. After 5 months of treatment with indinavir no virological response could be observed and indinavir was changed to nelfinavir, based on genotypic viral resistance analysis (resistance to AZT, 3TC and most protease inhibitors except nelfinavir). In November 1997 he was admitted to the hospital because of complaints of malaise, vomiting, abdominal pain and weight loss. On physical examination he was very cachectic and he had an enlarged liver. One day later his status deteriorated with a decreased consciousness, hypotension and a very low temperature. Although septic shock was assumed, all cultures remained negative. A severe metabolic acidosis (pH 7.10, bicarbonate 4.6 mmol/L) was found with an elevated lactate level of 16.8 mmol/L, maximally 31.2. Shortly after mechanical ventilation with dialysis and inotropic assistance,

complete liver failure developed. He died on the 6th day of admission. Autopsy showed severe hepatomegaly with microvesicular steatosis, cholestasis and extended pericentral liver cell necrosis.

Patient 4

A 37-year old Dutch man was found to be HIV positive in 1990. In 1996 Kaposi's sarcoma lesions were detected on the skin of the legs and thorax, and antiretroviral therapy with AZT (300 mg b.i.d.), 3TC (150 mg b.i.d.) and ritonavir (600 mg b.i.d.) was started (CD_4 count of 0.09 x 10^9/L). Kaposi's sarcoma treatment consisted of radiotherapy and cyclic bleomycin and vinblastine. Adverse events due to ritonavir (diarrhoea, nausea, and oral paraesthesia) resulted in discontinuation of ritonavir. Myalgia, which occurred after introduction of AZT, was temporary and disappeared after 2 months of treatment. In 1997 antiretroviral therapy failed (viral load 110.000 copies/ml, CD_4 count of 0.09 x 10^9/L) and was switched to d4T (40 mg b.i.d.), ddI (400 mg q.d.) and indinavir (800 mg t.i.d.). With this second antiretroviral regimen the patient developed d4T-related neuropathy after two months which progressed the following months with continuation of the drugs. Viral load became undetectable, while CD_4 count remained unchanged. In November 1998 the patient was admitted because of nausea, vomiting and tachypnoea. Metabolic acidosis was diagnosed (pH 7.25, bicarbonate 14.1 mmol/L; lactate 21.4 mmol/L) without signs of sepsis. A pulmonary X-ray was suspect for pneumonia but microbiological analysis of all cultures remained negative. Due to clinical deterioration the patient was transferred to the intensive care unit where mechanical ventilation, inotropic treatment and dialysis were started. Nevertheless, multiple organ failure with arrhythmias (supraventricular tachycardias and later ventricular tachycardias) developed and despite admission of riboflavin (50 mg) to treat possible nucleoside analogue toxicity, the patient died on the 10th day of admission. Autopsy was denied.

Discussion

The four cases of lactic acidosis described, show similarity with respect to clinical symptoms, antiretroviral therapy and the final fatal course *(table 1)*. The clinical features of this drug-induced toxicity are characterised by an episode of malaise, nausea and vomiting, often accompanied by abdominal pain and tachypnoea (Kussmaul breathing), finally resulting in liver failure and a fatal cardiovascular collapse with arrhythmias, secondary to metabolic acidosis. Similar symptoms at the time of presentation have been described for cases with hepatic failure and lactic acidosis related to treatment with AZT (n=14), ddI (n=3) [1;4;6;7;13] and also fialuridine, a nucleoside analogue which has been experimentally tested for hepatitis B treatment [14]. Nucleoside analogue induced mitochondrial toxicity is the underlying mechanism of lactic acidosis. The triphosphate compounds of the nucleoside analogues inhibit polymerase γ, an enzyme essential for mitochondrial replication [11]. In contrast, non nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) have no affinity for polymerase γ; this is due to difference in chemical structure. Therefore, they have no potency to interfere with

table 1 Patients' demographics.

	patient 1	patient 2	patient 3	patient 4
	female	female	male	male
	25 years	31 years	42 years	37 years
Presenting symptoms before fatal outcome	nausea vomiting tachypnoea	abdominal pain nausea vomiting flu-like syndrome	malaise vomiting abdominal pain weight loss	nausea vomiting tachypnoea
Laboratory parameters before fatal outcome				
Lactate (<1.8 mmol/L)	9.8-19.9 mmol/L	19 mmol/L	16.8-31.2 mmol/L	21.4 mmol/L
Bicarbonate (24-28 mmol/L)	4.8 mmol/L	8 mmol/L	4.6 mmol/L	14.1 mmol/L
pH (7.35-7.45)	7.04	7.17	7.10	7.25
Previous nucleoside analogues related toxicity				
AZT	anaemia	anaemia	leucopenia	myalgia
ddC	neuropathy	neuropathy		
ddI				
d4T				neuropathy*
Virological response	++	+	-	+
Therapy at time of event (duration)	d4T (6 months) 3TC (6 months) SQV (6 months)	d4T (18 months) ddI (18 months) NVP (3 months) hydroxy-urea (3 months)	d4T (20 months) ddI (7 months) NFV (1 month)	d4T (13 months) ddI (13 months) IDV (13 months)

AZT: zidovudine, ddC: zalcitabine, ddI: didanosine, d4T: stavudine, SQV: saquinavir, NVP: nevirapine, NFV: nelfinavir, IDV: indinavir
* neuropathy in this patient could also be due to vinblastine.

mitochondrial replication.
NRTI-related adverse events are rather common (incidences vary; e.g. neuropathy 12-46%, myopathy 17%, pancreatitis 0.5-7%) [15-20]. Fortunately lactic acidosis, the most severe toxic event, has a low incidence. Although incidence data are not exactly known, in a retrospective cohort of patients on antiretroviral therapy, the incidence was estimated around 1.3 per 1000 person-years [21].
At present, the reason why only some individuals suffer from these events is not clearly understood, although several factors might play a role in this mechanism of toxicity. As in congenital mitochondrial diseases this mitochondrial impairment might have a genetic basis. Patients with suboptimal mitochondrial function due to inherited mitochondrial DNA (mtDNA) mutations or deletions, with no symptoms yet, might be more susceptible to develop severe dysfunction of mitochondria on

NRTI therapy. In a comparable fashion, ageing will result in increasing mitochondrial dysfunction, since mutations in mtDNA accumulate in time and repair mechanisms in mitochondrial DNA replication are limited compared to nuclear DNA replication. Elder people might therefore be more susceptible than younger. A certain time span appears to be necessary for the accumulation of toxic effects. Consequently, long-term exposure to antiretroviral nucleoside analogues is likely to result in symptomatic mitochondrial dysfunction. Apparently, mitochondria have a rather long half-life: depletion of mtDNA will only be noticed when the existing mitochondria are substituted by new mitochondria. These new mitochondria are defective, due to the lack of mtDNA encoded enzymes. At present, there are no exact data about the life span of a mitochondrion. The influence of both factors, genetic predisposition and accumulative toxicity, is a possible explanation for the appearance of adverse events in the above cases. All patients with lactic acidosis reported adverse events to prior nucleoside analogues (e.g. neuropathy, myopathy and bone marrow suppression) and all patients developed this severe acidosis after at least several months of antiretroviral therapy *(table 1)*. The neuropathy in patient 4 might also have been related to synergistic toxicity of vinblastine, as this agent might cause mitochondrial toxicity as well. Another predisposing factor might be the patient's nutritional state. Biochemical reactions in mitochondria depend on a large scale of vitamins, co-factors and substrates. In malnourished patients there might be a deficiency for these co-factors, in this way contributing to impaired mitochondrial function.

In our four cases no specific diseases in the medical history of these patients could be kept responsible for the occurrence of the presenting symptoms. None of the patients had a history of chronic hepatitis B or C infection. However, concomitant diseases or co-medication in the period of admission, might have aggravated the mitochondrial toxicity. A precipitating factor in patient 2 might have been sepsis, which certainly enhanced the oxidative stress. Almost all fatal events described in literature occurred during monotherapy with a nucleoside analogue. In the patients discussed in this article, there might have been a toxic synergism between the nucleoside analogues, especially in patient 2, 3 and 4.

In patient 2, hydroxyurea might even have enhanced this synergism.

Up to now, fatal adverse reactions have not been reported for either d4T or 3TC. Recently reversible hepatic steatosis with lactic acidosis was related to the use of d4T [8-10]. Known side effects of d4T are peripheral neuropathy and pancreatitis and as with AZT induced myopathy and ddI/ddC induced neuropathy, these side effects are believed to be caused by acquired mitochondrial disease [11;12]. Although the above described patients had used several nucleoside analogues for a long time, they were all on a d4T containing regimen at the presentation of the symptoms. In patients 2, 3 and 4 ddI might have contributed to the toxicity, as discussed above. Furthermore, in patient 1 3TC use might have contributed to the toxicity, although in contrast to the other nucleoside analogues, 3TC shows little inhibition of mitochondrial DNA synthesis in vitro [22;23].

Since antiretroviral therapy will be used as long-term treatment in many HIV-infected patients, the occurrence of serious adverse events will increase. The chance that prolonged use of any antiretroviral nucleoside analogue might

precipitate this (irreversible) mitochondrial dysfunction will seriously hamper the possibility to control HIV-infection with HAART. More research is necessary to define predisposing factors responsible for this severe drug toxicity more clearly and screening methods to detect this toxicity in an early phase have to be developed. Moreover, information about mitochondria is required in order to gain a clear understanding of their regeneration capacity and to identify whether there is a point of no return. It is not known whether administration of oxidative phosphorylation substrates (i.e. vitamins) might be useful to prevent or treat severe mitochondrial damage. Therefore, the use of L-carnitine is advised in treatment with adefovir, a currently investigated NRTI. As long as predisposing factors and treatment in this syndrome remain unclear, two categories of patients should be monitored frequently: the ones with adverse events to previously used nucleoside analogues and the ones on long-term antiretroviral therapy.

As a result of mitochondrial dysfunction, pyruvate can only be metabolised into lactate, which leads to an increased lactate and lactate/pyruvate ratio. Therefore, measurements of serum lactate and pyruvate demonstrating elevated lactate/pyruvate ratios, might indicate early mitochondrial dysfunction [24;25].

These parameters might also be useful to detect mitochondrial damage in an early stage, when it is still asymptomatic and possibly reversible. Deviant bicarbonate levels and acid-base status will only become apparent in a later stage when lactic acidosis arises. It is crucial to act promptly when the above described symptoms start to occur since a fatal course will follow shortly. Antiretroviral therapy should be stopped immediately and supportive care has to be started to prevent complications of lactic acidosis and to reduce the potential risk of death. In addition, possible beneficial effect in hereditary mitochondrial diseases has been achieved after supplementary treatment with vitamins and co-factors. For example, Coenzyme Q_{10} (CoQ_{10}) and riboflavin have been used in patients with mitochondrial disorders. However, varying degrees of success have been reported in individual cases. It should be stressed that with these therapeutic options used in mitochondrial disorders, only modest improvement has been observed. Unfortunately, the course of the toxicity symptoms and prognosis are difficult to predict and depend largely on the type of tissue affected. In HIV-patients successful recovery of nucleoside analogue-induced lactic acidosis has been attributed to treatment with CoQ_{10} and riboflavin [8;26], supporting the hypothesis that metabolic deficiencies precipitate this toxicity. Adverse effects from co-factors and vitamins are rare, so despite the controversy about efficacy it is advisable to use riboflavin, thiamine, CoQ_{10} and L-carnitine in an attempt to improve the clinical status of a patient with lactic acidosis in order to enhance survival chances. Dosage regimens are uncertain and vary in case reports; therefore a recommendation cannot be given at present. Because the effect of these co-factors remains uncertain, additional support and immediate cessation of the NRTIs is required as well. In summary, treatment started early in the course of mitochondrial toxicity will achieve better survival [27;28].

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References

1 Aggarwal A, al Talib K, Alabrash M. Type B lactic acidosis in an AIDS patient treated with zidovudine. **Md Med J** 1996; 45:929-931.
2 Freiman JP, Helfert KE, Hamrell MR, Stein DS. Hepatomegaly with severe steatosis in HIV-seropositive patients. **AIDS** 1993; 7:379-385.
3 Stein DS, Helfert KE. A new syndrome of hepatomegaly with severe steatosis in HIV seropositive patients. **AIDS Clin Care** 1994; 6:17-20.4 Sundar K, Suarez M, Banogon PE, Shapiro JM. Zidovudine-induced fatal lactic acidosis and hepatic failure in patients with acquired immunodeficiency syndrome: report of two patients and review of the literature. **Crit Care Med** 1997; 25:1425-1430.
5 Chattha G, Arieff AI, Cummings C, Tierney Jr. LM. Lactic acidosis complicating the acquired immunodeficiency syndrome. **Ann Intern Med** 1993; 118:37-39.
6 Lai KK, Gang DL, Zawacki JK, Cooley TP. Fulminant hepatic failure associated with 2'3'-dideoxyinosine (ddI). **Ann Intern Med** 1991; 115:283-284.
7 Bissuel F, Bruneel F, Habersetzer F, et al. Fulminant hepatitis with severe lactate acidosis in HIV-infected patients on didanosine therapy. **J Intern Med** 1994; 235: 367-371.
8 Lenzo NP, Garas BA, French MA. Hepatic steatosis and lactic acidosis associated with stavudine treatment in an HIV patient: a case report. AIDS 1997; 11:1294-1296.
9 Boles RG, Chun N, Senadheera D, Wong LJ. Cyclic vomiting syndrome and mitochondrial DNA mutations. **Lancet** 1997; 350:1299-1300.
10 Luzatti R, Del Bravo P, Di Perri G, Luzanni A, Concia E. Riboflavine and severe lactic acidosis. **Lancet** 1999; 353:901-902.
11 Brinkman K, ter Hofstede HJM, Burger DM, Smeitink JAM, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. **AIDS** 1998; 12:1735-1744.
12 Lewis W, Dalakas MC. Mitochondrial toxicity of antiviral drugs. **Nat Med** 1995; 1:417-422.
13 Olano JP, Borucki MJ, Wen JW, Haque AK. Massive hepatic steatosis and lactic acidosis in a patient with AIDS who was receiving zidovudine. **Clin Infect Dis** 1995; 21:973-976.
14 Lewis W, Perrino FW. Severe toxicity of fialuridine (FIAU). **N Engl J Med** 1996; 334:1136-1138.
15 Peters BS, Winer J, Landon DN, Stotter A, Pinching AJ. Mitochondrial myopathy associated with chronic zidovudine therapy in AIDS. **Q J Med** 1993; 86:5-15.
16 Hoetelmans RM, Burger DM, Meenhorst PL, Beijnen JH. Pharmacokinetic individualisation of zidovudine therapy. Current state of pharmacokinetic-pharmacodynamic relationships. **Clin Pharmacokinet** 1996; 30:314-327.
17 Wilde MI, Langtry HD. Zidovudine. An update of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. **Drugs** 1993; 46:515-578.
18 Lea AP, Faulds D. Stavudine: a review of its pharmacodynamic and pharmacokinetic properties and clinical potential in HIV-infection. **Drugs** 1996; 51:846-864.

19 Perry CM, Balfour JA. Didanosine. An update on its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV-disease. **Drugs** 1996; 52:928-962.
20 Adkins JC, Peters DH, Faulds D. Zalcitabine. An update of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in the management of HIV-infection. **Drugs** 1997; 53:1054-1080.
21 Fortgang IS, Belitsos PC, Chaisson RE, Moore RD. Hepatomegaly and steatosis in HIV-infected patients receiving nucleoside analog antiretroviral therapy. **Am J Gastroenterol** 1995; 90:1433-1436.
22 Gray NM, Marr CL, Penn CR, Cameron JM, Bethell RC. The intracellular phosphorylation of (-)-2'-deoxy-3'-thiacytidine (3TC) and the incorporation of 3TC 5'-monophosphate into DNA by HIV-1 reverse transcriptase and human DNA polymerase gamma. **Biochem Pharmacol** 1995; 50:1043-1051.
23 Magnani M, Brandi G, Casabianca A, et al. 2',3'-Dideoxycytidine metabolism in a new drug-resistant cell line. **Biochem J** 1995; 312:115-123.
24 Chariot P, Monnet I, Mouchet M, et al. Determination of the blood actate:pyruvate ratio as a noninvasive test for the diagnosis of zidovudine myopathy. **Arthritis Rheum** 1994; 37:583-586.
25 Munnich A, Rustin P, Rötig A, et al. Clinical aspects of mitochondrial disorders. **J Inher Metab Dis** 1992; 15:448-455.
26 Fouty B, Frerman F, Reves R. Riboflavin to treat nucleoside analogue-induced lactic acidosis. **Lancet** 1998; 352:291-292.
27 Przyrembel H. Therapy of mitochondrial disorders. **J Inher Dis** 1999; 10:129-146.
28 Taylor RW, Chinnery PF, Clark KM., Lightowlers RN, Turnbull DM. Treatment of mitochondrial disease. **Journal of Bioenergetics and biomembranes** 1997; 29:195-205.

Chapter 3

Serum L-lactate and pyruvate in HIV-infected patients with and without presumed NRTI-related adverse events compared to healthy volunteers

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Abstract

Nucleoside reverse transcriptase inhibitors (NRTIs) used in antiretroviral therapy may cause mitochondrial toxicity. Mitochondrial dysfunction leads to disturbance of the glucose metabolism, resulting in an accumulation of L-lactate (L) and pyruvate (P), with an enhanced L/P ratio.
We analysed lactate and pyruvate blood samples of patients of our outpatient department. Aim of the analysis was to detect preliminary mitochondrial toxicity in patients on antiretroviral nucleoside analogues, which might result in disturbances of L, P, L/P ratio, bicarbonate (Bic) or β-hydroxybutyrate/aceto-acetate (β-HB/AA) ratios.
Blood samples of L, P, Bic, β-HB and AA were analysed in four groups of subjects. The first group (A) consisted of patients with presumed NRTI-related adverse events (n=21), the second group (B) consisted of patients without adverse events (n=28), the third group (C) were HIV-infected patients without antiretroviral therapy (n=6) and the last group (D) were healthy controls (n=12). The mean duration of NRTI treatment was 18 months (range 0 - 78 months).
The mean lactate level in group A was 2319 µmol/L (SD ± 1231, median 1741 µmol/L), in group B 1257 µmol/L (SD ± 607, median 1087), Group C 1285 µmol/L (SD ± 451, median 1245 µmol/L) and 951 µmol/L (SD ± 270, median 979) in the healthy controls. No significant differences in pyruvate, L/P, Bic and β-HB/AA were seen in the four groups. The mean lactate level in patients on stavudine was 1980 µmol/L (SD ± 1197) versus 1051 µmol/L (SD ± 395, p=0.01) in patients on zidovudine. All patients with lactate values above 2700 µmol/L (8) experienced adverse events.
Lactate levels were higher in patients with presumed NRTI-related adverse events. Furthermore, HIV-patients receiving a stavudine containing antiretroviral therapy had higher lactate values than patients without stavudine. Although routine lactate measurement in all patients on antiretroviral therapy is not recommended, lactate measurement might be useful for follow up of patients with presumed NRTI-related adverse events and in patients with lactate levels above 2500 µmol/L. These patients require extra surveillance to evaluate if discontinuation of the current antiretroviral therapy is needed.

Introduction

Although antiretroviral therapy in human immunodeficiency virus (HIV) infected patients has proven to be very effective in the suppression of HIV-infection [1] the efficacy of the drugs has not only resulted in almost complete inhibition of HIV replication, but also in severe side effects [2]. The incidences of these side effects are rather high, which has still been accepted because of the severity of HIV-infection itself. However, drug-safety becomes an even more important issue with the need for life-long treatment since HIV-infection can still only be suppressed and not cured.

The currently used so called highly active antiretroviral therapy (HAART) consists mostly of a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor (PI) or non nucleoside reverse transcriptase inhibitor (NNRTI). NRTIs play a prominent role in antiretroviral therapy. In the years of experience with NRTIs, the following adverse effects have been recorded: (cardio)myopathy (zidovudine, didanosine), neuropathy (stavudine, didanosine, zalcitabine), pancreatitis (stavudine, didanosine, zalcitabine), hepatic steatosis and hepatic failure with lactic acidosis (stavudine, zidovudine, didanosine) [3-5]. The underlying mechanism of these adverse effects is supposed to be mitochondrial toxicity [6;7]. In addition to inhibition of viral reverse transcriptase, the NRTIs inhibit human DNA polymerase γ, an enzyme essential for the mitochondrial DNA replication. In addition, NRTIs may also interfere with mitochondrial RNA formation, which has no repair mechanisms. This may prematurely terminate synthesis of mitochondrial messenger and transfer RNA, leading to impaired transcription and translation [8].

In paediatric literature it has been reported that patients with mitochondrial diseases have abnormal lactate levels due to a diminished function of the aerobe reaction. When the mitochondrial function is impaired, the oxidation of pyruvate cannot take place, and pyruvate will be turned to lactate because this is the only possible reaction outside mitochondria. A shift in the pyruvate to lactate equilibrium will occur, in favour of lactate production. Subsequently, metabolic acidosis might occur if the lactate production exceeds a certain threshold [6;7;9-14]. In HIV-infected patients on antiretroviral therapy these adverse events usually only occur after several months of therapy. There seems to be a certain threshold; passing this threshold results in irreversible damage. Since finally lactic acidosis might develop, which is potentially fatal and which happens to occur rather acutely, a laboratory test to detect mitochondrial toxicity in an early and still reversible phase, is needed.

In this comparative study we analysed the feasibility of lactate (L), pyruvate (P), L/P ratio, bicarbonate and β-hydroxybutyrate/aceto-acetate (β-HB/AA) ratio measurement to test mitochondrial damage. There were four groups: HIV–seropositive patients on antiretroviral therapy with presumed NRTI-induced adverse events related to mitochondrial toxicity, HIV-seropositive patients on antiretroviral therapy without adverse events, HIV–seropositive patients without antiretroviral therapy and healthy volunteers.

Patients and methods

Patients were selected from the clinic population who visited our outpatient department in the period November 1998 till December 1999. Selection of patients was at random. They were enrolled in one of the three HIV-subgroups depending on antiretroviral therapy use and presumed NRTI-related adverse events. Events were considered NRTI-related if all other causes could be excluded after laboratory measurements, cultures, radiologic imaging and if necessary electromyography and biopsy. Healthy volunteers were selected from the normal population and were screened (medical history, physical examination and laboratory) before entering the study.

Group A

The first group consisted of patients with presumed NRTI-related adverse events (n=21).
In the patients with presumed NRTI-related adverse events blood samples were collected within 3 months after the onset of adverse events. The mean duration of NRTI treatment was 20 months (range 3-78 months).

Group B

The second group consisted of patients on antiretroviral therapy without adverse events (n=28). The mean duration of treatment was 15 months (range 0-46 months).

Group C

The third group was a small group of 6 HIV-infected patients without antiretroviral therapy (the majority of our HIV-patients are treated with antiretroviral drugs).

Group D

The last group consisted of healthy volunteers (n=12), who served as controls. Written informed consent was obtained from all subjects.

Measurements

In all subjects we measured lactate, pyruvate, L/P ratio, bicarbonate and β-HB/AA ratios. In group A, B and C the samples were collected when patients visited the outpatient department. In the same period samples from 12 healthy volunteers were analysed. Population characteristics are depicted in *table 1*. A standard procedure for the venal puncture was followed in every subject. All blood samples were taken in the morning after a 5 minutes rest and without stowage of the vein to avoid lactate increase by the procedure itself.

L-Lactate and pyruvate assay in blood

For quantification of L-lactate and pyruvate in blood, enzymatic assays (Roche, Basel Switzerland) with L-lactate dehydrogenase (L-LDH EC 1.1.1.27) were performed. Before the assays were performed the samples were deproteinised

table 1 Population characteristics and L-lactate values.

	Group A	Group B	Group C	Group D
Number of patients	21	28	6	12
Age (years)	42±8	42±8	39±8	28±6
range	27-58	25-53	27-48	20-42
Gender	Male 17	Male 23	Male 4	Male 11
	Female 4	Female 5	Female 2	Female 1
Duration of therapy	20 months	15 months	-	-
range	3-78	0-46		
Median L (µmol/L)	1741	1087	1245	979
Mean L (µmol/L)	2319±1231	1257±607	1285±451	951±270
Range L (µmol/L)	824-5744	440-2644	755-1989	644-1286

L = lactate

with 6 g/L perchloric acid and for the pyruvate assay the perchloric solution was neutralised with 1.75 mol/L K_3PO_4 phosphate solution [15]. Because the reaction between L-lactate and pyruvate is an ongoing reaction by means of the lactate dehydrogenase enzyme (LDH), blood was immediately mixed in a tube containing perchloric acid (as stated above) to stop this reaction. Then the blood samples were placed on ice and instantly transported to the laboratory. The normal values of our laboratory are: L-lactate <1800 µmol/L, pyruvate levels <70 µmol/L, L/P ratio <18, bicarbonate 24-30 mmol/L, aceto-acetate/β-hydroxybutyrate ratio <0.3.

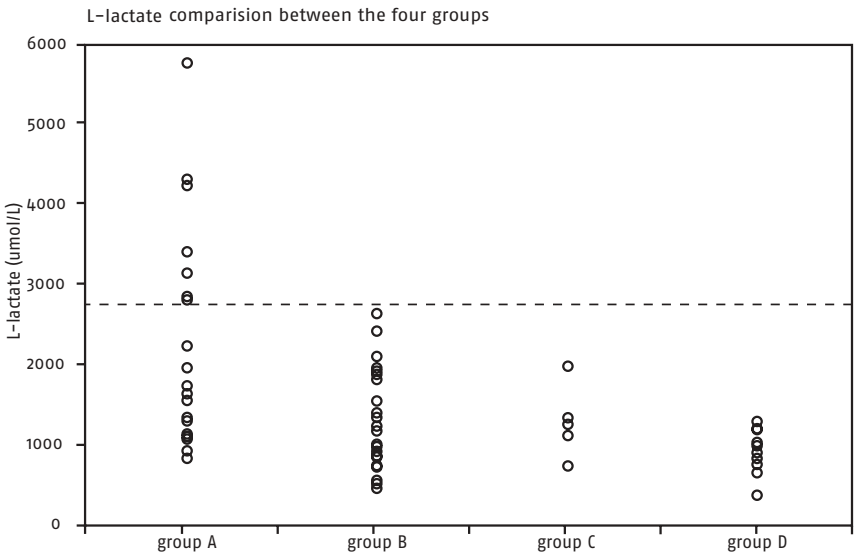
Statistics

Wilcoxon's rank sum test was used to detect statistical differences between the groups.

Results

A total of 67 samples were analysed, for bicarbonate, β-HB/AA ratio, lactate concentrations *(table 1 and 2, figure 1)*, pyruvate and L/P ratio. Bicarbonate levels were normal in the groups (data not shown). Also β-hydroxybutyrate/aceto-acetate ratios were within the normal range and did not show any differences between the groups (data not shown).
In the group of patients with presumed NRTI-related side effects (group A, *table 2*) 10 out of 21 patients (48%) had hyperlactataemia (L >1800 µmol/L). These patients are referred to as 'symptomatic hyperlactataemia'. In group B (patients with antiretroviral therapy without adverse events) 6 out of 28 patients (21%) had lactate levels above the upper limit of normal. In the group of HIV-infected patients still without antiretroviral therapy one patient had an elevated lactate level (1989 µmol/L). Hyperlactataemia was not found in any of the healthy

figure 1 L-Lactates in patients with and without adverse events, HIV-infected patients without antiretroviral therapy and healthy volunteers; individual levels. The line marks the lactate value of 2700 µmol/L. Patients with lactate levels ≥2700 µmol/L all experienced adverse events.



volunteers. Overall the lactate levels were the highest in the adverse event group, median lactate still within the normal range (1741 µmol/L) with an increased mean lactate (2319 µmol/L), but there were no statistical significant differences (p 0.07). The pyruvate levels did not show significant differences, although the mean pyruvate levels were slightly elevated (74 µmol/L) in group A. The highest L/P ratios were seen in group A, although group B and C also showed elevated ratios. Only one healthy volunteer had an elevated L/P ratio (23.1), the mean and median ratio were all normal in this group. Elevated L/P ratios were mostly due to a low pyruvate instead of hyperlactataemia, therefore we consider this measurement not useful.

Lactate levels in comparison with drug regimens and adverse events

Lactate values per drug regimen are depicted in *table 3*. The comparison between the antiretroviral drug regimens is striking. Of the 33 patients on stavudine, 17 had elevated L-lactate levels and 14 experienced adverse events (14/33; 42%). The mean lactate level was 1980 µmol/L (median 1832 µmol/L). None of the 12 zidovudine patients had hyperlactataemia, 7 of them experienced adverse events (7/12; 58%). In this treatment group the mean lactate level was 1051 µmol/L (median 963 µmol/L). Of the total population of HIV-infected subjects (55 patients) 18 patients had hyperlactataemia of whom 17 (17/18; 94%) patients were on a stavudine

table 2 Characteristics of group A.

	G	A	AEs	L	P	L/P	ART	Prior ART	Prior NRTI-tox
1	M	47	HS	3134	135	23.2	D4T, 3TC, IDV	AZT, ddI, ddC	
2	M	42	HS	4232	146	29.0	D4T, ddI, IDV	None	
3	M	37	HS	1952	45	43.3	D4T, ddI, RTV	AZT, ddC, 3TC	Np
4	M	36	HS	2219	94	23.6	D4T, ddC, IDV	AZT, 3TC	
5	M	25	HS	1065	20	53.3	D4T, ddI, IDV + RTV	AZT, ddC, RTV (alone)	
6	M	44	HS	1340	45	29.8	D4T, 3TC, NFV	AZT, ddI, ddC	Np
7	M	44	HS	1288	107	12.0	AZT, 3TC, IDV	None	
8	M	53	Np	4313	75	57.5	D4T, ddI, NVP	None	
9	M	52	Np	2790	129	21.6	D4T, SQV, RTV	None	
10	M	43	Np	2832	106	26.7	D4T, 3TC, NVP	AZT, ddC	Np
11	F	26	Np	5744	117	49.1	D4T, 3TC, SQV, RTV	AZT, ddI	
12	M	52	Mp	1545	50	30.9	D4T, ddI, IDV	AZT, 3TC	Pp
13	M	45	Mp	925	34	27.2	AZT, 3TC, IDV	None	
14	M	35	Mp	901	44	20.5	AZT, 3TC, RTV	None	
15	M	32	Mp	1613	113	14.3	AZT, 3TC, IDV	None	
16	F	42	Mp	1122	40	28.1	AZT, 3TC, IDV	None	
17	M	44	Mp	824	35	23.5	AZT, 3TC, IDV	None	
18	M	44	Am	1103	25	44.1	AZT, 3TC, IDV	None	
19	F	33	Np, HS	2787	45	61.9	D4T, 3TC, IDV	AZT, ddC, ddI	Np
20	M	44	Np, HS	3400	47	72.3	D4T, 3TC, IDV	AZT, ddC, ddI	Np
21	F	37	Np, Am	1721	55	31.3	D4T, 3TC, NFV	AZT	Am

HS = Hepatic Steatosis, Np = Neuropathy, Mp = Myopathy, Pp = Pancytopenia, Am = Anaemia G = gender *(M = male, F = female)*, A = age (years), AEs = adverse events, L = lactate (µmol/L), P = pyruvate (µmol/L) Prior ART = Prior antiretroviral therapy Prior NRTI-tox = Prior NRTI toxicity

table 3 Lactate values per drug regimen.

Drug regimens	Number of patients	Mean L (µmol/L)	Median L (µmol/L)
D4T combinations	33	1980	1832
AZT combinations	12	1051	963
2 PI, no NRTI	3	1061	921
HIV with no ART	6	1222	1176

containing regimen and one patient did not use any antiretroviral therapy at all. Seven of these hyperlactataemia patients had hepatic steatosis (ultrasound or biopsy). Laboratory evaluation did not reveal an underlying hepatic disease. Patients were all using NRTIs in combination with PI of NNRTI. Elevated transaminase levels in one patient with severe liver dysfunction decreased to normal values after a period of discontinuing HAART.

table 4 Lactate values per adverse event.

AEs	Number of patients	Mean L (µmol/L)	Median L (µmol/L)
Neuropathy	7	3644	3116
Hepatic Steatosis	9	2314	2086
Myopathy	6	1155	1024
Anaemia	2	1412	1412
NRTI			
D4T	14	2791	2789
AZT	7	1111	1103

Looking at the drug regimens more closely, demonstrated that stavudine/didanosine combinations revealed the highest lactate levels. There were two patients on a drug regimen with one NRTI. They received stavudine in combination with two PIs. One of these patients had an elevated lactate (2790 µmol/L) and suffered from neuropathy. The mean lactate level in the stavudine treated patients was 1980 µmol/L (SD ± 1197) versus 1051µmol/L (SD ± 395) in the zidovudine treated patients (p=0.01).
The values of lactate per adverse event group are represented in *table 4*. Striking are the highest levels of lactate in the neuropathy patients, who were all on stavudine. Symptomatic hyperlactataemia was mainly seen in stavudine patients. All patients with lactates ≥2700 µmol/L (8 patients) experienced adverse events and they were all on a stavudine containing regimen *(figure 1)*. In group C there were 2 patients with lactates ≥2400 µmol/L. They were both treated with stavudine, one of them died of an AIDS-defining disease nine months later and the other one developed stavudine induced neuropathy one year later.

Discussion

Lactate values showed differences between HIV-infected patients and healthy volunteers, in contrast to bicarbonate, β-HB/AA, pyruvate and L/P ratios, which did not provide additional information. β-HB/AA ratios stayed within the normal range and pyruvate L/P ratios were often elevated only due to a low pyruvate (although still within the normal range) and not as a result of hyperlactataemia. Only mild to moderate hyperlactataemia occurred, which did not result in low bicarbonate levels. There is a difference in hyperlactataemia and lactic acidosis, in the latter the lactate values are severe (mostly >10.000 µmol/L) and result in acidosis, which does not occur in mild to moderate lactate increases. In our study no subject had a lactate level above 6000 µmol/L.
Measurements of lactate demonstrated hyperlactataemia mainly in the NRTI-related adverse event group. Symptomatic hyperlactataemia was found in all patients (8) with a lactate value above 2700 µmol/L *(figure 1)*. Two patients had hepatic steatosis, four were suffering from neuropathy and two of them experienced both

hepatic steatosis and neuropathy. They were all on a stavudine containing regimen. Hyperlactataemia in patients on stavudine is noticeable and not always directly related to adverse events [8;16-23]. Interestingly, the separation of adverse events demonstrated the highest lactate values in patients with presumed NRTI-related neuropathy. This is consistent with a recent study using serum lactate levels in distinguishing between HIV- and NRTI-associated associated neuropathy [24].

Asymptomatic mild hyperlactataemia is a rather common feature of antiretroviral therapy. In recent publications the use of lactate to monitor complications of antiretroviral therapy has been discussed [8;13;20-23;25;26]. Routine lactate measurement is not recommended; a difference has to be made between symptomatic and asymptomatic hyperlactataemia and lactic acidosis. Mild asymptomatic hyperlactataemia requires careful monitoring but no immediate action. However, symptomatic hyperlactataemia and lactate levels above 5000 µmol/L are clinically relevant and need intervention. From our data and literature we suggest that lactate levels of ≥2500 µmol/L need attention and might indicate therapeutic problems in the near future [11;19-21;26-28]. It is advisable to check lactates in patients with NRTI-related neuropathy, hepatic steatosis or elevated transaminases, myopathy and in case of extreme fatigue, unexplained nausea, vomiting, dyspnoea or abdominal pain. These latter and non specific symptoms might be the first signs of sudden (and often fatal) lactic acidosis [11;18;19;29;30]. Patients with hyperlactataemia should be closely monitored and in case of increasing lactates or lactic acidosis, nucleoside analogues should be discontinued [8-10;31]. We would advice to pay especially attention to patients with stavudine-related neuropathy since we found the highest lactate levels in these patients.

In our study the duration of antiretroviral therapy was longer in patients with presumed NRTI-related adverse events than patients without side effects. Symptomatic patients were more often receiving their second (or more) regimen with antiretroviral drugs. Both factors are understandable because, mitochondrial toxicity due to NRTIs is correlated with duration of therapy, prior NRTI therapy and possibly combined use of PI or NNRTI [8;9;22;32].

The risk of hyperlactataemia in patients on antiretroviral therapy increases in patients with drug combinations, which are individually able to induce lactataemia. The risk of lactic acidosis and occurrence of hyperlactataemia is known for all approved nucleoside analogues in HIV-treatment [4;16;20;29;33-34]. Combinations with stavudine and didanosine might be of special concern regarding hyperlactataemia [6;22]. Although the group is rather small we also found higher lactate levels in antiretroviral drug combinations containing both stavudine and didanosine.

Anecdotal reports indicate that the use of NRTI in combination with PI or NNRTI contribute to hyperlactataemia [8;22;35]. The widely use of HAART, with simultaneous use of NRTI(s) with PI or NNRTI might be an additional risk factor in the occurrence of hyperlactataemia. Although the mechanism is not fully explained, a possible explanation might be that PIs and NNRTIs interfere with liver function. NNRTIs are known to cause liver function disorders. PIs are able to interfere with glucose and lipid metabolism resulting in insulin resistance, hyperglycaemia and hyperlipidaemia and finally hepatic steatosis. Lactate clearance depends on liver function and to a lesser extend on the kidneys. Thus, hyperlactataemia can be

due to all antiretroviral medication by increased extra-mitochondrial production in case of NRTI related mitochondrial toxicity or by decreased clearance in case of hepatic or kidney dysfunction [2;32;34-37]. All our patients with hyperlactataemia were treated with combinations of NRTI(s) with PI or NNRTI. We realise that PI and NNTRI use might have contributed to hyperlactataemia in case of hepatic steatosis. Hyperlactataemia due to impaired kidney clearance was not demonstrated; no abnormalities in creatinine were seen (data not shown).
Besides the influence of NRTIs on mitochondria, other factors might also influence mitochondrial function resulting in hyperlactataemia. Biochemical reactions in mitochondria depend on a large scale of vitamins, co-factors and substrates. Case reports have been described treating lactic acidosis due to severe NRTI-induced mitochondrial damage with co-enzyme Q, thiamine, L-carnitine and riboflavin. Although it is likely that metabolic deficiencies may provoke mitochondrial toxicity, it is not clear what effect can be expected by administering these substrates, since antiretroviral therapy was always discontinued in these cases [6;9;10;24;38-40].

In conclusion, hyperlactataemia might indicate early mitochondrial toxicity, however other factors might also play a role in this mechanism. More experience from prospective trials will be needed in order to evaluate the meaning and feasibility of L-lactate measurements in HIV-infected patients on antiretroviral therapy.

References

1 Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. **N Engl J Med** 1998;338:853-60.

2 Carr A, Cooper DA. Adverse effects of antiretroviral therapy. **Lancet** 2000;356:1423-30.

3 Dalakas MC, Illa I, Pezeshkpour GH, Laukaitis JP, Cohen B, Griffin JL. Mitochondrial myopathy caused by long-term zidovudine therapy. **N Engl J Med** 1990;322:1098-105.

4 Sundar K, Suarez M, Banogon PE, Shapiro JM. Zidovudine-induced fatal lactic acidosis and hepatic failure in patients with acquired immunodeficiency syndrome: report of two patients and review of the literature. **Crit Care Med** 1997;25:1425-30.

5 Lai KK, Gang DL, Zawacki JK, Cooley TP. Fulminant hepatic failure associated with 2',3'-dideoxyinosine (ddI). **Ann Intern Med** 1991;115:283-84.

6 Brinkman K, ter Hofstede HJM, Burger DM, Smeitink JA, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. **AIDS** 1998;12:1735-44.

7 Lewis W, Dalakas MC. Mitochondrial toxicity of antiviral drugs. **Nat Med** 1995;1:417-22.

8 Lonergan JT, Behling C, Pfander H, Hassanein TI, Mathews WC. Hyperlactatemia and hepatic abnormalities in 10 human immunodeficiency virus-infected patients receiving nucleoside analogue combination regimens. **Clin Infect Dis** 2000;31:162-66.

9 Brinkman K, ter Hofstede HJ. Mitochondrial toxicity of nucleoside analogue reverse transcriptase inhibitors: Lactic acidosis, risk factors and therapeutic options. **AIDS rev** 1999;1:140-146.

10 ter Hofstede HJM, Brinkman K. Mitochondrial toxicity owing to nucleoside reverse transcriptase inhibitors: importance of clinical features and early diagnosis. **Int Antiviral News** 1999;7:148-51.
11 ter Hofstede HJM, de Marie S, Foudraine NA, Danner SA, Brinkman K. Clinical features and risk factors of lactic acidosis following long-term antiretroviral therapy: 4 fatal cases. **Int J STD AIDS** 2000;11:611-16.
12 Chariot P, Monnet I, Mouchet M, Rohr M, Lefaucheur JP, Dubreuil-Lemaire ML, Chousterman M, Gherardi R. Determination of the blood lactate:pyruvate ratio as a non-invasive test for the diagnosis of zidovudine myopathy. **Arthritis Rheum** 1994;37:583-86.
13 Boffito M, Marietti G, Audagnotto S, Raiter R, Di PG. Lactacidemia in asymptomatic HIV-infected subjects receiving nucleoside reverse-transcriptase inhibitors. **Clin Infect Dis** 2002;34:558-59.
14 Stacpoole PW. Lactic acidosis and other mitochondrial disorders. **Metabolism** 1997;46:306-21.
15 Hohorst H J. In: Bergmeyer, ed. Methoden der enzymatischen analyse. **Weinheim: verleg Chemie**, 1970:1425.
16 Lenzo NP, Garas BA, French MA. Hepatic steatosis and lactic acidosis associated with stavudine treatment in an HIV patient: a case report. **AIDS** 1997;11:1294-96.
17 Vrouenraets SM, Treskes M, Regez RM, Troost N, Smulders YM, Weigel HM, Frissen PH, Brinkman K. Hyperlactataemia in HIV-infected patients: the role of NRTI treatment. **Antivir Ther** 2002;7:239-44.
18 Miller KD, Cameron M, Wood LV, Dalakas MC, Kovacs JA. Lactic acidosis and hepatic steatosis associated with use of stavudine: report of four cases. **Ann Intern Med** 2000;133:192-96.
19 Mokrzycki MH, Harris C, May H, Laut J, Palmisano J. Lactic acidosis associated with stavudine administration: a report of five cases. **Clin Infect Dis** 2000;30:198-200.
20 John M, Moore CB, James IR, Nolan D, Upton RP, McKinnon EJ, Mallal SA. Chronic hyperlactatemia in HIV-infected patients taking antiretroviral therapy. **AIDS** 2001;15:717-23.
21 Gerard Y, Maulin L, Yazdanpanah Y, De LT, X, Amiel C, Maurage CA, Robin S, Sablonniere B, Dhennain C, Mouton Y. Symptomatic hyperlactataemia: an emerging complication of antiretroviral therapy. **AIDS** 2000;14:2723-30.
22 Boubaker K, Flepp M, Sudre P, Furrer H, Haensel A, Hirschel B, Boggian K, Chave JP, Bernasconi E, Egger M, Opravil M, Rickenbach M, Francioli P, Telenti A. Hyperlactatemia and antiretroviral therapy: the Swiss HIV Cohort Study. **Clin Infect Dis** 2001;33:1931-37.
23 Delgado J, Harris M, Tesiorowski A, Montaner JS. Symptomatic elevations of lactic acid and their response to treatment manipulation in human immunodeficiency virus-infected persons: a case series. **Clin Infect Dis** 2001;33:2072-74.
24 Brew BJ, Tisch S, Law M. Lactate concentrations distinguish between nucleoside neuropathy and HIV neuropathy. **AIDS** 2003;17:1094-96.
25 Brinkman K. Editorial response: hyperlactatemia and hepatic steatosis as features of mitochondrial toxicity of nucleoside analogue reverse transcriptase inhibitors. **Clin Infect Dis** 2000;31:167-69.
26 Brinkman K. Management of hyperlactatemia: no need for routine lactate measurements. **AIDS** 2001;15:795-97.
27 Bartley PB, Westacott L, Boots RJ, Lawson M, Potter JM, Hyland VJ, Woods ML. Large hepatic mitochondrial DNA deletions associated with L-lactic acidosis and highly active antiretroviral therapy. **AIDS** 2001;15:419-20.

28 Chariot P, Drogou I, de Lacroix-Szmania I, Eliezer-Vanerot MC, Chazaud B, Lombes A, Schaeffer A, Zafrani ES. Zidovudine-induced mitochondrial disorder with massive liver steatosis, myopathy, lactic acidosis, and mitochondrial DNA depletion. **J Hepatol** 1999;30:156-60.

29 Aggarwal A, al-Talib K, Alabrash M. Type B lactic acidosis in an AIDS patient treated with zidovudine. **Md Med J** 1996;45:929-31.

30 Falco V, Rodriguez D, Ribera E, Martinez E, Miro JM, Domingo P, Diazaraque R, Arribas JR, Gonzalez-Garcia JJ, Montero F, Sanchez L, Pahissa A. Severe nucleoside-associated lactic acidosis in human immunodeficiency virus-infected patients: report of 12 cases and review of the literature. **Clin Infect Dis** 2002;34:838-46.

31 Cote HC, Brumme ZL, Craib KJ, Alexander CS, Wynhoven B, Ting L, Wong H, Harris M, Harrigan PR, O'Shaughnessy MV, Montaner JS. Changes in mitochondrial DNA as a marker of nucleoside toxicity in HIV-infected patients. **N Engl J Med** 2002;346:811-20.

32 Carr A, Samaras K, Chisholm DJ, Cooper DA. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. **Lancet** 1998;351:1881-83.

33 Bissuel F, Bruneel F, Habersetzer F, Chassard D, Cotte L, Chevallier M, Bernuau J, Lucet JC, Trepo C. Fulminant hepatitis with severe lactate acidosis in HIV-infected patients on didanosine therapy. **J Intern Med** 1994;235:367-71.

34 Carr A, Miller J, Law M, Cooper DA. A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. **AIDS** 2000;14:F25-F32.

35 Moyle G. Mitochondrial toxicity hypothesis for lipoatrophy: a refutation. **AIDS** 2001;15:413-15.

36 Dube MP, Johnson DL, Currier JS, Leedom JM. Protease inhibitor-associated hyperglycaemia. **Lancet** 1997;350:713-14.

37 Dube MP. Disorders of glucose metabolism in patients infected with human immunodeficiency virus. **Clin Infect Dis** 2000;31:1467-75.

38 Fouty B, Frerman F, Reves R. Riboflavin to treat nucleoside analogue-induced lactic acidosis. **Lancet** 1998;352:291-92.

39 Semino-Mora MC, Leon-Monzon ME, Dalakas MC. Effect of L-carnitine on the zidovudine-induced destruction of human myotubes. Part I: L-carnitine prevents the myotoxicity of AZT in vitro. **Lab Invest** 1994;71:102-12.

40 Matthews PM, Ford B, Dandurand RJ, Eidelman DH, O'Connor D, Sherwin A, Karpati G, Andermann F, Arnold DL. Coenzyme Q10 with multiple vitamins is generally ineffective in treatment of mitochondrial disease. **Neurology** 1993;43:884-90.

Chapter 4

Oral glucose loading for detection of mitochondrial toxicity during HAART in HIV-infected patients

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Abstract

Nucleoside reverse transcriptase inhibitors used in antiretroviral therapy may cause mitochondrial toxicity. Mitochondrial dysfunction leads to disturbance of the glucose metabolism, resulting in an accumulation of L-lactate. We tested the hypothesis that an oral glucose tolerance test (OGTT) can be used to detect mitochondrial toxicity in patients on antiretroviral nucleoside analogues.
An OGTT was performed in 30 subjects: 16 HIV-infected treated patients without adverse events (group 1) and 14 HIV-infected patients with adverse events related to nucleoside reverse transcriptase inhibitor-induced mitochondrial toxicity (group 2). Lactate was measured at baseline and 60 and 120 min after glucose loading.
At all time points the lactate levels were higher in the adverse events group compared to the other group, with the highest levels of lactate at t=60 min (mean 1912 µmol/L, SD ± 609); mean lactates in the group without adverse events was 1429 µmol/L (SD ± 464). When levels above the upper limit of normal of 1800 µmol/L were used as an indication for mitochondrial toxicity, the sensitivity and specificity were 57% and 81%, respectively. The area under the ROC curve was 0.75. For L-lactate levels >2000 µmol/L the specificity was 90%.
An OGTT with measurement of lactate at baseline and one hour after glucose loading can detect (occult) hyperlactataemia in patients with mitochondrial impairment. From our study we suggest to perform an OGTT as an additional test in patients with symptoms suspect for adverse events to discern mitochondrial toxicity.

Introduction

Highly active antiretroviral therapy (HAART) has altered the prognosis of HIV (human immunodeficiency virus) infected patients dramatically. This means that HAART, usually a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) as a backbone and one protease inhibitor (PI) or non nucleoside reverse transcriptase inhibitor (NNRTI), has converted lethal HIV-infection into a chronic disease. Unfortunately, antiretroviral therapy (ART) is often accompanied by severe adverse events, especially after long term use. Since HIV-infection can only be suppressed and not cured, patients need life-long treatment, which underscores the importance of drug safety.

NRTIs play a prominent role in antiretroviral therapy. After years of experience with these NRTIs, a series of adverse effects have been observed: myopathy, cardiomyopathy, pancreatitis, anaemia, hepatic steatosis, peripheral neuropathy, lactic acidosis and lipoatrophy/hypertrophy [3;5;10]. Mitochondrial toxicity has been identified as key factor in these adverse events [7;8;15;29]. Dysfunction of the OXPHOS system alters mitochondrial and cytoplasmic redox-status, leading among others, to a shift in the pyruvate to lactate equilibrium, in favour of lactate [5;17]. Subsequently, even metabolic acidosis might occur if the L-lactate level in serum, exceeds a certain threshold. Usually these adverse events only occur after several months of therapy. Since lactic acidosis tends to occur rather acutely, a test to detect mitochondrial toxicity in an early and perhaps still reversible state is needed. Normally glucose is metabolised to pyruvate. Oxidation of pyruvate takes place in the mitochondrion while the anaerobic reaction, producing lactate, takes place outside the mitochondrion. Therefore, in mitochondrial dysfunction pyruvate will be metabolised via the alternative pathway with production of lactate. Since in paediatric medicine glucose loading is used for early detection of inherited mitochondrial diseases, we wondered whether an oral glucose tolerance test (OGTT) could be useful in patients treated with antiretroviral therapy for detection of mitochondrial toxicity [16].

Materials and Methods

Study design

Between April 2001 and February 2002 57 HIV-patients (able to communicate in Dutch) visiting our outpatient department were asked for the OGTT study; 30 patients were enrolled. We investigated 2 groups of individuals (total of 30 subjects): 16 HIV-infected patients with antiretroviral therapy who did not suffer from adverse effects (group 1); and 14 HIV-infected patients with adverse effects (AEs) related to mitochondrial toxicity (group 2). At the time of the OGTT the side effects in group 2 were neuropathy (n=10) and hepatic steatosis (n=4).

At our outpatient department all patients are explicitly questioned about possible adverse events (i.e. paraesthesia, muscle pain, abdominal pain and body changes). Possible adverse events were evaluated by means of additional laboratory tests, radiological examination, endoscopy, electromyography or biopsy, if necessary (in patients with hepatic steatosis). Patients with infectious hepatitis,

alcohol abuse, diabetes or medication that might induce hyperlactataemia, were excluded. Events were recorded as a side effect if no other cause was identified. In the four patients with hepatic steatosis the suspected NRTI was finally stopped (after the OGTT) resulting in decrease of transaminases. One patient was rechallenged with the toxicity-associated NRTI, which resulted again in liver abnormalities. In six patients with neuropathy, stavudine was finally stopped (after the OGTT) resulting in diminishing paraesthesia, however not all patients experienced complete relieve of neurological symptoms. There was no difference in family history of diabetes or body mass index (BMI) between the two groups (nobody had a BMI >27 kg/m^2).
Subjects were screened by means of history taking, physical examination and blood tests. The research protocol was approved by the ethic committee of the University Medical Centre Nijmegen and written informed consent was obtained from all subjects. Characteristics of the groups are depicted in *table 1 and 2*.

Oral glucose tolerance test (OGTT)

The oral glucose tolerance tests (OGTTs) were performed at the outpatient clinic in the morning. The OGTT was performed after a 12 h overnight fast and at least 5 min after the placement of a peripheral intravenous catheter, in order to prevent enhanced lactate levels by stress. For the same reason no tourniquet was used. Patients were instructed not to use alcohol the day prior to the test and to avoid exercise prior to and during the test. After the first blood sample was taken (t=0), patients had to drink a solution of 75 g glucose in 250 ml water within 3 minutes, to create an effective glucose load. At t=0 minutes blood samples were taken for glucose, lactate, lipids and insulin. At t=60 and 120 minutes glucose and lactate were measured. Cholesterol and triglycerides were also measured at t=120 min. At the time of the OGTT patients were still suffering from the AEs.

L-Lactate

For the measurement of lactate special vacuum tubes with perchloric acid were made in the laboratory. Blood and perchloric acid (6 g/L) were mixed in a ratio of 1:1 in order to stop the otherwise ongoing reaction between pyruvate and lactate. The samples were immediately put on ice and transported to the laboratory in order to be measured directly after the test. Samples were analysed batch-wise. For quantification of L-lactate in blood, enzymatic assays (Roche, Basel Switzerland) with L-lactate dehydrogenase (L-LDH EC 1.1.1.27) were performed [22]. Lactate levels above the upper limit of normal (>1800 µmol/L) was referred to as hyperlactataemia. Symptomatic hyperlactataemia was defined as a combination of hyperlactataemia with AEs.

Statistics

Group characteristics and serum levels were compared using the Wilcoxon test. The Koziol test was used to compare serum levels that were measured more than once. Two sided p-values were calculated and a result with $p<0.05$ was considered statistically significant.

table 1 Characteristics of HIV-patients.

	Group 1 HIV-infected patients with antiretroviral therapy without adverse effects	Group 2 HIV-infected patients with antiretroviral therapy with adverse effects related to mitochondrial toxicity
Number	16	14
Gender	16 male / 0 female	11 male / 3 female
Mean age in years (range)	45 (40-52)	41 (27-52)
Mean duration of HIV in months	72 ± 19	92 ± 24
Mean CD_4 per mm^3 ± SD	424 ± 232	353 ± 275
Mean viral load copies/ml (range)	13.5 x 10^5 (<50-10^5)	3.0 x 10^4 (<50-10^5)
Mean duration of ART in months (range)	51 (10-72)	66 (11-78)
Hemoglobin (mmol/L) ± SD	8.8 ± 0.8	8.5 ± 0.9
Leucocytes (x 10^9/L) ± SD	6.2 ± 1.9	6.0 ± 2.3
Platelets (x 10^9/L) ± SD	227 ± 74	231 ± 75
Creatinine (µmol/L) ± SD	93 ± 20	90 ± 16

In order to evaluate the ability of the OGTT to detect mitochondrial toxicity, we determined the sensitivity and specificity when the upper limit of normal of 1800 µmol/L for the 60 minutes lactate level was used as cut-off value: higher levels were assumed to be an indication for toxicity.
In addition, we calculated the area under the ROC curve (AUC) and we determined the cut-off value for which the sum of the sensitivity and specificity was maximal. We used multiple logistic regression to evaluate the impact of other variables on the result and to evaluate whether other variables or combinations of variables, possibly in combination with lactate levels, would lead to a higher AUC. For this purpose we carried out a stepwise model selection procedure with the variables lactate level, age, BMI, disease duration, duration of therapy and type of therapy.

Results

An OGTT was performed in 30 subjects. Measurement of haemoglobin, leukocytes, platelets, creatinine and insulin before the OGTT did not show significant differences between the two study groups *(table 1)*. Mean time between AEs and the OGTT was 11 months (range 6-20).

Serum levels

The mean baseline glucose levels were in the range between 4.9 and 6.2 mmol/L. At t=60 min the glucose levels increased to mean levels of 8.6 mmol/L in the group of patients with adverse effects and 8.5 mmol/L in group 1. At 120 min the glucose levels declined again to baseline levels.

At baseline hyperlactataemia was only seen in three patients with AEs. The highest lactate levels were found in the AEs group at all time points compared to the group without AEs. An increase of lactate occurred in both groups at t=60 min.

Lactate levels and adverse events

With 1800 µmol/L as cut-off for the 60 minutes lactate levels, the sensitivity was 57%: eight patients in group 2 had high levels (1900-2990 µmol/L). The specificity was 81%: elevated lactates (>1800) were found in three patients in group 1 (resp 1830, 2100 and 2160 µmol/L; 3/16 = 19%).
The area under the ROC curve was 0.75 and a cut off value of 1660 µmol/L lead to slightly better results: a sensitivity of 64% and a specificity of 81%.
No statistically significant relation between age, BMI, disease duration, and type of therapy on the one hand and adverse events on the other hand was found. Patients with adverse events, however, had longer duration of medication (p=0.04). The combination of lactate level and treatment duration lead to an AUC of 0.80.
Patients with neuropathy had higher lactates during the OGTT than patients with hepatic steatosis. This was reflected in the use of stavudine; 80% of patients with hyperlactataemia were on a stavudine although a statistically significant difference could not be reached.

Discussion

Our results demonstrate that an OGTT might be useful to detect (occult) hyperlactataemia as a result of mitochondrial impairment. At all time points mean L-lactate levels in the AEs group were higher compared to the other group, with the highest value at t=60 min. One hour after glucose loading 8 (57%) of the patients with AEs had hyperlactataemia (>1800 µmol/L) compared to 3 (19%) in the group without AEs, corresponding to a sensitivity of 57% and a specificity of 81%. Althought the ROC analysis suggested 1660 µmol/L as a cut-off point, this did not lead to higher specificity and the sensitivity only improved from 57% to 64%. The combination of duration of medication gave an AUC of 0.80.
Symptomatic hyperlactataemia with mitochondrial abnormalities in muscle biopsies has been demonstrated by Gérard et al [18]. They found an incidence of 0.8% per year in HIV-infected adults on antiretroviral therapy, presenting with unexplained clinical symptoms. Brew at all showed a significant correlation between serum lactate and the development of nucleoside related neuropathy [6]. At random measurement of lactate in patients from our out-patient department revealed overall higher lactate levels in patients suffering from ART-related neuropathy [41].
The question remains if symptomatic hyperlactataemia due to mitochondrial toxicity affects only a minority of patients or might affect every individual in the long term as a result of cumulative drug exposure [24;29]. From our data we found that the mean duration of antiretroviral therapy was longer in patients from group 2 (66 months) compared to patients from group 1 (51 months).
Most patients with elevated lactate levels in both groups were on stavudine (80%). Similar to our results, other studies found elevated lactate levels more

table 2a Treatment regimens of HIV-patients (group 1).

Group 1	Gender	Age	Medication
1	M	46	D4T + 3TC + NVP
2	M	49	AZT + 3TC + NVP
3	M	51	D4T + ddI
4	M	48	D4T + 3TC + IDV + RTV
5	M	54	AZT + 3TC + NFV
6	M	44	D4T + 3TC + NVP
7	M	39	D4T + 3TC + IDV
8	M	42	AZT + 3TC + NVP
9	M	49	D4T + 3TC + NVP + LPV + RTV
10	M	37	D4T + 3TC + IDV + RTV
11	M	48	D4T + ddI + NVP
12	M	39	D4T + 3TC + NVP
13	M	42	AZT + 3TC + IDV
14	M	51	D4T + 3TC + IDV
15	M	43	D4T + 3TC + IDV + RTV
16	M	47	D4T + 3TC + NVP

table 2b Treatment regimens of HIV-patients (group 2).

Group 2	Gender	Age	Medication	Adverse event	Duration of AEs at time of OGTT (months)
1	M	48	D4T + 3TC + LPV + RTV	neuropathy	10
2	M	49	D4T + ddI + IDV + RTV	neuropathy	7
3	M	55	D4T + ddI + EFV	neuropathy	7
4	M	37	D4T + 3TC + IDV + RTV	neuropathy	20
5	M	38	D4T + 3TC + NVP	neuropathy	8
6	M	27	D4T + ddI + SQV + RTV	neuropathy	10
7	F	39	D4T + 3TC + NFV	neuropathy	12
8	F	35	D4T + ddI + EFV	neuropathy	16
9	M	41	D4T + 3TC + NVP	neuropathy	11
10	M	46	D4T + ABC + APV	neuropathy	14
11	M	32	D4T + 3TC + LPV + APV	hepatic steatosis	9
12	M	47	AZT + 3TC + NVP	hepatic steatosis	6
13	M	47	AZT + 3TC + SQV + RTV	hepatic steatosis	14
14	F	43	AZT + 3TC + NFV	hepatic steatosis	16

M = male, F = female
D4T = stavudine, 3TC = lamivudine, AZT = zidovudine, ddI = didanosine, ABC = abacavir, EFV = efavirenz, NVP = nevirapine, LPV = lopinavir, APV = amprenavir, IDV = indinavir, RTV = ritonavir, SQV = saquinavir.

frequently in stavudine containing regimens [21;25;27;28;30;31;36;40;42]. Routine lactate measurement in patients on ART has not been recommended because low grade stable hyperlactataemia or intermittent hyperlactataemia is of poor predictive value for impending lactic acidosis [26;37]. However, lactate measurement in combination with an OGTT can be used to detect NRTI-related toxicity in patients suspected of mitochondrial related events, especially when lactate levels >2000 µmol/L are found. Although the sensitivity is not very high (around 50%), the specificity is 80% for lactates >1800 µmol/L and reaching 90% specificity for levels >2000 µmol/L. Future tests have to be performed in order to improve the diagnositic value of the test.

Elevated L-lactate levels can also occur in liver and kidney failure due to impaired clearance by these organs [17;32]. Looking at our population with elevated L-lactate levels after glucose loading, only 2 patients had elevated transaminases without impairment of liver function. No other causes could be identified that might have induced the transaminase levels except the antiretroviral drugs. There were no patients with hepatitis B or C co-infection and none of the patients had impaired kidney function [1].

An OGTT with measurement of lactate one hour after glucose loading can be used as additional test in patients with suspected drug-induced mitochondrial impairment to detect (occult) hyperlactataemia. Based on our study and literature we would not recommend measurement of lactate more then one hour after glucose loading. Lactate levels >1800 µmol/L during the OGTT were associated with antiretroviral therapy related AEs. We suggest performing an OGTT as an additional test in patients with symptoms suspect for AEs to discern NRTI-related mitochondrial toxicity. Depending on the severity of symptoms and the possibility to start other drugs, antiretroviral therapy should be switched in patients with toxicity. Additional data will be necessary to evaluate the clinical importance of an OGTT as early predictor for the development of side effects.

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References

1 Becker S. Liver toxicity in epidemiological cohorts. **Clin Infect Dis** 2004; 38:S49-S55.

2 Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. **Trends Endocrinol Metab** 2000; 11:351-356.

3 Bissuel F, Bruneel F, Habersetzer F, Chassard D, Cotte L, Chevallier M, Bernuau J, Lucet JC, Trepo C. Fulminant hepatitis with severe lactate acidosis in HIV-infected patients on didanosine therapy. **J Internal Med** 1994; 235:367-371.

4 Blanco F, Carr A. (). Lipodystrophy syndrome: Diagnostic, clinic and therapeutic aspects. **AIDS rev** 2004; 3:98-105.

5 Boles R G, Chun N, Senadheera D, Wong L J. Cyclic vomiting syndrome and mitochondrial DNA mutations. **Lancet** 1997; 350:1299-1300.

6 Brew B J, Tisch S, Law M. Lactate concentrations distinguish between nucleoside neuropathy and HIV neuropathy. **AIDS** 2003; 17:1094-1096.

7 Brinkman K. Editorial response: hyperlactatemia and hepatic steatosis as features of mitochondrial toxicity of nucleoside analogue reverse transcriptase inhibitors. **Clin Infect Dis** 2000; 31:167-169.

8 Brinkman K, ter Hofstede H J, Burger DM, Smeitink J A, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. **AIDS** 1998; 12:1735-1744.

9 Carr A. HIV protease inhibitor-related lipodystrophy syndrome. **Clin Infect Dis** 2000; 30:S135-S142.

10 Carr A, Cooper D A. Adverse effects of antiretroviral therapy. **Lancet** 2000; 356:1423-1430.

11 Carr A, Law M. An objective lipodystrophy severity grading scale derived from the lipodystrophy case definition score. **J Acquir Immune Defic Syndr** 2003; 33:571-576.

12 Carr A, Miller J, Law M, Cooper D A. A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. **AIDS** 2000; 14:F25-F32.

13 Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, Cooper DA. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. **AIDS** 1998; 12:F51-F58.

14 Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. **Lancet** 1999; 353:2093-2099.

15 Chariot P, Monnet I, Mouchet M, Rohr M, Lefaucheur JP, Dubreuil-Lemaire ML, Chousterman M, Gherardi R. Determination of the blood lactate:pyruvate ratio as a noninvasive test for the diagnosis of zidovudine myopathy. **Arthritis Rheum** 1994; 37:583-586.

16 Chi CS, Mak SC, Shian WJ, Chen CH. Oral glucose lactate stimulation test in mitochondrial disease. **Pediatr Neurol** 1992; 8:445-449.

17 Chinnery PF, Turnbull DM. Vomiting, anorexia, and mitochondrial DNA disease. **Lancet** 1998; 351:448.

18 Gerard Y, Maulin L, Yazdanpanah Y, De LT, Amiel C, Maurage CA, Robin S, Sablonniere B, Dhennain C, Mouton Y. Symptomatic hyperlactataemia: an emerging complication of antiretroviral therapy. **AIDS** 2000; 14:2723-2730.

19 Grinspoon S. Mechanisms and strategies for insulin resistance in acquired immune deficiency syndrome. **Clin Infect Dis** 2003; 37:S85-S90.

20 Grunfeld C, Tien P. Difficulties in understanding the metabolic complications of acquired immune deficiency syndrome. **Clin Infect Dis** 2003; 37:S43-S46.
21 Harris M, Tesiorowski A, Montaner J. Screening for nucleoside-associated lactic acidosis. **XIIII international AIDS Conference** 2000; Abstract
22 Hohorst HJ, In: Bergmeyer, ed. Methoden der enzymatischen analyse. **Weinheim: verleg Chemie** 1970;1425.
23 Jain RG, Furfine ES, Pedneault L, White AJ, Lenhard JM. Metabolic complications associated with antiretroviral therapy. **Antiviral Res** 2001; 51:151-177.
24 John M, Mallal S. Hyperlactatemia syndromes in people with HIV infection. **Curr Opin Infect Dis** 2002; 15:23-29.
25 John M, Moore CB, James IR, Nolan D, Upton RP, McKinnon EJ, Mallal SA. Chronic hyperlactatemia in HIV-infected patients taking antiretroviral therapy. **AIDS** 2001; 15:717-723.
26 John M, Nolan D, Mallal S. Antiretroviral therapy and the lipodystrophy syndrome. **Antivir Therapy** 2001; 6:9-20.
27 Le Bras P, D'Oiron R, Quertainmont Y, Halfon P, Caquet R. Metabolic, hepatic and muscular changes during zidovudine therapy: a drug-induced mitochondrial disease? **AIDS** 1994; 8:716-717.
28 Lenzo NP, Garas BA, French MA. Hepatic steatosis and lactic acidosis associated with stavudine treatment in an HIV patient: a case report. **AIDS** 1997; 11:1294-1296.
29 Lewis W. Mitochondrial dysfunction and nucleoside reverse transcriptase inhibitor therapy: experimental clarifications and persistent clinical questions. **Antiviral Res** 2003; 58:189-197.
30 Lewis W, Dalakas MC. Mitochondrial toxicity of antiviral drugs. **Nat Med** 1995; 1:417-422.
31 Lonergan J T, Havlir D V, Barber E. Incidence and outcome of hyperlactataemia associated with clinical manifestations in HIV-infected adults receiving NRTI-containing regimens. **8th Conference on retroviruses and opportunistic infections** Chicago USA 2001; Abstract
32 Madias NE. Lactic acidosis. **Kidney Int** 1986; 29:752-774.
33 McComsey G. Update on mitochondrial toxicity of antiretrovirals and its link to lipodystrophy. **AIDS Rev** 2002; 4:140-147.
34 McComsey G, Lonergan JT. Mitochondrial Dysfunction: Patient Monitoring and Toxicity Management. **J Acquir Immune Defic Syndr** 2004; 37:S30-S35.
35 McComsey GA, Ward DJ, Hessenthaler SM, Sension MG, Shalit P, Lonergan JT, Fisher RL, Williams VC, Hernandez JE. Improvement in lipoatrophy associated with highly active antiretroviral therapy in human immunodeficiency virus-infected patients switched from stavudine to abacavir or zidovudine: the results of the TARHEEL study. **Clin Infect Dis** 2004; 38:263-270.
36 Montaner JS, Cote HC, Harris M, Hogg RS, Yip B, Harrigan PR, O'Shaughnessy MV. Nucleoside-related mitochondrial toxicity among HIV-infected patients receiving antiretroviral therapy: insights from the evaluation of venous lactic acid and peripheral blood mitochondrial DNA. **Clin Infect Dis** 2004; 38:S73-S79.
37 Moyle GJ, Datta D, Mandalia S, Morlese J, Asboe D, Gazzard BG. Hyperlactataemia and lactic acidosis during antiretroviral therapy: relevance, reproducibility and possible risk factors. **AIDS** 2002; 16:1341-1349.
38 Nolan D, Hammond E, James I, McKinnon E, Mallal S. Contribution of nucleoside-analogue reverse transcriptase inhibitor therapy to lipoatrophy from the population to the cellular level. **Antivir Ther** 2003; 8:617-626.
39 Saint-Marc T, Partisani M, Poizot-Martin I, Rouviere O, Bruno F, Avellaneda R, Lang J M,

Gastaut JA, Touraine JL. Fat distribution evaluated by computed tomography and metabolic abnormalities in patients undergoing antiretroviral therapy: preliminary results of the LIPOCO study. **AIDS** 2000; 14:37-49.

40 Sundar K, Suarez M, Banogon PE, Shapiro JM. Zidovudine-induced fatal lactic acidosis and hepatic failure in patients with acquired immunodeficiency syndrome: report of two patients and review of the literature. **Crit Care Med** 1997; 25:1425-1430.

41 ter Hofstede HJM, Willems HL, Koopmans PP. Serum L-lactate and pyruvate in HIV-infected patients with and without presumed NRTI-related adverse events compared to healthy volunteers. **J Clin Virol** 2004; 29:44-50.

42 Vrouenraets SM, Treskes M, Regez RM, Troost N, Smulders YM, Weigel HM, Frissen P H, Brinkman K. Hyperlactataemia in HIV-infected patients: the role of NRTI-treatment. **Antivir Ther** 2002; 7:239-244.

Section

Lipid and glucose disturbances

Chapter 5

Metabolic parameters in lopinavir use and after switch to abacavir

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Abstract

Protease inhibitors are known to interfere with lipid and glucose metabolism resulting in among others insulin resistance with hyperglycaemia, hypertriglyceridaemia and elevated total and LDL-cholesterol. In this study we investigated metabolic effects in antiretroviral therapy-naive HIV-patients.
This was a substudy of a prospective clinical trial of HIV antiretroviral therapy-naive patients who started on a regimen of AZT/3TC and LPV/RTV and were randomised to either continuation or switch to AZT/3TC/ABC at week 24. Prospectively we measured plasma lipids, apolipoproteins, glucose, insulin, leptin and hsCRP concentrations from baseline to week 72 in 21 treatment-naive HIV-seropositive patients.
In the first 24 weeks after start of LPV/RTV treatment there was an increase of serum lipids: total cholesterol 25%, LDL and VLDL-cholesterol 16% and 43% respectively, triglycerides 32% and VLDL triglycerides 40% and lp(a) 36%. The lipid values stabilised after week 24 in the LPV/RTV users and decreased to baseline levels in the group that switched to AZT/3TC/ABC; decrease in total cholesterol of 15% ($p<0.001$), in LDL-cholesterol 13% ($p=0.03$) and 31% decrease in triglycerides ($p=0.003$).
Antiretroviral therapy with LPV/RTV is associated with an atherogenic lipoprotein profile. Switch to AZT/3TC/ABC induced recovery of these atherogenic changes. Antiretroviral treatment with AZT/3TC/ABC has no negative metabolic effects.

Introduction

The metabolic syndrome consisting of impaired glucose tolerance, insulin resistance and dyslipidaemia is considered as one of the most important side effects of antiretroviral therapy (ART) in HIV-infection [1;2]. The pattern of dyslipidaemia is especially seen in patients on protease inhibitors (PIs) and is characterised by elevation in total and low density lipoprotein (LDL) cholesterol and triglycerides, together with a decrease in high density lipoprotein (HDL) cholesterol [3-6]. This lipid profile is known as the atherogenic lipoprotein profile which is associated with increased cardiovascular risk [7;8]. In contrast to PIs, the non nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine and efavirenz have a positive effect on HDL-cholesterol [9-11]. In general NNRTIs are associated with a more favourable lipid profile than the PIs. Of the PIs nelfinavir has the most favourable lipid profile [12].

Incidence rates vary but up to 40% of HIV-infected patients on ART may experience dyslipidaemia [13-15]. Hypercholesterolaemia and hypertriglyceridaemia after lopinavir and ritonavir use was found to occur in 17% and 40% respectively [16]. Elevated triglycerides presented in small very low density lipoprotein (VLDL) particles are also considered to be atherogenic, although it is not clear if this is the predominant form of VLDL in HIV-patients. Next to antiretrovirals, genetic factors such as apolipoprotein C (apoC) and apolipoprotein E (apoE) variants play a role in ART-associated dyslipidaemia [17-20].

The pathogenesis of insulin resistance is complex, including direct effects of antiretroviral medication (particularly PIs) on glucose uptake and indirect effects of dyslipidaemia, inflammation and body composition. Laboratory parameters that have been studied in this process are leptin and high sensitive C-reactive protein (hs-CRP) [21;22]. Until now, extensive measurement of these cardiovascular-related metabolic parameters has not been combined in a prospective trial comparing a PI-containing regimen versus switch to a non-PI-containing regimen.

Since HIV-patients have to endure long term ART, reduction of side effects is very welcome. Ritonavir-boosted lopinavir (LPV/RTV) is a very potent PI combination, but due to the relative unfavourable metabolic side effects, its use in the last years has been changed from first to second line treatment, at least in most of the Dutch centres for the treatment of HIV-infection. The FREE study is a randomised controlled trial with the aim to explore whether the metabolic side effects of LPV/RTV could be avoided if it is replaced by a triple nucleoside regimen. In this trial all patients started with zidovudine and lamivudine (given as the combination drug combivir®; AZT/3TC) and LPV/RTV (given as the combination drug kaletra®), When the viral load was below 50 copies/ml between week 12 and week 24 after start of treatment, the patients were randomised to continuation of this regimen or to the combination drug trizivir® (AZT/3TC/ABC), which contains 3 nucleoside reverse transcriptase inhibitors (NRTIs); abacavir 300 mg (ABC), zidovudine 300mg (AZT) and lamivudine 150 mg (3TC) b.i.d.

We report the results of a substudy of this clinical trial in which we determined several biochemical parameters of glucose and lipid metabolism and inflammation.

Methods

Study design

The FREE study (ClinicalTrials.gov NCT00405925) is a prospective, multi centre cohort. Two centres joined this substudy: UMC st Radboud Nijmegen and Rijnstate hospital Arnhem.

Inclusion criteria for the FREE study were: male or females older than 18 years, able and willing to sign informed consent, CD_4 count ≤350/mm^3 and HIV-1 RNA ≥30.000 copies/ml. The following metabolic parameters were required for inclusion: fasting glucose ≤7 mmol/L (non-fasting <11 mmol/L), fasting triglycerides ≤2 mmol/L and LDL-cholesterol ≤4 mmol/L or LDL/HDL ratio ≤4.1. Patients were excluded if metabolic parameters were not within the range requested in the inclusion.

Initially 28 patients were screened. A total of 21 patients started antiretroviral therapy between March 2003 and January 2006 and were consecutively observed up to week 72.

Follow-up visits were carried out at fixed time points: at baseline, and week 12, 24, 48 and 72. Venous blood samples were drawn for the measurement of the following metabolic parameters: total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, triglycerides, VLDL-triglycerides, lipoprotein (a) (Lp(a)), apolipoprotein B (apoB), fasting glucose, fasting insulin, oxidised LDL (oxLDL), serum autoantibodies against oxLDL (ab oxLDL), High sensitive CRP (hsCRP) and leptine. ApoE phenotyping was only investigated at baseline.

Laboratory measurements

Venous blood was drawn by venipuncture after an overnight fast. Plasma total cholesterol and triglycerides concentrations were determined using commercially available enzymatic reagents (Hitachi 747, Roche, Almere, The Netherlands). VLDL-cholesterol was isolated from whole plasma by ultracentrifugation at density (d) = 1.006 g ml^{-1} for 16 h at 36,000 rpm in a fixed angle rotor (TFT 45.6 rotor, Kontron), in a Beckman L7-55 ultracentrifuge. HDL-cholesterol was determined by the polyethylene glycol 6000 method [23]. LDL-cholesterol was calculated by subtraction of VLDL-cholesterol and HDL-cholesterol from plasma triglycerides. Enzymatic, commercially available reagents (Roche Molecular Biochemicals, Germany, catalog no. 237574 and Sera Pak, Miles, Belgium, catalog no. 6639, respectively) determined cholesterol and triglycerides. Total plasma apoB concentration was determined by immunonephelometry [24]. Both hsCRP (Imtec Immun-diagnostica, Berlin, Germany) and plasma oxLDL (Mercodia, Uppsala, Sweden) were determined by ELISA. Plasma glucose was determined by a commercially available glucose oxidation method (GOD-PAP, Hitachi 747; Roche Molecular Biochemicals, Indianapolis, IN). Plasma insulin concentrations were assessed by means of radioimmunoassay (in-house RIA [interassay coefficient of variation (CV), 10.3%]) [25]. Insulin resistance was assessed by the homeostasis model assessment (HOMA). The HOMA-index was calculated from the fasting concentrations of insulin and glucose using the following formula: HOMA-index = fasting serum insulin (mU l^{-1}) x fasting plasma glucose (mmol l^{-1})/22.5 [26].

table 1 Patients' characteristics.

	AZT/3TC+LPV/RTV	AZT/3TC+ABC
Number of patients enrolled	16	12
Sex (%)	15 males	8 males
	1 female	4 females
Race (%)	11 Caucasian	9 Caucasian
	3 Black	2 Black
	2 Asian	1 Asian
Only baseline data	3	
Data till week 24	4	
Data till week 72	9	12
Number of eligible patients	9	12
CDC classification A	5	10
CDC classification B	3	2
CDC classification C	1	0
Mean HIV-RNA level (log copies/mL)	5.92	5.35
CD_4 cell count (cells/mm^3) ± SD	183 ± 124	161 ± 87
BMI (kg/m^2) ± SD	22 ± 4	23 ± 3
Age (years) ± SD	42 ± 10	39 ± 10

Statistics

In order to remove the skewness in the laboratory data, we first applied a logarithmic transformation. The transformed variables were then analyzed using a mixed linear model with random factor patient and fixed factors time and treatment. We calculated 95% confidence intervals (CI) on the logarithmic scale and we transformed these back into percentages for interpretation.

Ethics

The research protocol was approved by the regional Medical Ethic Committee Arnhem-Nijmegen and written informed consent was obtained from all subjects.

Results

A total of 28 patients were initially screened for this study and 21 were consecutively observed up to week 72. In 3 patients only baseline data were available, they did not meet the criteria for the main (FREE) study and in 4 patients we could only collect data till week 24.
During the study period five patients presented with adverse events classified as grade 2 hypercholesterolaemia (>6.2 mmol/L; WHO toxicity scale) whereas no patients experienced grade 2 hypertriglyceridaemia (>4.5 mmol/L).

table 2 Laboratory parameters at baseline, at randomisation and at week 72 and differences in metabolic parameters after switch to AZT/3TC/ABC.
(Group A = Week 72 AZT/3TC +LPV/RTV group, Group B = Week 72 AZT/3TC/ABC group)

Parameter	Baseline	Rs	Change Baseline-Rs		Group A	Group B	Differences after switch to Group B compared to Group A	
Total chol (mmol/L)	4.39	5.49	25%	(p<0.001)	5.39	4.67	-15%	(p<0.001)
LDL-chol (mmol/L)	2.87	3.33	16%	(p=0.02)	3.20	2.90	-13%	(p=0.03)
HDL-chol (mmol/L)	0.94	0.97	33%	(p<0.001)	1.28	0.99	2%	(p=0.62)
VLDL-chol (mmol/L)	0.53	1.39	43%	(p=0.06)	0.91	0.97	-30%	(p=0.12)
TG (mmol/L)	1.57	2.07	32%	(p=0.009)	2.19	1.42	-31%	(p=0.002)
VLDL-TG (mmol/L)	0.99	1.39	40%	(p=0.05)	1.63	1.28	-8%	(p=0.70)
Lp(a) (mg/L)	400	544	36%	(p<0.001)	643	533	-2%	(p=0.84)
ApoB (mg/L)	891	1042	17%	(p<0.001)	1068	916	-12%	(p=0.008)
Glucose (mmol/L)	4.8	4.9	2%	(p=0.36)	5.3	4.9	0%	(p=0.91)
Insuline (mU/ml)	10.0	8.9	-11%	(p=0.31)	9.9	10.5	18%	(p=0.43)
oxLDL (U/L)	72	78	8%	(p=0.28)	76	73	-6%	(p=0.27)
hsCRP (mg/L)	3.9	2.15	-45%	(p=0.01)	1.20	3.70	72%	(p=0.18)
Leptine (ng/ml)	9.3	11.9	26%	(p=0.05)	6.0	14.0	18%	(p=0.24)
HOMA-index	2.1	1.9	-11%	(p=0.33)	2.4	2.0	4%	(p=0.86)

Rs = randomisation, TG = triglycerides, chol = cholesterol.
At baseline and randomisation all patients are on AZT/3TC+LPV/RTV.

Baseline values are depicted in table 1. In the first weeks (mostly 24) till randomisation total cholesterol increased with 25% (CI 14%-37%, P<0.0001) due to increase in LDL-cholesterol (16%) and VLDL-cholesterol (43%) and HDL-cholesterol (33%). The mean total cholesterol at baseline was 4.39 mmol/L and increased to 5.49 mmol/L (range 2.47-6.50 mmol/L). Triglycerides increased with 32% due to increase in VLDL triglycerides (40%). Mean triglycerides changed from 1.57 mmol/L at baseline (range 0.78-2.91) to a mean value of 2.07 mmol/L at randomisation (range 1.01-4.10 mmol/L). Increase of 36% was seen in lp(a). ApoB increased (17%) due to increase in LDL and VLDL particles. Overall patients with low baseline levels had a slightly larger increase in lipids compared to patients with rather high baseline levels although the difference was not statistically significant.
No effect on glucose (2% increase), insulin and HOMA-score (both 11% decrease) was found. This was also true for LDL/HDL ratio. No effect of LPV/RTV and AZT/3TC/ABC was found on the inflammatory parameters oxLDL and ab ox LDL.
Leptin increased with 26% and no correlation was found with body mass index (BMI). HsCRP decreased significantly with 45% (CI 12-66%). However, after six months hsCRP stabilised and no differences between the two treatment arms were observed.
After the randomisation to either continuation with AZT/3TC + LPV/RTV or switch to AZT/3TC/ABC, several differences in the metabolic parameters between both treatment arms were found. The total cholesterol levels in the AZT/3TC/ABC arm were 15% lower (CI 9-20%) than in the group continuing LPV/RTV. The same pattern was seen for the

figure 1 Serum total cholesterol levels in lopinavir (LPV) treatment versus switch to AZT/3TC/ABC.

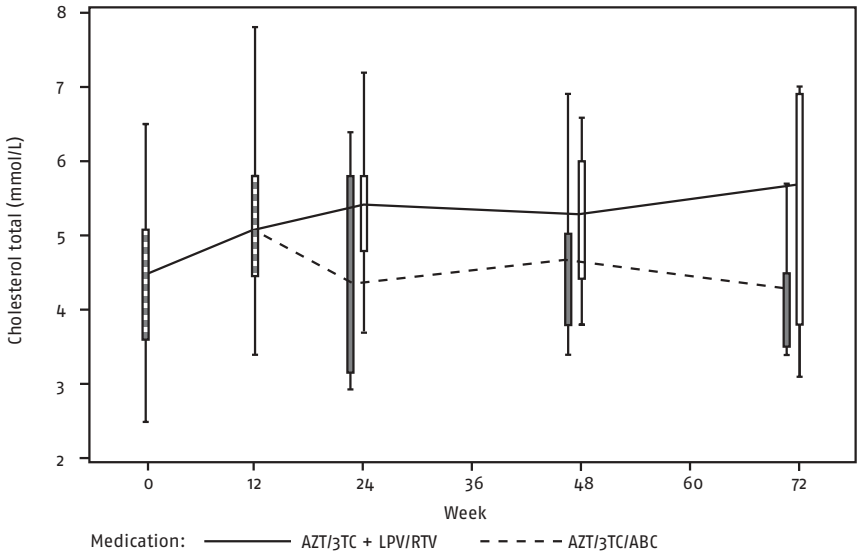
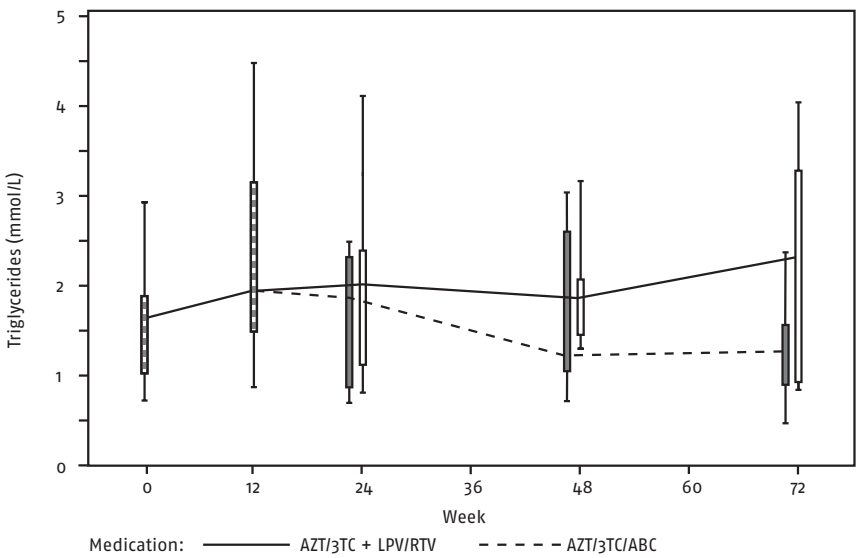


figure 2 Serum triglycerides levels in lopinavir (LPV) treatment versus switch to AZT/3TC/ABC.



other serum lipids and lipoproteins; apoB, LDL and VLDL-cholesterol, triglycerides (12%, 13%, 30% and 31% lower respectively). The major difference after switching to AZT/3TC/ABC was a decrease of 31% in triglycerides (CI 13-45%, p=0.002). In the group that continued LPV/RTV treatment, the increase in lipid values stabilised after six months in contrast to the AZT/3TC/ABC group where total cholesterol and triglycerides returned mostly to baseline values. No differences in glucose and insulin were found. (*table 2*, *figure 1 and 2*). Moreover, the other parameters did not change after 24 weeks of treatment with LPV/RTV. Changes in metabolic parameters were not related to age, sex, baseline CD_4 or HIV-RNA.
The baseline ratio of apoCII/CIII was normal in all patients. The apoE phenotyping results were as follows: apoE 3/2 was found in 7 patients, apoE 3/3 in 6 patients, apoE 4/2 1 patient, apoE 4/3 in 4 patients and apoE 4/4/ in 1 patient (data of 2 patients concerning apoE phenotyping were missing). There was no relation between the ApoE phenotyping and (changes in) serum lipids.

Discussion

We demonstrated that LPV/RTV is associated with the development of an atherogenic lipid and lipoprotein profile with exception of the rise in HDL-cholesterol. Switch to AZT/3TC/ABC can reverse the atherogenic lipid profile as lipid and lipoprotein levels decreased to baseline values.
Antiretroviral therapy with AZT/3TC/ABC decreased triglycerides, VLDL and LDL-cholesterol. No change in glucose was seen.
PIs are known to interfere with lipid and glucose metabolism resulting in, among others, insulin resistance with hyperglycaemia, hypertriglyceridaemia and elevated total and LDL-cholesterol. Like other investigators we found that already shortly after the start of antiretroviral drug use changes in metabolism of lipids and glucose can be observed [14-16]. Within 24 weeks of treatment with LPV/RTV and 2 NRTIs a rise in total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, VLDL-triglycerides lp(a), and apoB was observed. After week 24 the increased serum lipids stabilised in those who continued LPV/RTV use. This was in contrast to the patients that were randomised to AZT/3TC/ABC , in whom serum lipids decreased mostly to baseline levels. The present data suggest that lipid increase will reach its maximum between 24-48 weeks. A retrospective analysis of HIV-infected patients on LPV/RTV also revealed increase of total cholesterol and triglycerides early after introduction of LPV which remained subsequently stable for a period of 15 months [15].
Deficiency of the adipocyte hormone leptin is associated with insulin resistance and hypertriglyceridaemia [27]. It is suggested that initiation with PIs may lead to leptin deficiency [28], however we found the opposite i.e. an increase in leptin in patients on lopinavir. Obese patients might have increased leptin levels due to leptin resistance. However this would not be the case in our study since the body mass index (BMI) of our population was within the normal range. Recently investigations in HIV-infected children also did not demonstrate any difference in serum leptin in patients on PIs or on non-PI ART or therapy-naive patients [29]. Furthermore, we did found no difference between leptin levels in lopinavir and AZT/3TC/ABC users, although our study population was rather small.

Increased baseline levels of serum cholesterol and triglycerides have been associated with a higher risk of developing clinically significant hyperlipidaemia [16]. This was not found in our study, however this might be due to the strict inclusion criteria for lipids in this study. There was even a tendency that patients with low baseline levels had a larger increase in lipids even more than patients with rather high baseline levels, although this was not statistically significant.
Apo lipoproteins are known to be the best markers to predict cardiovascular heart disease. Our data show an initial increase in apoB during the first 24 weeks of LPV treatment, which stabilised in the next months and decreased when patients were switched to a PI-free regimen. In a recent published report no difference between apoB levels was found between HIV-infected patients on ART and healthy age- and gender matched controls [30]. Variants of ApoE genotypes have been associated with severity of ART-induced dyslipidaemia and with lipodystrophy induction. The ApoE2 genotype associated with increased triglycerides was not found in our patients. Additionally, apoC3 increases the risk of fat redistribution [14;31]. If more insight is gained in the genetic pathogenesis of ART-induced dyslipidaemia, pharmacogenomic stratification might be helpful to prevent serious lipid problems in HIV-patients starting with HAART.
The risk for cardiovascular disease in HIV is known to be multifactorial and includes, stage of HIV-infection, ART, nutritional status, exercise and genetic parameters [32;33]. A multifactorial origin of dyslipidaemia is also suggested by the fact that the degree of hyperlipidaemia varies widely. Furthermore, higher lipids are observed in patients with the lipodystrophy syndrome [34]. HIV affects the physiology of the endothelium. Endothelial dysfunction and or injury have been seen in young HIV-patients without cardiovascular risk factors in autopsy studies [35]. Hypo-alpha-lipoproteinaemia is not only hypothesised to be drug-related but also secondary to HIV-infection itself [36]. Additionally, advanced stage HIV-infection is associated with an atherogenic lipid profile including a high prevalence of small density LDL (sdLDL), which can be reflected by apoB [37]. It is still remarkable that patients switching from PI to AZT/3TC/ABC showed a significant amelioration of serum lipids. Even average increase of 28% total cholesterol and 96% triglycerides compared to pre-treatment values have been published in large series of PI-naive HIV-patients [38]. The DAD study reported an increased risk of myocardial infarction in patients exposed to abacavir and didanosine within the preceding 6 months [39]. This risk disappeared 6 months after drug cessation. There were no cardial events during our study.
Insulin resistance with impaired glucose tolerance (without frank diabetes) may also be associated with HIV-infection itself as this phenomenon is also observed in patients not receiving PI therapy, perhaps resulting from the direct effect of the virus on pancreatic beta cell function and insulin secretion [40].
In a large cohort of HIV-infected subjects the 10-year predicted risk of developing coronary heart disease was estimated. Overall, 17% of males and 12% of females met criteria for high predicted cardiovascular heart disease risk [41]. The predicted rate of vascular events was higher for treated HIV-infected patients compared to untreated HIV-patients. Earlier studies also showed these risks. In one cohort of HIV-patients, ART was associated with a 27% relative increase in the rate of myocardial infarction per year of exposure over the first 7 year of use [7].
Recently, in a large cohort of women lower HDL-cholesterol and higher

triglyceride levels were observed in HIV-infected women who used PIs or untreated patients with HIV-infection compared to HIV-negative controls [42]. In addition, LDL-cholesterol levels were higher in the PI users than in the untreated HIV-positive women, but the latter had lower LDL-cholesterol levels compared to HIV-negative women. Lamivudine, didanosine, nevirapine and efavirenz were independently associated with higher HDL-cholesterol levels. Ritonavir, indinavir/ritonavir and nelfinavir were associated with higher LDL-cholesterol levels. Stavudine, abacavir and all ritonavir-containing regimens were associated with higher triglyceride levels. Tenofovir was associated with lower triglycerides. There are only a few prospective studies in homogenous patient populations that have examined the usefulness of serum lipid measurement in the treatment of HIV-infection. More prospective data are necessary to evaluate the clinical importance of metabolic evaluation as an early predictor for the development of side effects and its risk for cardiovascular disease in the future.

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References

1 Wohl DA, McComsey G, Tebas P, Brown TT, Glesby MJ, Reeds D, Shikuma C, Mulligan K, Dube M, Wininger D, Huang J, Revuelta M, Currier J, Swindells S, Fichtenbaum C, Basar M, Tungsiripat M, Meyer W, Weihe J, Wanke C. Current concepts in the diagnosis and management of metabolic complications of HIV-infection and its therapy. **Clin Infect Dis** 2006;43:645-53.

2 Jain RG, Furfine ES, Pedneault L, White AJ, Lenhard JM. Metabolic complications associated with antiretroviral therapy. **Antiviral Res** 2001;51:151-77.

3 Periard D, Telenti A, Sudre P, Cheseaux JJ, Halfon P, Reymond MJ, Marcovina SM, Glauser MP, Nicod P, Darioli R, Mooser V. Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors. The Swiss HIV Cohort Study. **Circulation** 1999;100:700-705.

4 Lee GA, Seneviratne T, Noor MA, Lo JC, Schwarz JM, Aweeka FT, Mulligan K, Schambelan M, Grunfeld C. The metabolic effects of lopinavir/ritonavir in HIV-negative men. **AIDS** 2004;18:641-49.

5 Carpentier A, Patterson BW, Uffelman KD, Salit I, Lewis GF. Mechanism of highly active anti-retroviral therapy-induced hyperlipidemia in HIV-infected individuals. **Atherosclerosis** 2005;178:165-72.

6 Rimland D, Guest JL, Hernandez-Ramos I, Del RC, Le NA, Brown WV. Antiretroviral therapy in HIV-positive women is associated with increased apolipoproteins and total cholesterol. **J Acquir Immune Defic Syndr** 2006;42:307-13.

7 Aberg JA, Gallant JE, Anderson J, Oleske JM, Libman H, Currier JS, Stone VE, Kaplan JE. Primary care guidelines for the management of persons infected with human immunodeficiency virus: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. **Clin Infect Dis** 2004;39:609-29.

8 Friis-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio MA, Pradier C, Morfeldt L, Mateu S, Law M, El-Sadr W, De WS, Sabin CA, Phillips AN, Lundgren JD. Cardiovascular disease risk factors in HIV-patients--association with antiretroviral therapy. Results from the DAD study. **AIDS** 2003;17:1179-93.

9 van de Valk M, Kastelein JJ, Murphy RL, van Leth F, Katlama C, Horban A, Glesby M, Behrens G, Clotet B, Stellato RK, Molhuizen HO, Reiss P. Nevirapine-containing antiretroviral therapy in HIV-1 infected patients results in an anti-atherogenic lipid profile. **AIDS** 2001;15:2407-14.

10 van de Valk M, Reiss P. Lipid profiles associated with antiretroviral drug choices. **Curr Opin Infect Dis** 2003;16:19-23.

11 Pereira SA, Branco T, Corte-Real RM, Germano I, Lampreia F, Caixas U, Monteiro EC. Long-term and concentration-dependent beneficial effect of efavirenz on HDL-cholesterol in HIV-infected patients. **Br J Clin Pharmacol** 2006;61:601-4.

12 Young J, Weber R, Rickenbach M, Furrer H, Bernasconi E, Hirschel B, Tarr PE, Vernazza P, Battegay M, Bucher HC. Lipid profiles for antiretroviral-naive patients starting PI- and NNRTI-based therapy in the Swiss HIV cohort study. **Antivir Ther** 2005;10:585-91.

13 Martinez E, Domingo P, Galindo MJ, Milinkovic A, Arroyo JA, Baldovi F, Larrousse M, Leon A, de LE, Gatell JM. Risk of metabolic abnormalities in patients infected with HIV receiving antiretroviral therapy that contains lopinavir-ritonavir. **Clin Infect Dis** 2004;38:1017-23.

14 Calza L, Manfredi R, Farneti B, Chiodo F. Incidence of hyperlipidaemia in a cohort of 212 HIV-infected patients receiving a protease inhibitor-based antiretroviral therapy. **Int J Antimicrob Agents** 2003;22:54-59.

15 Lafeuillade A, Hittinger G, Philip G, Lambry V, Jolly P, Poggi C. Metabolic evaluation of HIV-infected patients receiving a regimen containing lopinavir/ritonavir (Kaletra). **HIV Clin Trials** 2004;5:392-98.

16 Montes ML, Pulido F, Barros C, Condes E, Rubio R, Cepeda C, Dronda F, Antela A, Sanz J, Navas E, Miralles P, Berenguer J, Perez S, Zapata A, Gonzalez-Garcia JJ, Pena JM, Vazquez JJ, Arribas JR. Lipid disorders in antiretroviral-naive patients treated with lopinavir/ritonavir-based HAART: frequency, characterization and risk factors. **J Antimicrob Chemother** 2005;55:800-804.

17. Oh J, Hegele RA. HIV-associated dyslipidaemia: pathogenesis and treatment. **Lancet Infect Dis** 2007;7:787-96.

18 Petit JM, Duong M, Florentin E, Duvillard L, Chavanet P, Brun JM, Portier H, Gambert P, Verges B. Increased VLDL-apoB and IDL-apoB production rates in nonlipodystrophic HIV-infected patients on a protease inhibitor-containing regimen: a stable isotope kinetic study. **J Lipid Res** 2003;44:1692-97.

19 Bard JM, Lassalle R, Capeau J, Bach-Ngohou K, Nazih-Sanderson F, Remy G, Reynes J, Ecobichon JL, Saves M, Raffi F. Association of apolipoproteins C3 and E with metabolic changes in HIV-infected adults treated with a protease-inhibitor-containing antiretroviral therapy. **Antivir Ther** 2006;11:361-70.

20 Behrens G, Schmidt HH, Stoll M, Schmidt RE. ApoE genotype and protease-inhibitor-associated hyperlipidaemia. **Lancet** 1999;354:76.

21 Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. **JAMA** 2001;286:327-34.

22 Pepys MB, Hirschfield GM. C-reactive protein: a critical update. **J Clin Invest** 2003;111:1805-12.

23 Demacker PN, Hijmans AG, Vos-Janssen HE, van't LA, Jansen AP. A study of the use of polyethylene glycol in estimating cholesterol in high-density lipoprotein. **Clin Chem** 1980;26:1775-79.

24 Demacker PN, Veerkamp MJ, Bredie SJ, Marcovina SM, de GJ, Stalenhoef AF. Comparison of the measurement of lipids and lipoproteins versus assay of apolipoprotein B for estimation of coronary heart disease risk: a study in familial combined hyperlipidemia. **Atherosclerosis** 2000;153:483-90.

25 de Galan BE, Tack CJ, Lenders JW, Pasman JW, Elving LD, Russel FG, Lutterman JA, Smits P. Theophylline improves hypoglycemia unawareness in type 1 diabetes. **Diabetes** 2002;51:790-796.

26 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. **Diabetologia** 1985;28:412-19.

27 Jazet IM, Pijl H, Meinders AE. Adipose tissue as an endocrine organ: impact on insulin resistance. **Neth J Med** 2003;61:194-212.

28 Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P, Garg A. Leptin-replacement therapy for lipodystrophy. **N Engl J Med** 2002;346:570-578.

29 Dzwonek AB, Novelli V, Schwenk A. Serum leptin concentrations and fat redistribution in HIV-1-infected children on highly active antiretroviral therapy. **HIV Med** 2007;8:433-38.

30 Catzin-Kuhlmann A, Castillo-Martinez L, Colin-Ramirez E, Valles V, guilar-Salinas CA, Sierra J, Calva JJ. No evidence of increased risk for certain highly atherogenic lipoprotein phenotypes in HIV-infected patients. **Arch Med Res** 2008;39:84-91.

31 Bonnet E, Genoux A, Bernard J, Fauvel J, Massip P, Perret B. Impact of genetic polymorphisms on the risk of lipid disorders in patients on anti-HIV therapy. **Clin Chem Lab Med** 2007;45:815-21.

32 Rose H, Woolley I, Hoy J, Dart A, Bryant B, Mijch A, Sviridov D. HIV-infection and high-density lipoprotein: the effect of the disease vs the effect of treatment. **Metabolism** 2006;55:90-95.

33 Mallon PW. Pathogenesis of lipodystrophy and lipid abnormalities in patients taking antiretroviral therapy. **AIDS Rev** 2007;9:3-15.

34 Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, Cooper DA. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. **AIDS** 1998;12:F51-F58.

35 d'Amati G, di Gioia CR, Gallo P. Pathological findings of HIV-associated cardiovascular disease. **Ann N Y Acad Sci** 2001;946:23-45.

36 Fitch KV, Anderson EJ, Hubbard JL, Carpenter SJ, Waddell WR, Caliendo AM, Grinspoon SK. Effects of a lifestyle modification program in HIV-infected patients with the metabolic syndrome. **AIDS** 2006;20:1843-50.

37 Badiou S, Merle De BC, Dupuy AM, Baillat V, Cristol JP, Reynes J. Decrease in LDL size in HIV-positive adults before and after lopinavir/ritonavir-containing regimen: an index of atherogenicity? **Atherosclerosis** 2003;168:107-13.

38 Galli M, Ridolfo AL, Gervasoni C. Cardiovascular disease risk factors in HIV-infected patients in the HAART era. **Ann N Y Acad Sci** 2001;946:200-213.

39 Sabin CA, Worm SW, Weber R, Reiss P, El-Sadr W, Dabis F, De WS, Law M, d'Arminio MA, Friis-Moller N, Kirk O, Pradier C, Weller I, Phillips AN, Lundgren JD. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the DAD study: a multi-cohort collaboration. **Lancet** 2008;371:1417-26.

40 Dube MP, Stein JH, Aberg JA, Fichtenbaum CJ, Gerber JG, Tashima KT, Henry WK, Currier JS, Sprecher D, Glesby MJ. Guidelines for the evaluation and management of dyslipidemia in human immunodeficiency virus (HIV)-infected adults receiving antiretroviral therapy: recommendations of the HIV Medical Association of the Infectious Disease Society of America and the Adult AIDS Clinical Trials Group. **Clin Infect Dis** 2003;37:613-27.

41 Kaplan RC, Kingsley LA, Sharrett AR, Li X, Lazar J, Tien PC, Mack WJ, Cohen MH, Jacobson L, Gange SJ. Ten-year predicted coronary heart disease risk in HIV-infected men and women. **Clin Infect Dis** 2007;45:1074-81.

42 Anastos K, Lu D, Shi Q, Tien PC, Kaplan RC, Hessol NA, Cole S, Vigen C, Cohen M, Young M, Justman J. Association of serum lipid levels with HIV serostatus, specific antiretroviral agents, and treatment regimens. **J Acquir Immune Defic Syndr** 2007;45:34-42.

Section

Pharmacokinetics

Chapter 6

Stavudine plasma concentrations and lipoatrophy

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Abstract

This study was to determine the correlation between plasma stavudine concentrations and lipoatrophy (LA), one of the major adverse events in patients on stavudine and one of the major reasons to discontinue stavudine. Plasma drug concentrations were retrospectively analysed in patients who were on a stavudine-containing regimen for at least 12 months. We defined two groups of patients: 21 patients with LA and 15 patients without LA or other stavudine-related side effects (i.e. neuropathy).
We analysed stavudine concentrations in 212 plasma samples; 87 in the control group and 125 in the LA group, with a mean of 4 plasma samples per person at least 2 a year). Demographics were comparable in LA patients and controls, except the duration of stavudine use which was longer in the LA group: 55 versus 42 months in the control group. Overall LA patients had higher drug exposure to stavudine compared to the controls, this was seen in the geometric concentration ratios (CR) respectively 0.978 and 0.741 ($p=0.04$) and also a higher percentage of CR values >1.0, representing a drug concentration above the normal population curve (46% versus 23%, $p=0.02$). In addition, the duration of stavudine therapy was independently associated with lipoatrophy ($p=0.05$). In the multivariate analysis both duration of stavudine ($p=0.05$) and CR >1.0 ($p=0.02$) were independently correlated with LA.
Monitoring of plasma stavudine concentrations can be useful to prevent stavudine-related lipoatrophy.

Introduction

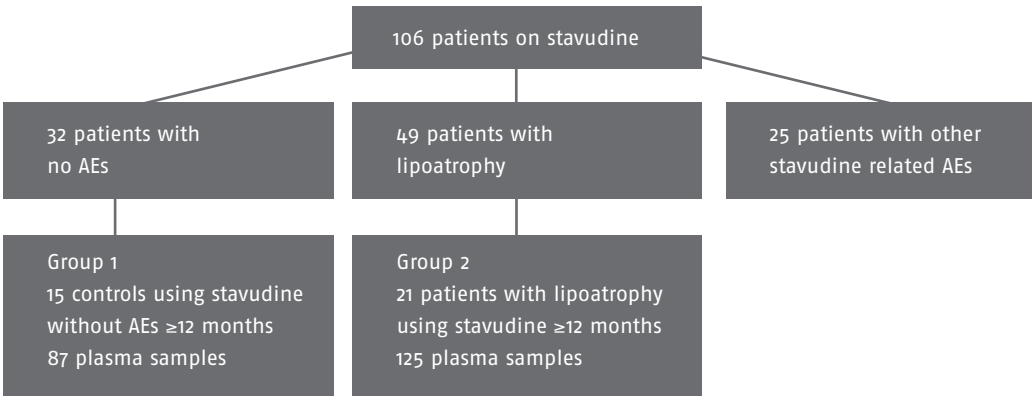
Thymidin nucleoside reverse transcriptase inhibitors (tNRTIs) containing regimens have been associated with various adverse events such as lipoatrophy, peripheral polyneuropathy and lactic acidosis. These adverse events seem to be class specific; eventhough the pathophysiology is not yet fully elucidated. Especially the development of lipoatrophy is one of the major concerns for patients and physicians when choosing drugs for a first line antiretroviral regimen. Several prospective studies have shown that the use of stavudine and to a lesser extend zidovudine are major contributors in the development of lipoatrophy. Stavudine is therefore no longer recommended by the international guidelines for first line treatment of previously antiretroviral naive patients in North America and Europe. However, a substantial amount of HIV-patients in countries with limited resources use stavudine [1]. For instance, in Uganda and Kenia a cohort of respectively 1073 and 284 patients were treated with antiretroviral therapy containing stavudine. Another report from South Africa analysed toxicity in 1700 patients receiving antiretroviral therapy, consisting of either stavudine or zidovudine [2-7].
Several trials in HIV-treated subjects have showed evidence that side effects are dependent on plasma drug concentrations [8-12]. There is conflicting data about the best representative of NRTI levels; intracellular or plasma [13]. To assess the clinical use of plasma stavudine concentrations and its relation with the development of toxicity, we performed a study regarding plasma stavudine levels in relation to lipoatrophy (LA). A major impediment for the success of HIV treatment is poor adherence, which is often a result of side effects. In this respect lipoatrophy is the most feared event by the patient. With this in mind, we wanted to investigate the relationship between stavudine plasma levels and lipoatrophy appearance.
The purpose of this study was to verify whether the side effect lipoatrophy, which often occurs after at least several months of therapy, could be related to elevated plasma concentrations of stavudine. In this way drug monitoring can be helpful in the decision making of changing a probable toxic antiretroviral regimen to a less toxic regimen which can be continued for a prolonged time, resulting in chronic suppression of HIV-infection.

Patients and methods

Study design

Plasma concentrations are routinely measured in all HIV-patients on antiretroviral therapy visiting our outpatient department. We analysed data of all patients that used or had used stavudine in the period 1996-2006. We analysed our database to find all patients on a stavudine-containing regimen. All patients had to be on their first antiretroviral therapy regimen. From our database we selected 106 patients with a stavudine-containing regimen. Patients were divided into two groups based on the development of adverse events (AEs) due to stavudine. Patients that used or had used stavudine without experiencing AEs were enrolled in group 1 (32 controls). Patients that experienced lipoatrophy due to stavudine were enrolled in group 2 (49 LA patients). Patients with other AEs due to stavudine

figure 1 Flow diagram: Inclusion of patients.



were not enrolled. Because body shape changes with fat accumulation (lipohypertrophy) have been largely associated with protease inhibitors, we excluded patients who experienced only lipohypertrophy [14;15].
Because lipoatrophy mostly occurs after at least several months of therapy, we decided to enrol only patients that used or had used stavudine for at least 12 months [6-18]. After correction for duration of therapy 36 patients were left from the initially selected 81 patients on a stavudine-containing regimen *(figure 1)*.
Of all the patients meeting the criteria we retrospectively measured 2 samples a year with an interval of at least 4 months between the samples.
Lipoatrophy was defined as peripheral fat loss (face, buttocks and extremities) and lipohypertrophy as central fat accumulation (abdominal, neck and breasts) both reported by the patient and confirmed by physical examination (i.e. waist to hip ratio measurements, lipodystrophy scoring system) [19;20]. Patients, who were clinically suspected of severe abdominal fat accumulation, had indeed increased intra-abdominal fat tissue (evaluated by DEXA or CT scan).

Concentration Ratio (CR)

A concentration ratio (CR) was calculated for every plasma sample, comparing the stavudine plasma level of the patient with time-adjusted stavudine plasma concentration for the population. Based on multiple measurements over time, for every individual the geometric mean value of the stavudine CR value was calculated. In addition we looked at CR levels >1.0, which represent higher exposure when compared to the overall population [21]. For a normal distribution we used the geometric mean CR.

Drug monitoring

Stavudine plasma concentrations were determined by a high-performance liquid chromatography (HPLC) assay as previously reported [22]. The volume of the plasma sample was 500 microL. The lower limit of quantification is 0.015 mg/L. Average accuracy ranged from 98-101% and precision ranged from 1.3-2.2%.

table 1 Patients' characteristics.

	Controls	Lipoatrophy
Number of patients	15	21
Sex (%)	10 males (67)	15 males (71)
	5 females (33)	6 females (29)
Race (%)	9 Caucasian (60)	18 Caucasian (86)
	4 Black (27)	2 Black (10)
	1 Asian (7)	1 Asian (5)
	1 Hispanic (7)	
Number of samples	87	125
Mean number of samples per person (range)	4.3 (2–10)	4.6 (2–9)
Mean HIV-RNA level (log copies/mL) at start stavudine ± SD	4.63 ± 0.93	4.63 ± 0.90
CD_4 cell count at initiation of stavudine (cells/mm^3) ± SD	210 ± 125	205 ± 128
Weight (kg) ± SD (range)	68 ± 10 (52–82)	70 ± 10 (48–85)
Mean age (years) at start stavudine (range)	40 (23–57)	39 (25–58)
Duration of stavudine in months ± SD (range)	42 ± 21 (15–86)	55 ± 18 (30–84)
Mean stavudine concentration (mg/L) ± SD	0.174 ± 0.102	0.270 ± 0.172
Mean CR ± SD	0.799 ± 0.309	1.126 ± 0.514
Median CR ± SD	0.833 ± 0.414	0.952 ± 0.288
Geometric mean ± SD	0.741 ± 0.295	0.978 ± 0.348
% CR >1.0 ± SD	23 ± 27	46 ± 29

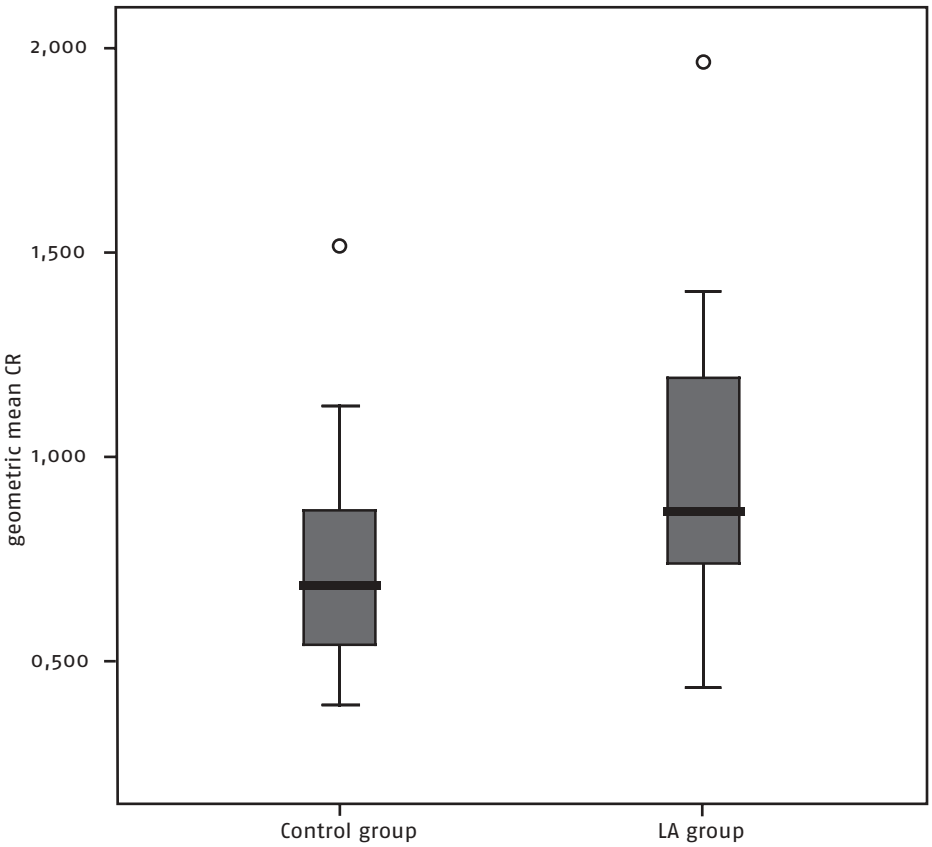
Statistical Analysis

Statistical evaluation was performed with SPSS for windows, version 11.0. Geometric means with 95% confidence intervals were calculated for all samples in each individual. For the analysis, a p-value of ≤0.05 was regarded as significant. We performed a logistic multiple regression analysis with lipoatrophy (present or not present) as the outcome variable versus demographic factors, co-medication in HAART (except NRTI because this was lamivudine in the majority of the patients) and CRs. In addition, the variable duration of treatment was analysed as an independently factor for lipoatrophy.

Ethics

All our HIV-infected outpatients are asked for informed consent to use their demographic data in an anonymous database. Maintenance of our database and its use for retrospective analysis of patient's outcomes are approved by the ethics committee.

figure 2 Geometric means of CR in patients with lipoatrophy and controls.

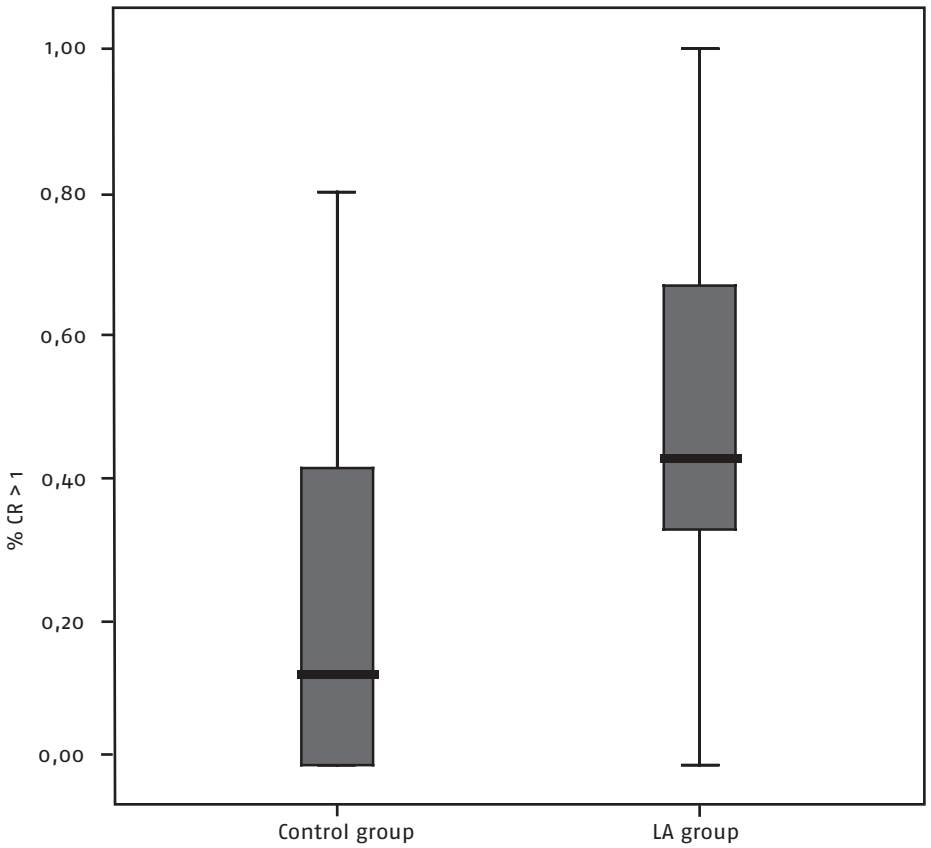


In both groups, there was one value far above the other geometric means, which did not lead to statistical significant differences. The median values are marked in the box-plots.

Results

Retrospectively we analysed plasma samples in 36 patients using stavudine. Plasma stavudine concentrations were measured in 15 controls (10 males and 5 females) and 21 lipoatrophy patients (15 males and 6 females). A total of 212 samples were analysed: 87 in the control group and 125 in the LA group. The range of samples per patients varied from 2 to 10 with a mean of 4 samples per individual in both groups. The mean age at the start of stavudine was 40 and 39 years in the control and LA group, respectively. There was an equal percentage of females between the two groups (33% in controls and 29% in LA patients). In both groups the majority of patients were Caucasian, 60% and 86% in the control and LA group, respectively. In the control group there were 4 Black patients (27%), 1 Asian (7%) and 1 Hispanic patient (7%). Furthermore, the LA group consisted of 2 Black patients (10%) and 1 Asian patient (5%) (*table 1*). The mean CD_4 cell count at

figure 3 Percentage of CR >1 in patients with lipoatrophy and controls.



The median values are marked in the box-plots.

initiation of stavudine was 210 and 205 cells/mm^3 in the control group and LA group, respectively (p=0.84). In the control group 10 out of 15 patients were on a NNRTI containing regimen and the other 5 used a PI versus 10 out of 21 patients using a NNRTI and 11 patients using a PI in the LA group (p=0.33). The concomittant medication consisted in both groups of lamivudine (only one patient in every group was on didanosine). Except for duration of therapy, which was longer in the LA group, there were no statistically significant differences in patient characteristics between the groups. The duration of stavudine in the LA group was 55 months (range 30-84) in the LA group and 42 months (range 15-86) in the control group. The dosage of stavudine was 40 mg twice daily, only one patient in each group used 30 mg twice daily due to low bodyweight (<60 kg).
The overall drug exposure to stavudine expressed in the geometric mean values was higher in the LA group compared to the controls, respectively 0.978 and 0.741 (p=0.04, *figure 2*). In both groups there was one value far above the other geometric means *(figure 2)*, which did not lead to statistical significant

differences. In addition, CR values >1.0, representing a drug concentration above the normal population curve, were more often seen in the LA group than in the controls (46% versus 23%, p=0.02, *figure 3*). *Table 1* provides an overview of the results.
In the multivariate regression analysis duration of stavudine therapy (p=0.05) and CR level >1.0 (p=0.02) were both independently associated with lipoatrophy. No association between body weight and stavudine levels were found.

Discussion

Since clinicians and patients are often confronted with side effects, we wanted to evaluate drug exposure in patients on antiretroviral therapy. Our analysis showed higher plasma stavudine levels in patients with LA compared to the patients without adverse events. Besides, these patients had more often a CR >1.0, representing a drug concentration above the normal population curve. Yet, no such analysis concerning NRTIs has been reported. Our results confirm earlier data reporting longer duration of stavudine use in patients experiencing lipoatrophy, but we show that high exposure to stavudine is an independent predictive factor [16-18].
Untill now TDM has been successfully applied to NNRTIs and PIs and to a lesser extend for NRTIs. Efficacy of NRTIs has not been correlated to plasma drug levels because these drugs must be converted to active intracellular metabolites to become active. Intracellular concentrations of NRTI-triphosphate compounds have been related to plasma HIV-RNA levels and CD_4 cell counts [23]. There is only limited experience with the use of NRTI drug monitoring. There are two studies by Fletcher et al. that investigated concentration-controlled NRTI management in zidovudine and lamivudine therapy. They especially compared antiviral response in fixed-dose regimens versus concentration-controlled regimens, however the trials did not assess NRTI-associated toxicity in relation to plasma levels [13;24].
For dose-related AEs, patients who experience adverse events are hypothesised to be those with higher plasma concentrations. Possible reasons for this phenomenon might be greater absorption, slower metabolism, change in drug transporters or reduced body weight and thereby a small volume of distribution [25-28]. For example, Gatti et al discovered a clear relationship between ritonavir plasma concentrations and side effects [8]. In addition, Dieleman et al found elevated indinavir plasma concentrations in patients with cristalluria [9]. Recently, a correlation was found between neuropsychiatric adverse events and efavirenz plasma levels in HIV-patients receiving long-term therapy with efavirenz [12]. Patients with higher efavirenz plasma levels were at risk for central nervous system toxicity. Additionally, increased bilirubin levels are associated with elevated concentrations of lopinavir, indinavir and atazanavir [10;11].
Our data provide interesting insights into possible uses for TDM and/or lower doses of stavudine. Our data suggest that the occurrence of LA can be predicted by higher plasma drug concentrations of stavudine. In this way tolerance of antiretrovirals can be improved by avoiding excessive plasma concentrations. By monitoring drug levels of stavudine or starting with a lower dose of stavudine,

toxicity can be found in an early phase when irreversible damage still could be prevented. A lower dose of stavudine with or without drug monitoring may contribute to the safe use of stavudine in therapy-naive and NRTI-experienced patients that need this antiretroviral drug. Eventually, stavudine plasma monitoring can be applied in stavudine-naive patients to prevent LA by adapting the stavudine dose in case the CR exceeds the normal level of 1.0. In the same way, stavudine can be applied in patients experiencing AEs from other NRTIs. Few studies looked at the use of lower dose of stavudine to reduce side effects [29-31] Under strict control of viral load it was possible to reduce stavudine dosage resulting in less side effects compared to standard dose regimens. Recently, an interesting study investigated the role of lowering stavudine dose or switching to tenofovir compared with standard stavudine dose in patients on long-term stavudine therapy [32]. Lowering stavudine dose was associated with improvement of lipoatrophy. No virological failures were reported in both groups. However, none of these studies looked at plasma stavudine concentrations. Combining these data and our results we suggest that reduction to a twice-daily dose of 30 mg stavudine might be safer and even effective in a majority of patients. In addition, we suggest that a plasma stavudine concentration ratio (CR) <1.0 (for example 0.8-1.0) might be a safe level for treatment of HIV-patients to prevent lipoatrophy. Although the above mentioned studies described efficacious control of HIV, detailed studies have to be performed to prove this hypothesis. Interesting, a recent addendum to the WHO guidelines on antiretroviral therapy for HIV-infection in adults and adolescents recommends now a lower dosage for stavudine (30 mg twice daily).

Due to genetic and environmental factors, there is a wide inter-patient variability when measuring drug exposure to a standard dose. Earlier studies have proven a relationship between drug exposure and efficacy or toxicity [9;26;33-37]. This inter-individual variability makes drug monitoring useful in these specific situations to predict not only virological response but also toxicity. Because symptoms are not always present or appear only until an irreversible phase has been reached, timely identification of toxicity is essential for the optimal treatment of HIV-patients.

Although it is frequently stated that plasma concentrations do not correlate with intracellular active NRTI-triphosphate metabolites, our data show that plasma NRTI concentrations give a good reflection of toxicity [23;35;38]. Therefore, high plasma drug levels of stavudine are probably a useful reflection of its intracellular concentrations. Till now less attention has been paid to the monitoring of plasma concentrations of NRTIs in contrast to PIs and NNRTIs. In the absence of easier techniques for measuring NRTI in drug monitoring, plasma NRTI levels can be well used as a replacement of intracellular measurement.

It has not clearly been elucidated which factors additionally might have played a role in the elevated plasma drug levels in the LA group. Enhanced plasma concentrations may have a multifactorial origin; absorption, elimination, Cytochrome P450 system (isoenzyme CYP3A4), gender, liver or urinary clearance, drug-drug interaction or co-medication for other diseases [8;39;40-43]. This study was not intended to look at these factors although we compared the latter two factors between the groups and did not find significant differences. In addition,

in the controls as well as the LA patients one third were females. Another explanation for the differences in plasma drug levels between the groups might be adherence. However, we looked also at levels of concomitant used PIs or NNRTIs and these levels were overall within the normal range and there were no differences between the groups.
Our study was a retrospective analysis. There are only a few prospective studies that have examined the utility of plasma drug concentrations in the treatment of HIV-infection [36;44]. More prospective studies have to be performed in order to get more insight in the use of plasma drug monitoring for antiretroviral therapy and its decision making in case of toxicity. It also would be helpful to investigate the use of lower dose of stavudine more closely, given the increasing number of HIV-infected patients in developing countries were stavudine is used widely, owing to low costs and inclusion in generic fixed-dose combination drugs. Decrease of stavudine dosing might result in additional cost- and side effect reduction.

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References

1 Hammer SM, Saag MS, Schechter M et al. Treatment for adult HIV-infection: 2006 recommendations of the International AIDS Society-USA panel. **JAMA** 2006; 296:827-843.
2 WHO. Recommendations for a public health approach. 2006. Guideline Antiretroviral therapy for HIV-infection in adults and adolescents. **http://www.who.int/hiv/art/ARTadultsaddendum.pdf**.
3 WHO. WHO Project: ICP HIV 001. The Use of Antiretroviral Therapy:A Simplified Approach for Resource-Constrained Countries. **http://www.searo.who.int/LinkFiles/Publications_aids-133.pdf.**
4 Carr A, Cooper DA. Adverse effects of antiretroviral therapy. **Lancet** 2000; 356:1423-1430.
5 McComsey GA, Ward DJ, Hessenthaler SM et al. Improvement in lipoatrophy associated with highly active antiretroviral therapy in human immunodeficiency virus-infected patients switched from stavudine to abacavir or zidovudine: the results of the TARHEEL study. **Clin Infect Dis** 2004; 38:263-270.
6 Mallal SA, John M, Moore CB et al. Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV-infection. **AIDS** 2000; 14:1309-1316.
7 Saint-Marc T, Partisani M, Poizot-Martin et al. A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy. **AIDS** 1999; 13:1659-1667.

8 Gatti G, Di BA, Casazza R et al. The relationship between ritonavir plasma levels and side effects: implications for therapeutic drug monitoring. **AIDS** 1999; 13:2083-2089.

9 Dieleman JP, Gyssens IC, van der Ende ME et al. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. **AIDS** 1999; 13:473-478.

10 Rodriguez NS, Barreiro P, Rendon A et al. Plasma levels of atazanavir and the risk of hyperbilirubinemia are predicted by the 3435C->T polymorphism at the multidrug resistance gene 1. **Clin Infect Dis** 2006; 42:291-295.

11 Dicenzo R, Luque A, Larppanichpoonphol P et al. Association of total bilirubin with indinavir and lopinavir plasma concentrations in HIV-infected patients receiving three different double-boosted dosing regimens. **J Antimicrob Chemother** 2006; 58:393-400.

12 Gutierrez F, Navarro A, Padilla S et al. Prediction of neuropsychiatric adverse events associated with long-term efavirenz therapy, using plasma drug level monitoring. **Clin Infect Dis** 2005; 41:1648-1653.

13 Fletcher CV, Anderson PL, Kakuda TN et al. Concentration-controlled compared with conventional antiretroviral therapy for HIV-infection. **AIDS** 2002; 16:551-560.

14 Carr A. HIV protease inhibitor-related lipodystrophy syndrome. **Clin Infect Dis** 2000; 30 Suppl 2:S135-S142.

15 Carr A, Miller J, Law M et al. A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. **AIDS** 2000; 14:F25-F32.

16 Bogner JR, Vielhauer V, Beckmann RA et al. Stavudine versus zidovudine and the development of lipodystrophy. **J Acquir Immune Defic Syndr** 2001; 27:237-244.

17 Carr A, Samaras K, Thorisdottir A et al. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. **Lancet** 1999; 353:2093-2099.

18 Dieleman, J. P. Risk of lipodystrophy is highest after prolonged exposure to stavudine containing antiretroviral treatment. **The ATHENA cohort.** 108-122. Thesis 2003.

19 Carr A, Emery S, Law M et al. An objective case definition of lipodystrophy in HIV-infected adults: a case-control study. **Lancet** 2003; 361:726-735.

20 Carr A, Law M. An objective lipodystrophy severity grading scale derived from the lipodystrophy case definition score. **J Acquir Immune Defic Syndr** 2003; 33(5):571-576.

21 Horton CM, Dudley MN, Kaul S et al. Population pharmacokinetics of stavudine (d4T) in patients with AIDS or advanced AIDS-related complex. **Antimicrob Agents Chemother** 1995; 39:2309-2315.

22 Verweij-van Wissen CP, Aarnoutse RE, Burger DM. Simultaneous determination of the HIV nucleoside analogue reverse transcriptase inhibitors lamivudine, didanosine, stavudine, zidovudine and abacavir in human plasma by reversed phase high performance liquid chromatography. **J Chromatogr B Analyt Technol Biomed Life Sci** 2005; 816:121-129.

23 Fletcher CV, Kawle SP, Kakuda TN et al. Zidovudine triphosphate and lamivudine triphosphate concentration-response relationships in HIV-infected persons. **AIDS** 2000; 14:2137-2144.

24 Fletcher CV, Acosta EP, Henry K et al. Concentration-controlled zidovudine therapy. **Clin Pharmacol Ther** 1998; 64:331-338.

25 Kim RB. Drug transporters in HIV Therapy. **Top HIV Med** 2003; 11:136-139.

26 Acosta EP, Kakuda TN, Brundage RC et al. Pharmacodynamics of human immunodeficiency virus type 1 protease inhibitors. **Clin Infect Dis** 2000; 30 Suppl 2:S151-S159.

27 Gandhi M, Aweeka F, Greenblatt RM et al. Sex differences in pharmacokinetics and pharmacodynamics. **Annu Rev Pharmacol Toxicol** 2004; 44:499–523.
28 Angel JB, Khaliq Y, Monpetit ML et al. An argument for routine therapeutic drug monitoring of HIV-1 protease inhibitors during pregnancy. **AIDS** 2001; 15:417–419.
29 Sanchez-Conde M, de MC, Jimenez-Nacher I, Barreiro P et al. Reductions in stavudine dose might ameliorate mitochondrial-associated complications without compromising antiviral activity. **HIV Clin Trials** 2005; 6:197–202.
30 Hanvanich M, Prasanthai V Riengschan R et al. Reduction of d4T dosage improves lipoatrophy without virological failure. **Antivir Ther** 2003; 8 Suppl 1:S392–S393.
31 Hill A, Ruxrungtham K, Hanvanich M et al. Systematic review of clinical trials evaluating low doses of stavudine as part of antiretroviral treatment. **Expert Opin Pharmacother** 2007; 8:679–688.
32 Milinkovic A, Martinez E, López S et al. The impact of reducing stavudine dose versus switching to tenofovir on plasma lipids, body composition and mitochondrial function in HIV-infected patients. **Antivir Ther** 2007; 12:407–415.
33 Burger DM, Hugen PW, Aarnoutse RE et al. Treatment failure of nelfinavir-containing triple therapy can largely be explained by low nelfinavir plasma concentrations. **Ther Drug Monit** 2003; 25:73–80.
34 Marzolini C, Telenti A, Decosterd LA et al. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. **AIDS** 2001; 15:71–75.
35 Ford J, Khoo SH, Back DJ. The intracellular pharmacology of antiretroviral protease inhibitors. **J Antimicrob Chemother** 2004; 54:982–990.
36 Burger D, Hugen P, Reiss P et al. Therapeutic drug monitoring of nelfinavir and indinavir in treatment-naive HIV-1-infected individuals. **AIDS** 2003; 17:1157–1165.
37 Bossi P, Peytavin G, it-Mohand H et al. GENOPHAR: a randomized study of plasma drug measurements in association with genotypic resistance testing and expert advice to optimize therapy in patients failing antiretroviral therapy. **HIV Med** 2004; 5:352–359.
38 Piliero PJ. Pharmacokinetic properties of nucleoside/nucleotide reverse transcriptase inhibitors. **J Acquir Immune Defic Syndr** 2004; 37 Suppl 1:S2–S12.
39 Burger DM, Aarnoutse RE, Hugen PW. Pros and cons of therapeutic drug monitoring of antiretroviral agents. **Curr Opin Infect Dis** 2002; 15:17–22.
40 Burger DM, Hoetelmans RM, Koopmans PP et al. Clinically relevant drug interactions with antiretroviral agents. **Antivir Ther** 1997; 2:149–165.
41 Lucas GM, Chaisson RE, Moore RD. Highly active antiretroviral therapy in a large urban clinic: risk factors for virologic failure and adverse drug reactions. **Ann Intern Med** 1999; 131:81–87.
42 Durant J, Clevenbergh P, Garraffo R et al. Importance of protease inhibitor plasma levels in HIV-infected patients treated with genotypic-guided therapy: pharmacological data from the Viradapt Study. **AIDS** 2000; 14:1333–1339.
43 Clark R. Sex differences in antiretroviral therapy-associated intolerance and adverse events. **Drug Saf** 2005; 28:1075–1083.
44 Gerber JG, Acosta EP. Therapeutic drug monitoring in the treatment of HIV-infection. **J Clin Virol** 2003; 27:117–128.

Chapter 7

Lopinavir plasma concentrations and serum lipids in therapy-naive HIV-patients: a subanalysis of the FREE study

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Submitted

Abstract

The aim of the study was to investigate if higher plasma lopinavir concentrations lead to increase of serum lipids, one of the major adverse events in patients on lopinavir. Plasma drug concentrations were analysed up to week 24 in a prospective cohort of HIV antiretroviral therapy-naive patients who started on a regimen of zidovudine, lamivudine and ritonavir-boosted lopinavir.

Prospectively we measured plasma lopinavir concentrations from baseline to week 24 in 72 naive HIV-patients starting on lopinavir (59 males and 13 females). A total of 210 samples were analysed, with at least two samples in every patient. Mean LPV trough concentration was 4.3 mg/L (± 2.1). The median intra-subject variation in LPV level was 38% (range 4-111%).

Serum lipids were not correlated to LPV plasma concentrations possibly due to the wide intra-individual variability in LPV trough levels. Monitoring of plasma lopinavir and subsequent dose adjustment of LPV will not be useful to prevent hyperlipidaemia.

Introduction

The high incidence of the metabolic syndrome in human immunodeficiency virus (HIV)-patients on antiretroviral therapy (ART) containing protease inhibitors (PIs) is an increasing clinical problem in HIV treatment. Lopinavir (LPV) is a potent PI, which is recommended in therapy-naive as well as heavily pre-treated HIV-patients (co-formulation with a low dose ritonavir; LPV/RTV).
Together with abdominal discomfort and diarrhoea, hyperlipidaemia are among the most described adverse events in patients receiving lopinavir. Grade 3 or higher hypercholesterolaemia and hypertriglyceridaemia have been reported in around 10% of antiretroviral therapy-naive patients and in up to 30% of patients with prevous PI-experience [1-4]. Patients with initially high lipid levels are at higher risk of developing (severe) hyperlipidaemia. The pathogenic mechanism of this dyslipidaemia is multifactorial and includes effects of the virus itself and antiretroviral use [5].
Measurement of antiretroviral plasma concentrations has been proven to be useful for guidance in HIV-therapy, not only for adequate virus control but also for reducing adverse events. A series of trials have demonstrated the relationship between plasma levels of antiretrovirals and the presence of side effects [6-8]. For example, Gatti et al discovered a clear relationship between ritonavir plasma concentrations and side effects [9]. Our own investigation demonstrated higher stavudine levels in patients experiencing lipoatrophy.[10] Previous data showed that higher plasma lopinavir concentrations are associated with a higher risk of elevated cholesterol levels [11;12].
The free study (ClinicalTrials.gov NCT00405925) is a prospective multicenter cohort of HIV-patients starting with ART. In this study therapy-naive patients started on zidovudine, lamivudine and ritonavir boosted lopinavir to achieve an undetectable viral load in short term. Patients with an undetectable viral load between week 12 and 24, were randomised to either continuation of the initial therapy or switch to 3 NRTIs (trizivir® b.i.d.: each tablet contains 300 mg zidovudine, 150 mg lamivudine and 300 mg abacavir) in order to simplify treatment, facilitate drug adherence and prevent potential long term PI-toxicity. Within the FREE study we investigated whether serum lipid elevation was correlated to LPV exposure.

Methods

Study design

The FREE study is a prospective, multi center cohort in which ART-naive HIV-infected patients were initially treated with zidovudine, lamivudine and ritonavir boosted lopinavir. When patients received undetectable viral load between week 12 till week 24, they were randomised to either continuation of this drug regimen or treatment with zidovudine, lamivudine and abacavir as a fixed-dose combination drug (trizivir®). In this substudy we measured lopinavir plasma levels in all patients up to randomisation (maximum week 24).
A total of 146 patients who started antiretroviral therapy between March 2003 and January 2006 were consecutively observed up to week 24.

Measurement of lopinavir plasma drug concentrations

Lopinavir plasma concentrations were determined by a high-performance liquid chromatography (HPLC) assay as previously reported. The lower limit of quantification is 0.07 mg/L. Average accuracy ranged from 97-106% and precision ranged from 2.4-8.1%, with inter-assay coefficient of variation 2.3-5.9% [13].

Study population

Inclusion criteria in the cohort included: ≥18 years of age, HIV-seropositivity, necessity to begin treatment with ART and informed consent. Inclusion criteria were: male or females older than 18 years, able and willing to sign informed consent, CD_4 count ≤350/mm^3 and HIV-1 RNA ≥30.000 copies/ml. The following metabolic parameters were required for inclusion: Fasting glucose ≤7 mmol/L (non-fasting <11 mmol/L), fasting triglycerides ≤2 mmol/L and LDL-cholesterol ≤4 mmol/L or LDL/HDL ration ≤4.1. Patients with lipids or glucose levels increasing above the inclusion values (triglycerides >8 mmol/L) could be switched to trizivir® after week 18 if their viral load had become undetectable (<50 copies/ml). Otherwise (no undetectable viral load at week 24 or lipid increase before randomisation) they were defined as failures and discontinued the original study, however their lipid and LPV data could still be used for our subanalysis. Patients enrolled in this ongoing trial comparing LPV/RTV with abacavir were selected for this analysis. Every patient started with LPV/RTV in combination with zidovudine and lamivudine. Follow-up visits were carried out by each individual center at fixed timepoints, according to the study scheme. Nine hospitals enrolled patients for this trial. Triglycerides and cholesterol were measured at baseline, week 4, week 8, week 12, week 18 and week 24. Plasma lopinavir levels were measured in all patients up to randomisation (week 4, 8, 12, 18 and 24 dependent on whether the patient was randomised to the LPV/RTV ongoing arm). In order to get a representative impression of the overall LPV exposure patients with at least 2 LPV trough levels were included in this study.

Patients were not allowed to use hypolipidemic medication. Lopinavir/ritonavir was given in a fixed dose combination of 133/33 mg three capsules twice daily (the former formula kaletra® capsules). For the evaluation of HIV-infection CD_4 cell count and viral load were measured at baseline and at week 12 and 24.

Statistics

Statistical evaluation was performed with SPSS for windows, version 14.0. Mean and median values with 95% confidence intervals were calculated for all samples in each individual. For the analysis, a p-value of ≤0.05 was regarded as significant.

Ethics

The research protocol was approved by the National Medical Ethic Committee and written informed consent was obtained from all subjects.

table 1 Baseline characteristics of patients.

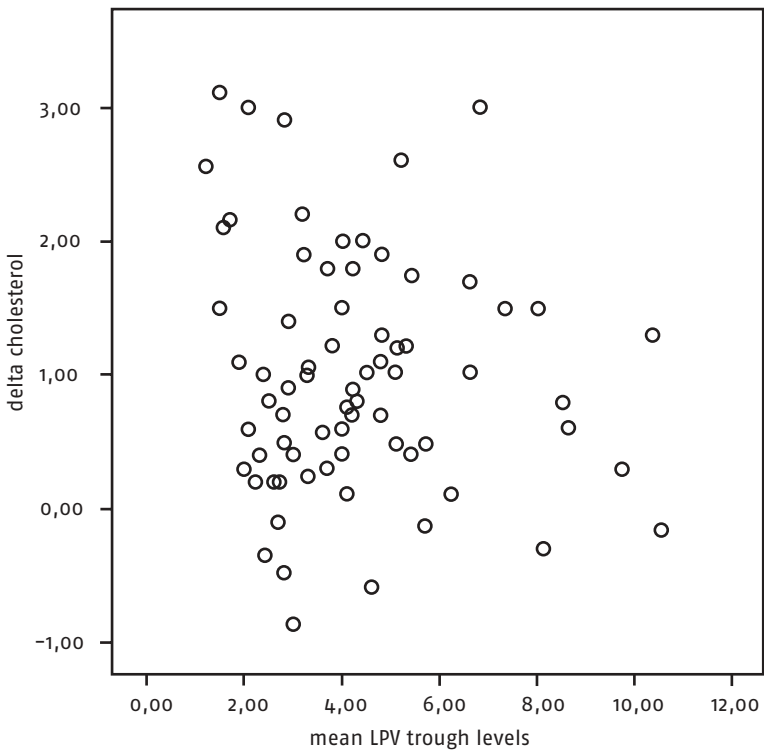
Patients	72
Gender	
Male	59 (82%)
Female	13 (18%)
Mean age (years)	44 ± 9
Mean CD_4 cells (cells/mm^3)	170 ± 94
Mean HIV-RNA (log copies/mL)	4.3
Race	
Caucasian	49 (68%)
Black	19 (26%)
Asian	4 (6%)
Total samples	210
2 samples	32 patients
3 samples	22 patients
4 samples	10 patients
5 samples	8 patients
BMI (kg/m^2)	22.9 ± 3.5
Data until week 12	5 patients
Data until week 18	3 patients
Data until week 24	64 patients

Results

From the beginning of the study in March 2003 to January 2006 146 patients were included, in 132 patients LPV plasma samples were collected, in 93 patients lopinavir trough levels could be calculated and were enrolled for this analysis. Because LPV levels within 4 hours after dosing do not allow a representative calculation of trough concentrations, we did not use these levels for the analysis. All data of patients were collected from baseline till randomisation. Four patients were not enrolled due to incomplete lipid data. Since an elevated plasma concentration can be obtained during one of the visits, whereas the overall drug levels would be adequate, we enrolled only patients with at least two plasma LPV trough levels to get a representative overall impression of the mean individual drug levels. There were 17 patients left out of the study due to <2 LPV samples. Prospectively we measured plasma lopinavir concentrations in 72 naive HIV-patients starting therapy with zidovudine, lamivudine and ritonavir boosted lopinavir (59 males and 13 females) until randomisation (between week 12-24). Five patients used LPV until week 12, three patients until week 18 and of 64 patients we had LPV plasma data until week 24.

A total of 210 samples were analysed; 32 patients had 2, 22 patients had 3, 10 had 4 and 8 patients had 5 LPV trough levels. The range of samples per patients varied from 2 to 5 with a mean of 2.9 samples per individual. The mean age at the start of

figure 1 Cholesterol changes and lopinavir trough concentrations.

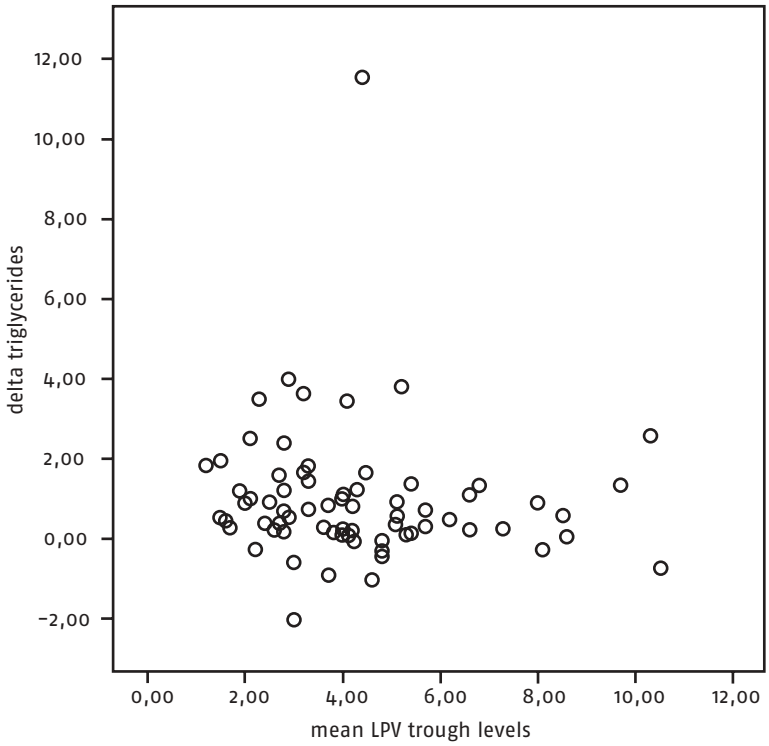


the study was 44 ± 9 years. The majority of patients were Caucasian (n=49, 68 %), there were 19 Black patients (26%) and 4 Asian (6%). The mean CD_4 cell count at initiation of the study was 170 ± 94 cells/mm^3. Characteristics of the groups are depicted in *table 1*.

During the study period 64 (89%) patients experienced an increase in cholesterol. An increase of triglycerides compared to baseline was seen in 61 (85%) patients. None of these patients were treated with lipid lowering drugs. Grade 2 hypercholesterolaemia (>6.2 mmol/L; WHO toxicity scale) and hypertriglyceridaemia (>4.5 mmol/L) was seen in 10 (14%) and 6 (8%) patients respectively, of whom 4 (6%) had even grade 3 hypercholesterolaemia (>7.8 mmol/L) and only 1 (1.4%) had grade 3 hypertriglyceridaemia (>8.5 mmol/L). LDL-cholesterol >4.8 mmol/L is an indication for lipid lowering drugs. Only one patient had a serum LDL-cholesterol above this threshold, which was 6.4 mmol/L 24 weeks after start of LPV. This patient had a median LPV trough level of 4.6 mg/L compared to a median trough level of 4.0 mg/L for all patients.

Mean LPV trough concentration was 4.3 mg/L (± 2.1) and median 4.0 mg/L. Mean and median trough levels of LPV >8 mg/L were seen in 6 and 5 patients respectively. Mean and median trough levels of LPV >8 mg/L were seen in 6 and 5 patients respectively.

figure 2 Triglycerides changes and lopinavir trough concentrations.



We did not found a correlation between lipids (cholesterol and triglycerides) and LPV trough concentrations (figure 1 and 2). We found a remarkable wide inter-subject variation concerning the LPV trough levels with a median of 38% (range 4-111%).
As far as risk factors are concerned, slightly higher lopinavir through levels were observed in females compared to males however without statistical significant difference (4.8 and 3.4 mg/L in females and males respectively). In addition, no relation between LPV levels and race, age, BMI, CD_4 cell count, HIV-RNA and CDC classification could be detected.

Discussion

Our data confirm results of previous studies that revealed dyslipidaemia in patients on LPV. However, we could not demonstrate a correlation between lopinavir trough levels and dyslipidaemia. In addition, no other factors influencing lipid levels were defined.
There is a wide inter- and intra-patient variability when measuring drug exposure to a standard dose. In our study inter-subject variation in LPV trough levels

ranged from 4-111% with a median of 38%. This phenomenon might explain why no relation between LPV levels and serum lipids were detected. High variability in LPV concentrations has been found in several studies ranging from 15-54%; median of 35% [14;15]. This phenomenon might be an explanation why no correlation between LPV trough levels and serum lipids could be detected. An alternative explanation might be that lipid alterations in LPV use is an intrinsic adverse event of LPV, independent of LPV concentrations.

There have been a number of studies looking at different aspects of PI drug levels and the influence on serum lipids. Results from these studies show conflicting data about relations between drug concentrations and lipids. González De Requena et al investigated lipids in HIV-patients on salvage therapy with LPV/RTV from baseline to month 3. They found a positive correlation between the percentage increase in triglycerides and LPV trough levels, but no correlation was found between LPV trough levels and the percentage of increase in cholesterol levels [12]. In a small population all patients with higher LPV trough (especially trough levels >8 mg/mL) levels were the ones at risk of dyslipidaemia [16;17]. Moreover, in a population of 142 ART-experienced patients high triglycerides were found in subjects with high LPV residual concentrations [11]. In contrast to the PharmaAdapt study, where no relationship was demonstrated between PI drug concentrations and lipids between week 0 and 32 in 252 patients [18]. At week 24 increase of triglycerides and total cholesterol could not be correlated with LPV plasma levels by Leon et al in a population of 26 patients [19]. Additionally, in a study by Torti et al no correlation between lipid abnormalities and LPV plasma concentrations were seen [20]. These were all trials in heavily pre-treated HIV-patients.

Of the PI-group lopinavir has been the most frequently associated with hyper-lipidaemia. A retrospective study comparing patients on an indinavir/ritonavir (IDV/RTV) with a LPV/RTV containing regimen, noted higher serum cholesterol levels in the IDV/RTV group at 3 and 12 months [21]. Whereas, Antoniou et al could not detect a significant difference in lipid alterations between ritonavir boosted indinavir and ritonavir in a small cohort of HIV-patients [22]. However, these trials did not investigate plasma drug levels.

Elevated lipid values at baseline and high dietary fat intake are risk factors for hypertriglyceridaemia and hypercholesterolaemia [23]. These confounding factors could not have played a role in our study because of strict criteria for inclusion and the measurement of fasting serum lipids. Other advantages of this study are the prospective design, the homogenous study population, the sample size and no confounding by the use of statines or fibrates (lipid lowering drugs were only allowed after randomistation).

However, some limitations of our study should be noted. First, no RTV levels were measured therefore the influence of this low dose booster PI cannot be ruled out. Second, not from all patients there were 5 LPV samples taken up to randomisation. This study only analysed data till week 24. We might have missed the occurrence of hyperlipidaemia as a result of longer duration of treatment, independent of lopinavir plasma concentrations. However, the increase of serum lipids was already within 24 weeks of LPV use in our ART-naive patients. Others also found lipid elevations even after short term use of LPV [19;24].

Data of lipid changes and LPV plasma levels after week 24 are needed for a better knowledge of the effect of LPV levels on lipids. The total analysis of the FREE study might give more insight in this issue. Of note, a retrospective analysis of HIV-infected patients on LPV/RTV revealed increase of total cholesterol and triglycerides early after introduction of LPV which remained subsequently stable for a period of 15 months [3]. Finally, the overal incidence of grade 3 or more hyperlipidaemia might be underestimated due to the strict criteria for inclusion and continuation of the study. The percentage of patients presenting grade 2 or more hyper-lipidaemia was lower during the study period compared to other studies. However, the populations described in literature were heterogenous and even started with increased serum lipids due to previous therapy or stage of HIV-disease. Favourable changes in lipids and lipoproteins have been found in HIV-infected patients treated with a regimen of stavudine, didanosine and nevirapine [25]. An overall increase of HDL-cholesterol was seen in these patients compared to those treated with stavudine and didanosine combined with either lamivudine or indinavir. It is remarkable that some studies showed that NRTIs might have an additional effect on the development of lipid increase while others did not [26]. It is important to keep in mind that maybe LPV trough levels are not the best tool to investigate adverse events such as hyperlipidaemia. In order to gain further insight into the relevance of LPV plasma concentrations and lipid increase, additional studies have to be performed using peak levels. It might be possible that peak concentrations are the best representative to detect a correlation. As a consequence of lower volume of distribution female patients are tend to be more prone for side effects, which has been observed in some clinical trials [27]. Slightly higher lopinavir levels were observed in females compared to males however statistical significant difference could not be reached. Our study was not intended to look at risk factors or to define predictive factors of hyperlipidaemia, just to investigate the influence of LPV plasma levels. Ritonavir boosted lopinavir is frequently associated with a significant increase in triglyceride and/or cholesterol levels soon after starting therapy. It is hypothesised that this dyslipidaemia is multifactorial and includes a direct effect of the PI and a more complex mechanism involving immunologic, genetic and pharmacodynamic factors and HIV-infection itself [5;12;16;17;24]. To our knowledge this is the first prospective study analysing LPV levels with serum lipids in a cohort of only antiretroviral therapy-naive patients. Based on our data we cannot recommend frequent measurements of serum (trough) levels in lopinavir use in order to prevent dyslipidaemia.

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References

1 Martinez E, Domingo P, Galindo MJ, Milinkovic A, Arroyo JA, Baldovi F, Larrousse M, Leon A, de LE, Gatell JM. Risk of metabolic abnormalities in patients infected with HIV receiving antiretroviral therapy that contains lopinavir-ritonavir. **Clin Infect Dis** 2004; 38:1017-23.

2 Calza L, Manfredi R, Farneti B, Chiodo F. Incidence of hyperlipidaemia in a cohort of 212 HIV-infected patients receiving a protease inhibitor-based antiretroviral therapy. **Int J Antimicrob Agents** 2003; 22:54-59.

3 Lafeuillade A, Hittinger G, Philip G, Lambry V, Jolly P, Poggi C. Metabolic evaluation of HIV-infected patients receiving a regimen containing lopinavir/ritonavir (Kaletra). **HIV Clin Trials** 2004; 5:392-98.

4 Montes ML, Pulido F, Barros C, Condes E, Rubio R, Cepeda C, Dronda F, Antela A, Sanz J, Navas E, Miralles P, Berenguer J, Perez S, Zapata A, Gonzalez-Garcia JJ, Pena JM, Vazquez JJ, Arribas JR. Lipid disorders in antiretroviral-naive patients treated with lopinavir/ritonavir-based HAART: frequency, characterization and risk factors. **J Antimicrob Chemother** 2005; 55:800-804.

5 Oh J, Hegele RA. HIV-associated dyslipidaemia: pathogenesis and treatment. **Lancet Infect Dis** 2007; 7:787-96.

6 Dieleman JP, Gyssens IC, van der Ende ME, de MS, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. **AIDS** 1999; 13:473-78.

7 Gutierrez F, Navarro A, Padilla S, Anton R, Masia M, Borras J, Martin-Hidalgo A. Prediction of neuropsychiatric adverse events associated with long-term efavirenz therapy, using plasma drug level monitoring. **Clin Infect Dis** 2005; 41:1648-53.

8 Dicenzo R, Luque A, Larppanichpoonphol P, Reichman R. Association of total bilirubin with indinavir and lopinavir plasma concentrations in HIV-infected patients receiving three different double-boosted dosing regimens. **J Antimicrob Chemother** 2006; 58:393-400.

9 Gatti G, Di BA, Casazza R, De PC, Bassetti M, Cruciani M, Vella S, Bassetti D. The relationship between ritonavir plasma levels and side effects: implications for therapeutic drug monitoring. **AIDS** 1999; 13:2083-89.

10 ter Hofstede HJM, Koopmans PP, Burger DM. Stavudine plasma concentrations and lipoatrophy. **J Antimicrob Chemother** 2008; 61:933-38.

11 Valerio L, Fontas E, Pradier C, Lavrut T, Garraffo R, Dunais B, Cua E, Dellamonica P. Lopinavir/ritonavir combination and total/HDL-cholesterol ratio. **J Infect** 2005; 50:229-35.

12 Gonzalez de RD, Blanco F, Garcia-Benayas T, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Correlation between lopinavir plasma levels and lipid abnormalities in patients taking lopinavir/ritonavir. **AIDS Patient Care STDS** 2003; 17:443-45.

13 Droste JA, Verweij-van Wissen CP, Burger DM. Simultaneous determination of the HIV drugs indinavir, amprenavir, saquinavir, ritonavir, lopinavir, nelfinavir, the nelfinavir hydroxymetabolite M8, and nevirapine in human plasma by reversed-phase high-performance liquid chromatography. **Ther Drug Monit** 2003; 25:393-99.

14 Torti C, Quiros-Roldan E, Tirelli V, Regazzi-Bonora M, Moretti F, Pierotti P, Orani A, Maggi P, Cargnel A, Patroni A, De LA, Carosi G. Lopinavir plasma levels in salvage regimes by a population of highly active antiretroviral therapy-treated HIV-1-positive patients. **AIDS Patient Care STDS** 2004; 18:629-34.

15 Boffito M, Back DJ, Hoggard PG, Caci A, Bonora S, Raiteri R, Sinicco A, Reynolds HE, Khoo S, Di PG. Intra-individual variability in lopinavir plasma trough concentrations supports therapeutic drug monitoring. **AIDS** 2003; 17:1107-8.

16 Gutierrez F, Padilla S, Navarro A, Masia M, Hernandez I, Ramos J, Esteban A, Martin-Hidalgo A. Lopinavir plasma concentrations and changes in lipid levels during salvage therapy with lopinavir/ritonavir-containing regimens. **J Acquir Immune Defic Syndr** 2003; 33:594-600.

17 Gutierrez F, Padilla S, Navarro A, Masia M, Hernandez I. Lipid abnormalities in HIV-Infected patients and lopinavir plasma concentrations. **J Acquir Immune Defic Syndr** 2004; 36:1107-9.

18 Clevenbergh P, Garraffo R, Dellamonica P. Impact of various antiretroviral drugs and their plasma concentrations on plasma lipids in heavily pretreated HIV-infected patients. **HIV Clin Trials** 2003; 4:330-336.

19 Leon A, Martinez E, Sarasa M, Lopez Y, Mallolas J, De Lazzari E, Laguno M, Milincovic A, Blanco JL, Larrousse M, Lonca M, Gatell JM. Impact of steady-state lopinavir plasma levels on plasma lipids and body composition after 24 weeks of lopinavir/ritonavir-containing therapy free of thymidine analogues. J Antimicrob Chemother 2007; 60:824-30.

20 Torti C, Quiros-Roldan E, Regazzi-Bonora M, De LA, Lo CS, Di GS, Patroni A, Villani P, Micheli V, Carosi G. Lipid abnormalities in HIV-infected patients are not correlated with lopinavir plasma concentrations. **J Acquir Immune Defic Syndr** 2004; 35:324-26.

21 Bongiovanni M, Bini T, Chiesa E, Cicconi P, Adorni F, Monforte dA. Lopinavir/ritonavir vs. indinavir/ritonavir in antiretroviral naive HIV-infected patients: immunovirological outcome and side effects. **Antiviral Res** 2004; 62:53-56.

22 Antoniou T, Park-Wyllie L, Boyle E. A comparison of dyslipidemias associated with either lopinavir/ritonavir- or indinavir/ritonavir-based antiretroviral therapy. **J Acquir Immune Defic Syndr** 2004; 37:1666-67.

23 Bongiovanni M, Bini T, Cicconi P, Landonio S, Meraviglia P, Testa L, Di BA, Chiesa E, Tordato F, Biasi P, Adorni F, Monforte AD. Predictive factors of hyperlipidemia in HIV-infected subjects receiving lopinavir/ritonavir. **AIDS Res Hum Retroviruses** 2006; 22:132-38.

24 Schambelan M, Benson CA, Carr A, Currier JS, Dube MP, Gerber JG, Grinspoon SK, Grunfeld C, Kotler DP, Mulligan K, Powderly WG, Saag MS. Management of metabolic complications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. **J Acquir Immune Defic Syndr** 2002; 31:257-75.

25 van de Valk M, Kastelein JJ, Murphy RL, van Leth F, Katlama C, Horban A, Glesby M, Behrens G, Clotet B, Stellato RK, Molhuizen HO, Reiss P. Nevirapine-containing antiretroviral therapy in HIV-1 infected patients results in an anti-atherogenic lipid profile. **AIDS** 2001; 15:2407-14.

26 Anastos K, Lu D, Shi Q, Tien PC, Kaplan RC, Hessol NA, Cole S, Vigen C, Cohen M, Young M, Justman J. Association of serum lipid levels with HIV serostatus, specific antiretroviral agents, and treatment regimens. **J Acquir Immune Defic Syndr** 2007; 45:34-42.

27 Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF. Sex differences in pharmacokinetics and pharmacodynamics. **Annu Rev Pharmacol Toxicol** 2004; 44:499-523.

General discussion and Summary

General discussion

With the currently available antiretroviral drugs eradication of HIV-infection is still impossible. Even as a chronic disease, HIV continues to be a potentially life-threatening infection, as disease progression can occur rapidly if treatment is suboptimal or toxicity leads to discontinuation of medication. In addition, two other risks in chronic HIV-infection play an important role in morbidity nowadays. First, as the prognosis for HIV-infected patients steadily improves, these individuals will incur an increased risk for atherosclerosis resulting in cardiovascular disease. The cardiovascular risk profile caused by the HIV-infection and by antiretroviral therapy (ART) will even enhance this problem. A recent study collecting data of adverse events in ART, stated that ART was associated with a 26% relative increase in the rate of myocardial infarction per year of exposure during the first 4-6 years of use. An interesting report that was recently published by Sabin et al (DAD study), demonstrated an increased risk of myocardial infarction in patients exposed to abacavir and didanosine within the preceding 6 months and was not present beyond 6 months after drug cessation. Second, although ART can (partly) reconstitute the human immune system, it is still not optimal and patients are high risk for all kind of malignancies. Especially lymphomas and lung carcinoma are frequently diagnosed.
The management of HIV-infection is nowadays characterised by easier treatment schedules than in the beginning of highly active antiretroviral therapy (HAART). For example, a previously used drug scheme with saquinavir consisted of at least 6 tablets three times a day taken with high fat meals combined with 3 tablets of NRTIs twice a day. In sharp contrast, in 2008 a capsule with tenofovir-emtricitabine-efavirenz is on the market which reduces the burden of tablets to one o.i.d..
However drug interactions and a wide scale of adverse events are still present and often hamper continuation of chronic therapy. In salvage therapy, sometimes complex treatment schedules with fat intake are needed in order to attain effective plasma drug levels to suppress viral replication, with the additional risk of side effects.

Adherence to antiretroviral therapy remains the cornerstone of treatment success. The major reason for discontinuation of therapy in HIV-infected patients appeared to be toxicity. Analysis of data from the Dutch HIV monitoring database showed that in therapy-naive HIV-patients, adverse events were the major reason for switching ART (44-58%). Additionally, 56% of switches on subsequent regimens have been attributed to toxicity.
In management of patients on antiretroviral therapy, identification of possible factors that make patients prone for toxicity is essential. The benefits of HIV treatment have to outweigh the disadvantages of antiretroviral therapy. In this light the aim of this thesis is to analyse correlations between laboratory investigations, plasma drug concentrations and toxicity and to identify factors to prevent adverse events.

Several issues of toxicity are highlighted in this thesis. First of all, NRTI-related

toxicity, which has been recognised as mitochondrial dysfunction. Adverse events related to mitochondrial toxicity are (cardio)myopathy, neuropathy, pancreatitis, hepatic steatosis and lactic acidosis. Second, the metabolic syndrome with lipid alterations and insulin resistance is an important adverse event in PI-use, often seen in combination with body shape changes with fat loss (lipoatrophy) or fat accumulation (lipohypertrophy); the so-called lipodystrophy syndrome.

In **chapter 1** an overview is given on the emergence of adverse events in antiretroviral therapy of HIV-infected patients. Especially NRTI-related mitochondrial toxicity and lipoatrophy/lipodystrophy with lipid changes and impaired glucose tolerance are reviewed. Mitochondrial toxicity has been hypothesised as the mechanism underlying for the pathogenesis of various adverse events associated with the use of nucleoside reverse transcriptase inhibitors. NRTIs are supposed to interfere with an enzyme (polymerase γ), essential for the synthesis of mitochondrial DNA strands. The triphosphate compounds of the nucleoside analogues inhibit polymerase γ. Blocking of this enzyme results in mitochondrial damage and thereby mitochondrial dysfunction. By interference with this enzyme, NRTIs are build into a new mitochondrial DNA strand instead of the normal nucleosides, resulting in chain termination and thereby impaired DNA synthesis. Incidences of mitochondria-related toxic symptoms are not well known and vary, dependent on the analysis; neuropathy occurs in 12-46% (with stavudine and didanosine use), myopathy in 17% (with zidovudine use) and pancreatitis in 0.5-7% (didanosine and lamivudine). The incidence of lactic acidosis is estimated around 1.3 per 1000 person-years, based on retrospective analysis of a cohort of antiretroviral drug users.

Exposure to protease inhibitors has been associated with the development of significant metabolic adverse effects such as hyperlipidaemia, hyperglycaemia and insulin resistance, peripheral fat wasting and central adiposity (neck and visceral fat).
The prevalence of lipodystrophy varies widely. Previous data from the Dutch ATHENA cohort showed an incidence rate of 6.2 per 100 person years with a four-year cumulative incidence of 25%. Pathogenesis of the metabolic syndrome in HIV-patients is multifactorial and includes effects of inflammation and the virus itself, effects of antiretroviral drugs on metabolic pathways and drug-associated adipose repartitioning with subsequent development of insulin resistance and lipid alterations.

In **chapter 2** we describe four patients in whom fatal lactic acidosis occurred due to the use of NRTIs. The initial symptoms were nausea and vomiting, at this stage the lactic acidosis was not recognised. However when tachypnoea occurred to compensate the acidosis, the physicians discovered the reason of the symptoms, i.e. the lactic acidosis. However, despite extensive treatment in the intensive care departments the complications were fatal when liver failure and uncontrollable arrhythmias occurred. These four cases illustrate the importance of early recognition of lactic acidosis. Although co-enzyme Q, thiamine, L-carnitine and riboflavin have been used, there is no established treatment. The use of riboflavin in one of our cases was unsuccessful. Our report of these four cases of fatal lactic acidosis

revealed that all patients already experienced some kind of NRTI-related toxicity before this fatal event.

In order to investigate mitochondrial toxicity as the basis for NRTI-induced adverse events, we analysed serum lactate and pyruvate levels which is described in **chapter 3**. There were four groups: patients with presumed NRTI-related adverse events, patients without adverse events, HIV-infected patients without antiretroviral therapy and healthy controls. The mean duration of NRTI treatment was 18 months. The highest lactate levels were found in the patients experiencing NRTI-related adverse events, especially in patients with stavudine-induced neuropathy. The lowest levels were measured in the healthy controls. Remarkable was that all patients with a lactate value above 2700 µmol/L experienced adverse events and that the majority even suffered from more than one NRTI-related adverse event. Just like the fatal cases, these patients were all on a stavudine containing regimen. Looking at the adverse event neuropathy more closely, we found that the highest lactates were measured in patients with NRTI-related neuropathy. This is consistent with a study using serum lactate levels in distinguishing between HIV- and NRTI-associated neuropathy. Neuropathy is an important cause of switching HIV-treatment and one of the reasons for the unpopularity of stavudine and didanosine. Asymptomatic mild hyperlactataemia is a rather common feature of antiretroviral therapy. Mild asymptomatic hyperlactataemia requires careful monitoring but no immediate action. In case symptoms occur that might be due to side effects of NRTIs, hyperlactataemia might be of additional value to distinguish the cause of these symptoms. We advise to measure serum lactate concentrations in these patients. In addition, from our data we suggest that lactate levels of ≥2500 µmol/L (even in asymptomatic patients) need careful attention and eventually discontinuation if lactate concentrations stay high or if symptoms occur. Patients with stavudine-related neuropathy and long term antiretroviral therapy (mostly second or more regimens) are prone for symptomatic hyperlactataemia. Hyperlactataemia in patients on stavudine is noticeable and not always directly related to adverse events. We would not recommend routine measurement of lactate during antiretroviral therapy in all asymptomatic patients. However, serum lactate measurement is useful in patients with NRTI-related adverse events. In patients with serum lactates above 2500 µmol/L, we would advise frequent monitoring in order to evaluate if discontinuation of the current antiretroviral therapy is needed.

The other study measuring lactates in different groups of HIV-patients is described in **chapter 4**. This is an additional study to the previous trial. Since an oral glucose tolerance test (OGTT) can diagnose mitochondrial dysfunction in patients with inherited mitochondrial disorders, our aim was to test whether an OGTT is useful for the detection of (occult) hyperlactataemia as a result of NRTI-related toxicity. We performed an OGTT in HIV-patients with and without NRTI-related mitochondrial toxicity. By this approach we showed that an OGTT is useful as an additional test to find hyperlactataemia in mitochondrial related toxicity. On the basis of the sensitivity and specificity we illustrated that an OGTT in patients suspected of mitochondrial related toxicity is useful to distinguish toxicity from

other symptoms. However, an OGTT should not be used as a routine screening in asymptomatic patients on antiretroviral treatment. The use of an oral glucose tolerance test is worthwhile in symptomatic patients on antiretroviral therapy to confirm NRTI suspected mitochondrial toxicity. Hyperlactataemia after glucose loading reflected the patients with NRTI-related adverse events. Because of its low sensitivity but good specificity, an OGTT should be used as an additional test to verify NRTI-related toxicity rather than to search for NRTI-related toxicity. In both previously described studies we found that duration of therapy is an additional risk factor for the development of mitochondrial toxicity in NRTI-treatment.

One of the most expanding problems, which have been underestimated in the first years of highly active antiretroviral therapy, is the lipodystrophy syndrome, with lipohypertrophy and lipoatrophy. Exposure to PIs has been associated with the development of metabolic adverse effects such as hyperlipidaemia, insulin resistance and central adiposity. Peripheral fat wasting (lipoatrophy) is associated with NRTI and especially stavudine use. Given the multifactorial pathogenesis of this syndrome, we wanted to analyse metabolic changes in more detail, especially since the high incidence of the metabolic syndrome in ART-use means an increase in cardiovascular co-morbidity. In **chapter 5** we present the results of metabolic parameters in a prospective study treating antiretroviral naive patients. Since antiretrovirals are known to cause lipid alterations and glucose intolerance, it is becoming increasingly important to make an adequate risk profile for cardiovascular diseases in patients treated with antiretrovirals. Our results provide more insight into the existence of these metabolic changes. The data show a lipid increase and impaired insulin sensitivity in the first 24 weeks of ART (lopinavir use). After week 24 this elevation becomes stable in lopinavir users. In contrast to the subjects that continued on abacavir. There was a significant difference between the PI group and the triple NRTI group. The PI-free group (triple NRTI use) demonstrated a decrease of serum lipids and improved glucose tolerance after initiation, while in the PI containing regimen lipid and glucose increase stabilised. Like other investigators we demonstrated that PI regimens are associated with changes in lipoprotein profiles and thereby an increased risk of cardiovascular disease. However this is the first prospective trial investigating extensive metabolic parameters in naive patients starting on PI with randomisation to a PI free regimen (abacavir) or continuation of PI at week 24.

The lipohypertrophy/lipoatrophy syndrome has not only major psychosocial implications for patients suffering from this condition it also has impact on the life-expectancy by introducing cardiovascular risk factors both influencing treatment adherence. Miscellaneous reports highlight the fact that lipodystrophy, mainly the lipoatrophy component, is primarily linked to NRTI therapy, in particular stavudine, while dyslipidaemia and insulin resistance are associated with PI therapy. For these reasons we investigated the role of drug concentrations in the occurrence of lipoatrophy and dyslipidaemia. We performed two studies: one regarding the correlation between stavudine plasma concentrations and lipoatrophy, the other investigating plasma lopinavir concentrations in relation

to serum lipids. In **chapter 6** the relation between lipoatrophy and plasma stavudine concentrations is investigated. We retrospectively measured stavudine plasma concentrations in patients with and without lipoatrophy. Plasma stavudine concentrations were higher in the group with lipoatrophy compared to patients without lipoatrophy. Although it is stated that plasma NRTI concentrations do not correlate with the active triphosphate NRTI metabolite which is intracellular, our data show that measurement of NRTI plasma concentrations can be useful as a derivative of NRTI-induced toxicity. On the basis of recent published data and those of our study, we suggest that a plasma stavudine concentration ratio <1.0 is a safe level for treatment of HIV-patients to prevent lipoatrophy. Our findings are in line with a recent addendum to the WHO-guidelines on antiretroviral therapy for HIV-infection in adults and adolescents, which now recommends a lower dosage for stavudine (30 mg twice daily).

Hypercholesterolaemia and hypertriglyceridaemia are frequently seen in lopinavir use, incidence rates vary depending on the severity of the increase; grade 3-4 lipid rise has been reported in about 25% of patients. Especially the rise of LDL-cholesterol makes these patients more prone for cardiovascular diseases. A recent cohort showed a prevalence of high LDL in men of around 20% and in women of around 10%. The percentages were higher than in non HIV-infected subjects, with a further increased prevalence in patients on PIs.
To look more closely at lipid alterations as a result of toxicity, we analysed the plasma lopinavir (LPV) concentrations and lipid levels of the FREE study in **chapter 7**. This study provided a unique opportunity to address this issue as the initial treatment in all patients was zidovudine, lamivudine and ritonavir-boosted lopinavir. By using this database of patients in whom fasting serum lipids and lopinavir concentrations were collected, we could analyse 210 plasma samples in 72 patients. During the study period the majority of the patients experienced an increase in cholesterol and triglycerides, although a grade 2 hyperlipidaemia was only seen in a small population (<10%). This study supports that PI use contributes to the development of lipoprotein abnormalities. Lipid increase was already seen even after short-term PI use. However, serum lipids did not correlate to LPV plasma concentrations probably due to a remarkable wide intra-individual variability in LPV trough levels varying from 4-111% (median of 35%) and the intrinsic effect of LPV on lipid metabolism. We conclude that plasma LPV measurement and eventually dose adjustment of LPV will not be useful to prevent hyperlipidaemia.
The studies reported in **chapter 6** and **7** did not have the intention to determine causal factors of lipoatrophy and dyslipidaemia, we were just searching for a potential influence of high drug exposure as risk factor for the occurrence of metabolic complications and how therapeutic drug monitoring might influence this in the future.

The studies described in this thesis represent an overview of different types of toxicity in antiretroviral therapy in HIV-infected patients. These data show the clinical relevance of close monitoring of patients on antiretroviral therapy to prevent drug toxicity and achieve better adherence.

At present no combination of antiretroviral compounds meets all of the characteristics of an ideal antiretroviral regimen without any adverse events. All different kinds of agents have their advantages and disadvantages. In clinical practice, the choice of the best antiretroviral regimen should be made after careful evaluation of all aspects of HIV-treatment. The best antiretroviral therapy for this specific patient must be based on a careful balance between efficacy and safety. The tolerance profile of the individual drugs and the combination of them in particular should be taken into account when choosing antiretroviral medicines. With the help of therapeutic drug monitoring of NRTIs, NNRTIs and PIs toxicity can be prevented or detected in an early and still reversible phase. This individual approach makes HIV-treatment a challenging business for every physician treating this kind of chronic infections.
In the next years, we expect new agents in the already existing classes of NRTIs, NNRTIs and PIs, but also new drugs from the more recent introduced classes of antiretrovirals such as integrase and fusion inhibitors as well as chemokine receptor blockers. The type of toxicity occurring in antiretroviral therapy seems to be class specific, this might implicate that with the introduction of new antiretrovirals also new kinds of adverse events will be seen. As the future might bring us numerous other adverse events with the release of new drug classes, which deserve scientific attention for their severity or their effect on quality of life, we would welcome more studies in this field. An interesting new field in pharmacotherapy is pharmaco-genetics. Genetic constitution plays a role in adverse events. By exploring this field, more individualised therapy can be applied resulting is less side effects.

Samenvatting

Samenvatting

Met de huidige beschikbare medicijnen voor HIV-infectie (HIV: humaan immunodeficiëntie virus) is het mogelijk om deze potentieel dodelijke ziekte tot staan te brengen. Genezing is echter helaas niet mogelijk gebleken. De vermenigvuldiging van het virus is goed te onderdrukken, maar uitroeiing ervan lukt nog steeds niet. Door continu gebruik van HIV-onderdrukkende medicijnen is HIV-infectie een chronische ziekte geworden. Maar zelfs als chronische ziekte blijft HIV nog steeds een potentieel ernstige infectie met soms dodelijke afloop. Zo kan ziekteprogressie snel optreden als de behandeling niet optimaal is of als bijwerkingen leiden tot het staken van de medicijnen. Er spelen twee belangrijke klinische problemen in chronische HIV-infectie.
Allereerst, de ouder wordende HIV-patiënt zal net als individuen zonder HIV aderverkalking krijgen met het toenemen van de leeftijd, terwijl deze kans nog meer toeneemt door HIV-infectie zelf en door de antiretrovirale therapie. Een van de belangrijkste problemen op dit moment bij het chronisch gebruik van antiretrovirale medicijnen bij HIV zijn stijging van cholesterol en vetten in het bloed (triglyceriden) en neiging tot suikerziekte (ongevoeligheid voor insuline). Dit wordt met name gezien bij het gebruik van de zogenaamde protease remmers (Engels: protease inhibitors: PIs). Deze afwijkingen kunnen leiden tot hart- en vaatziekten. Recent toonde een onderzoek dat behandeling met HIV-medicijnen (antiretrovirale therapie) gepaard gaat met een relatieve toename van 26% in het aantal hartinfarcten per jaar in de eerste 4-6 jaar van antiretrovirale therapie. Een andere studie toonde een toename in het aantal hartinfarcten bij patiënten die abacavir en didanosine gebruikten. Dit toegenomen risico verdween 6 maanden na het staken van deze medicijnen. Met name omdat de leeftijd van patiënten met HIV-infectie steeds hoger wordt, mag verwacht worden dat dit probleem in de toekomst groter zal worden. Immers de kans op aderverkalking en daarmee de kans op hartinfarcten of een beroerte, wordt groter naarmate men ouder wordt.
Het tweede belangrijke probleem bij chronische HIV-infectie is het maar gedeeltelijk herstel van de afweer. Dat is waarschijnlijk de belangrijkste reden dat er bij HIV-patiënten meer kanker voorkomt dan bij de normale bevolking.
De huidige HIV-therapie bestaat in de regel uit een combinatie van drie geneesmiddelen uit tenminste twee klassen. De inname is een stuk eenvoudiger geworden in de afgelopen tijd. Een voorheen gebruikt medicatieschema met bijvoorbeeld de proteaseremmer saquinavir bestond uit 3 maal daags 6 tabletten die ingenomen moesten worden met een vetrijke maaltijd, gecombineerd met 2 maal daags 3 tabletten uit een andere klasse medicijnen; de nucleoside reverse transcriptase remmers (Engels: nucleoside reverse transcriptase inhibitors: NRTIs). Dit is een groot contrast met tegenwoordig. Onlangs is een combinatiemedicijn op de markt gekomen dat uit drie componenten bestaat: tenofovir, emtricitabine en efavirenz. Hiermee is het mogelijk geworden om HIV-infectie te behandelen met één keer per dag één tablet. Naast deze voordelen zijn er nog steeds veel nadelen. Interacties tussen medicijnen en bijwerkingen zijn een belangrijk probleem bij het chronisch gebruik van HIV-medicatie.

Niettemin wordt deze één maal daags behandeling niet door alle patiënten verdragen. Slaapstoornissen, duizeligheid, hallucinaties, afwijkingen in botten en nieren kunnen voorkomen. Ook zijn er nog onvoldoende lange-termijngegevens om te kunnen stellen dat het hier een perfect werkzame en veilige behandeling betreft. Daarom blijft er ook voor de andere antiretrovirale geneesmiddelen een plaats in de behandeling.
Dit proefschrift gaat over de bijwerkingen van antiretrovirale medicijnen bij HIV (met name de PIs en de NRTIs). Therapietrouw is de belangrijkste hoeksteen van succesvolle therapie. Dit was in elk geval tot voor kort een belangrijk probleem. De belangrijkste reden voor het staken van medicatie is het optreden van bijwerkingen. Een analyse van gegevens uit de Nederlandse HIV monitoring databank toonde dat 44-58% van de patiënten op hun eerste antiretrovirale therapie hun medicatie stopten vanwege bijwerkingen. Dit was 58% bij daarop volgende therapieën. Dit benadrukt hoe belangrijk veiligheid is. Bovendien is het van belang factoren op te sporen die een rol spelen bij het optreden van bijwerkingen. De voordelen van therapie moeten opwegen tegen de nadelen van bijwerkingen. Met dit doel heeft dit onderzoek zich gericht op de analyse van verbanden tussen laboratoriumonderzoek, plasmaspiegels van medicijnen en schadelijkheid (toxiciteit). Daarmee hebben we ook gekeken naar mogelijke factoren die een rol spelen bij het ontstaan van bijwerkingen die mogelijk kunnen worden toegepast voor preventie.

Verscheidene kanten van toxiciteit worden belicht in dit proefschrift. Ten eerste, de bijwerkingen van de klasse nucleoside reverse transcriptase remmers (NRTI-gerelateerde schade). NRTIs veroorzaken afwijkingen in de mitochondriën; de onderdelen van cellen die zorgen voor de energiehuishouding. Bijwerkingen gerelateerd aan mitochondriële schade zijn schade aan de hartspier, zenuwbeschadiging, alvleesklierontsteking, leververvetting en melkzuurvergiftiging. Ten tweede, hebben we studies verricht met betrekking tot het zogenaamde metabool syndroom. Dit syndroom bestaat uit een ongunstige verandering in de bloedvetten (lipiden) en de glucose (suiker) stofwisseling, waardoor er suikerziekte kan ontstaan bij de HIV-geïnfecteerde patiënt. Het metabole syndroom met lipidenveranderingen en insulineresistentie (neiging tot suikerziekte) is een belangrijke bijwerking van PI-gebruik. Daarnaast worden er tevens vaak lichaamsveranderingen gezien bij de patiënt, met verlies van vetweefsel in het gelaat, de armen en benen, en toename van vet in de buik en de nek (het zogenaamde lipodystrofie syndroom). Het perifere vetverlies wordt lipoatrofie genoemd en de centrale vetophoping wordt ook wel lipohypertrofie genoemd.

In **hoofdstuk 1** geven we een overzicht van de mogelijke bijwerkingen ten gevolge van HIV-behandeling. Met name de zogenaamde schade aan de mitochondriën ten gevolge van het gebruik van nucleoside reverse transcriptase remmers en het lipodystrofie syndroom met de lipidenveranderingen, de afwijkingen in de glucosestofwisseling en het lipodystrofiesyndroom komen aan de orde. Dit laatste syndroom werkt erg stigmatiserend, mensen worden herkend door hun uiterlijk. Het confronteert de patiënt dagelijks met de infectie en de schaduwzijde van de succesvolle therapie. Deze bijwerking werkt dan ook zeer demotiverend voor de

patiënt om de (in principe) levenslange therapie vol te houden. Mitochondriële schade is het mechanisme dat ten grondslag ligt aan het optreden van bepaalde bijwerkingen bij gebruik van NRTIs. Enkele voorbeelden hiervan zijn hierboven reeds genoemd; zoals het optreden van leververvetting, bloedarmoede, pijnlijke zenuwuiteinden van de handen en voeten (polyneuropathie) en spierpijnklachten. De chemisch structuur van deze NRTIs zorgt ervoor dat niet alleen de vorming van een nieuwe HIV-RNA-keten (erfelijk materiaal van het virus) wordt geremd, maar ook de opbouw van een nieuwe mitochondriële DNA-keten van de gastheer kan worden geblokkeerd. Normaliter worden fouten in het DNA (erfelijk materiaal) gecontroleerd en eruit geknipt. Dit mechanisme van reparatie werkt zeer goed in onze celkernen maar is minder effectief in onze mitochondriën. Daarom treedt er bij het maken van een nieuw mitochondrion eerder schade op dan bij de opbouw van een nieuwe celkern. Het percentage van deze bijwerkingen is niet goed bekend, deze variëren afhankelijk van het soort onderzoek; pijnlijke prikkelingen in de handen en voeten ten gevolge van zenuwschade (neuropathie) wordt gezien in 12-46%, spierpijnklachten in ca. 17% en ontsteking van de alvleesklier in 0.5-7%. Het voorkomen van lactaatacidose (verzuring van het bloed ten gevolge van overproductie van melkzuur) wordt geschat op te treden in 1.3 per 1000 persoonsjaren.
De blootstelling aan protease remmers (PIs) is de belangrijkste oorzaak van de stofwisselingsproblemen zoals toename van vetgehaltes in het bloed, een hoog suikergehalte, vetverlies op de armen, benen en deels ook voor de vetophoping in de buik en nek (lipodystrofie syndroom). Het verlies van vet in het gelaat en aan de armen en benen is vooral een bijwerking van NRTIs.
Het optreden van deze bijwerking (verandering van lichaamsvet) is wisselend. Eerdere data van het zogenaamde Nederlandse ATHENA cohort (huidige naam: Stichting HIV Monitoring) toonde een incidentie ratio van 6.2 per 100 persoonsjaren met een cumulatieve incidentie van 25%. Er zijn meerdere factoren die een rol spelen bij het ontstaan van dit metabool syndroom: ontsteking, het virus zelf en effecten van HIV-medicijnen op vet- en suikerhuishouding.

In **hoofdstuk 2** beschrijven we vier patiënten die een fatale melkzuurvergiftiging (lactaatacidose) ontwikkelden ten gevolge van NRTI-gebruik. De eerste symptomen bij deze patiënten waren misselijkheid en braken. In dit stadium was melkzuurvergiftiging als de oorzaak van het probleem nog niet onderkend. Dit werd gevonden toen hyperventilatie optrad als compensatiemechanisme voor de verzuring van het bloed. Ondanks intensieve zorg en het toedienen van diverse medicijnen, ging de leverfunctie achteruit en traden gevaarlijke hartritmestoornissen op. Uiteindelijk zijn alle patiënten overleden. Een van deze patiënten werd nog behandeld met riboflavin, een middel dat in enkele gevallen gebruikt is voor de behandeling van lactaatacidose in patiënten met mitochondriële schade. Het opvallende bij deze patiënten was dat zij allen tevoren al andere bijwerkingen hadden gehad ten gevolge van NRTI-therapie. Dit illustreert het belang van vroege herkenning van symptomen veroorzaakt door bijwerkingen.

Om mitochondriële schade door NRTIs aan te tonen, hebben we in het bloed melkzuur en pyruvaat (beide stoffen zijn van belang voor onze energiestof-

wisseling) gemeten in poliklinische patiënten en gezonde vrijwilligers. Glucose (suiker) wordt in de cel omgezet in pyruvaat, dit wordt in het mitochondrion via de citroenzuurcyclus verder omgezet met als eindresultaat energie (het zogenaamde ATP) en koolzuur (CO_2). Als de mitochondriën niet goed functioneren kan pyruvaat niet via de citroenzuurcyclus worden omgezet en wordt het buiten het mitochondrion via het enzym LDH (lactaat dehydrogenase) omgezet in lactaat. Deze reactie is omkeerbaar. Met behulp van het meten van lactaat en pyruvaat wilden we meer inzicht krijgen in het optreden van mitochondriële dysfunctie. De resultaten staan beschreven in **hoofdstuk 3**. Er waren vier groepen: patiënten met NRTI-gerelateerde bijwerkingen, patiënten zonder bijwerkingen van HIV-medicatie, HIV-patiënten die (nog) geen medicatie gebruikten en gezonde controles. De gemiddelde duur van NRTIs was 18 maanden. De hoogste lactaat-waarden werden gevonden bij patiënten met bijwerkingen door NRTIs (vooral de patiënten met zenuwirritatie) en bij patiënten met stavudine-gebruik. De laagste lactaatwaarden werden gevonden in de gezonde vrijwilligers. Alle patiënten met lactaatwaarden boven de 2700 µmol/L hadden last van bijwerkingen en de meerderheid had zelfs meer dan 1 NRTI-gerelateerde bijwerking. Net als de patiënten beschreven in **hoofdstuk 2**, gebruikten deze patiënten allemaal stavudine. Hoge lactaatwaarden bij patiënten met pijnlijke zenuwuiteinden (neuropathie) werden ook gevonden in een ander onderzoek. Hierbij werd melkzuur-meting toegepast om onderscheid te maken tussen neuropathie door HIV zelf of door HIV-medicijnen. Neuropathie is een belangrijke reden om therapie te veranderen. Het is ook een van de belangrijkste redenen waarom stavudine en didanosine niet veel meer gebruikt worden. Een verhoogd melkzuurgehalte in het bloed komt vaak voor. Routinematig meten hiervan wordt niet aanbevolen. Er moet onderscheid gemaakt worden tussen verhoogd melkzuur met en zonder klachten. Melkzuur-verhoging wordt nogal eens gezien bij stavudine-gebruik daarom zijn deze patiënten vatbaarder voor bijwerkingen zeker na langdurig gebruik hiervan. Op basis van onze bevindingen zouden we frequente controle van melkzuur adviseren bij mensen met waarden boven de 2500 µmol/L vooral om te zien of deze waarden niet toenemen en gaan leiden tot problemen. Bij toename van melkzuur of duidelijke klachten moet het betreffende medicijn gestaakt worden.

In **hoofdstuk 4** bespreken we de resultaten van een aanvullend onderzoek waarin we meer te weten wilden komen over de mitochondriële schade en om na te gaan of we deze bijwerking met een test in een vroeg stadium kunnen opsporen. Een orale glucose tolerantie test (OGTT; een test waarbij mensen een suikeroplossing drinken) wordt gebruikt voor het vaststellen van aangeboren afwijkingen van de mitochondriën bij kinderen. Wij vroegen ons af of het gebruik van deze test ook zinvol zou zijn voor onze patiëntengroep met bijwerkingen. Het bleek dat we met deze test verhoogde melkzuurconcentraties in een vroege fase konden vinden. De test is niet gevoelig genoeg als screeningstest, maar wel toepasbaar om een vermoeden op bijwerkingen te bevestigen.

Toen de protease remmers (PIs) op de markt kwamen waren een aantal lange termijn bijwerkingen nog niet duidelijk. Inmiddels is het lipodystrofie syndroom

met metabole veranderingen een van de grootste problemen op dit gebied. Gezien het feit dat meerdere factoren een rol spelen bij het ontstaan en het risico op hart- en vaatziekten, wilden we in meer detail hiernaar kijken. In **hoofdstuk 5** presenteren we de resultaten van onderzoek waarbij uitgebreid bloedonderzoek werd gedaan bij HIV-patiënten die net starten met hun eerste HIV-behandeling. In dit onderzoek werden alle patiënten eerst behandeld met zidovudine, lamivudine en de proteaseremmer lopinavir/ritonavir totdat de hoeveelheid virus in het bloed niet meer meetbaar was. Op dat moment werden de patiënten verdeeld in twee groepen: doorgaan op dezelfde manier of omzetting van lopinavir/ritonavir in een ander medicijn; abacavir. Protease-remmers staan bekend om hun verhoging van cholesterol en vetten in het bloed en neiging tot suikerziekte. Dit verhoogt weer het risico op hart- en vaatziekten. Het doel van deze studie was dan ook om na te gaan of de vet- en suikerstofwisseling in gunstige zin beïnvloed zou worden met het vervangen van lopinavir/ritonavir door abacavir (medicijn uit de NRTI-groep). Onze resultaten geven meer inzicht in het optreden van deze stofwisselingsveranderingen. We vonden een verhoging van het cholesterol en vetten in het bloed in de eerste 24 weken van de behandeling (lopinavir gebruik). Na 24 weken nam dit niet meer toe maar werd een stabiele waarde bereikt bij patiënten met lopinavir. In tegenstelling tot de patiënten die verder gingen met abacavir. Na de omzetting naar abacavir werd een daling van het cholesterol en de vetten in het bloed gezien. Bij de groep die ritonavir/lopinavir bleven gebruiken, traden er geen veranderingen op en ook geen verdere stijging. Andere bloedtesten die gedaan kunnen worden voor het bepalen van het risico op hart- en vaatziekten zijn: apoE, apoB, high sensitive CRP en leptine. Deze waarden toonden geen verschil in beide groepen.
Onze gegevens bevestigen de resultaten van andere onderzoekers. Ons onderzoek is echter het eerste onderzoek dat dit heeft bekeken in een groep die net start met medicijnen.

De lichaamveranderingen door het lipodystrofie syndroom hebben niet alleen grote psychosociale gevolgen maar zijn ook van groot belang voor de levens-verwachting van patiënten door het toegenomen risico op hart- en vaatziekten. Beide factoren hebben een zeer ongunstige invloed op het dagelijks innemen van de medicijnen (therapietrouw). Diverse artikelen laten zien dat het vetverlies in gelaat en armen/benen wordt veroorzaakt door NRTIs en vooral door langdurig gebruik van stavudine. Ontregeling van de vetten in het bloed en de neiging tot suikerziekte wordt vooral veroorzaakt door protease remmers. Wij wilden weten of patiënten met lipoatrofie wellicht te hoge concentraties van stavudine in het bloed hadden. In **hoofdstuk 6** wordt hiervan verslag gedaan. We hebben hiervoor concentraties van stavudine in het bloed gemeten bij 2 groepen patiënten: patiënten met en zonder vetverlies (lipoatrofie; zie foto's op pagina 22). Inderdaad bleken de stavudine concentraties hoger te zijn in de groep van patiënten met lipoatrofie vergeleken met die zonder lipoatrofie. Op basis van recent gepubliceerde gegevens en onze studie stellen we dat een concentratie ratio <1.0 veilig kan zijn voor de behandeling van HIV-patiënten om zo lipoatrofie te voorkomen. Onze bevindingen komen overeen met een onlangs aangepaste richtlijn van de WHO (World Health Organisation) voor het behandelen van

volwassenen met HIV. Deze richtlijn adviseert een lagere dosis van stavudine dan voorheen gebruikelijk was; 2 x per dag 30 mg in plaats van 40 mg.

Verhoogd cholesterol en vetten in het bloed worden vaak gezien bij gebruik van lopinavir. Ernstige stijging van o.a. cholesterol wordt gerapporteerd in 25% van de patiënten. Vooral de stijging van het 'slechte' cholesterol (LDL-cholesterol) geeft een toename in de kans op hart- en vaatziekten. Een onlangs gepubliceerd onderzoek toonde een hoog LDL-cholesterol in 20% van de mannen en 10% van de vrouwen met een HIV-infectie. Deze percentages waren hoger dan in gezonde personen. Ook werden hogere percentages gevonden in patiënten die protease remmers gebruikten. Om in meer detail te kijken naar vetveranderingen in het bloed als bijwerking, hebben we cholesterol concentraties gemeten in het bloed bij HIV-patiënten. Dit waren mensen die meededen aan de zogenaamde FREE studie (zie **hoofdstuk 5**). De resultaten staan beschreven in **hoofdstuk 7**. In deze patiëntengroep hebben we onderzocht of er een verband is tussen de veranderingen in de vetstofwisseling en de concentraties ritonavir/lopinavir in het bloed. Bij 72 patiënten (210 serum monsters) hebben we dit gemeten. Tijdens de studie-periode kreeg het merendeel van de patiënten een verhoging van het cholesterol in het bloed. Al na kortdurend gebruik van lopinavir zagen we stijging in het cholesterol optreden. We vonden echter geen verband tussen de blootstelling aan lopinavir (concentraties in bloed) en de cholesterolwaarden. Dit is waarschijnlijk het gevolg van de grote spreiding in lopinavir concentraties binnen de patiënten zelf.
De lopinavir concentraties varieerden binnen de patiënten (intra-individuele spreiding) van 4-111% met een gemiddelde van 35%. De hoeveelheid van het medicijn in het lichaam lijkt dus geen rol te spelen in deze cholerstoltoename.
Op basis van onze bevindingen zal verlaging van de dosis geen invloed hebben op het voorkomen van cholesterolstijgingen.
De studies in **hoofdstuk 6** en **7** waren niet bedoeld om te kijken naar de oorzaak voor vetveranderingen. Ze waren louter opgezet om de invloed van medicatie-blootstelling als risicofactor te bestuderen.

De studies beschreven in dit proefschrift geven een overzicht van de verschillende soorten bijwerkingen veroorzaakt door HIV-medicijnen. Deze gegevens laten zien dat het zinvol is patiënten goed te controleren. Op deze manier kunnen bijwerkingen vroeg worden opgespoord of zelfs worden voorkomen. Hierdoor is het gemakkelijker voor de patiënten om deze behandeling langdurig vol te houden.
Op dit moment kan elke behandeling bijwerkingen veroorzaken. Geen enkele combinatie van medicijnen is ideaal en zonder bijwerkingen. In de praktijk moet de keuze van de beste behandeling gemaakt worden na een zorgvuldige afweging tussen effectiviteit en veiligheid. Hierbij zal ook rekening gehouden moeten worden met individuele medische problemen van de betreffende patiënt. Met behulp van bloedonderzoek kunnen bijwerkingen in een vroege fase opgespoord worden. Deze individuele aanpak bij de behandeling van HIV-patiënten is een uitdaging voor elke internist die deze chronische infectie behandelt.
Inmiddels zijn er nieuwe klassen van geneesmiddelen op de markt gekomen: de chemokine receptor remmers en de integraseremmers. De veiligheid van deze

nieuwe groepen en ook de werkzaamheid op lange termijn staan nog niet vast. In de komende jaren zullen nieuwe medicijnen uit de reeds bekende klassen van PIs, NNRTIs (Engels: non nucleoside reverse transcriptase inhibitors) en NRTIs op de markt komen. Daarnaast zullen ook de nieuwere klasse medicijnen met andere aangrijpingspunten op het HIV-virus, uitgebreid worden. De soorten bijwerkingen die kunnen ontstaan zijn vaak klasse-specifiek. Dit betekent dat bij de komst van nieuwe medicijnen ook nieuwe bijwerkingen zullen worden gezien. De toekomst kan ons daarom, nog diverse nieuwe en onbekende bijwerkingen brengen die wetenschappelijk onderzoek noodzakelijk maken. Daarbij is zowel onderzoek naar de ernst als het effect op kwaliteit van leven van belang. Het is belangrijk om de mogelijke bijwerkingen goed te onderzoeken en onderliggende mechanismen te bestuderen. Een nog nauwelijks ontgonnen terrein is hierbij het onderzoeken van genetische aanleg voor bepaalde bijwerkingen. Mogelijk dat dit in de toekomst resulteert in een nog betere individuele HIV-behandeling.

List of publications

List of publications

1 ter Hofstede HJM, Koopmans PP, Burger DM. Stavudine plasma concentrations and lipoatrophy. **J Antimicrob Chemother** 2008; 61(4):933-938.

2 ter Hofstede HJM, Borm GF, Koopmans PP.Oral glucose loading for detection of mitochondrial toxicity during HAART in HIV-infected patients. **Curr HIV Res** 2007; 5(4):389-393.

3 van der Lee MJ, Dawood L, ter Hofstede HJM, de Graaff-Teulen MJ, van Ewijk-Beneken Kolmer EW, Caliskan-Yassen N, Koopmans PP, Burger DM. Lopinavir/ritonavir reduces lamotrigine plasma concentrations in healthy subjects. **Clin Pharmacol Ther** 2006; 80(2):159-168.

4 ter Hofstede HJM, Willems HL, Koopmans PP. Serum L-lactate and pyruvate in HIV-infected patients with and without presumed NRTI-related adverse events compared to healthy volunteers. **J Clin Virol** 2004; 29(1):44-50.

5 ter Hofstede HJM, Burger DM, Koopmans PP. Antiretroviral therapy in HIV patients: aspects of metabolic complications and mitochondrial toxicity. **Neth J Med** 2003; 61(12):393-403.

6 ter Hofstede HJM, Telgt DS, Koopmans PP. Therapy of HIV infection: how early and how intensive? **Ned Tijdschr Geneeskd** 2001; 145(5):201-204. Erratum in: **Ned Tijdschr Geneeskd** 2001; 145(14):712.

7 Reijers MH, Weverling GJ, Jurriaans S, Roos MT, Wit FW, Weigel HM, Ten Kate RW, Mulder JW, Richter C, ter Hofstede HJM, Sprenger H, Hoetelmans RM, Schuitemaker H, Lange JM. The ADAM study continued: maintenance therapy after 50 weeks of induction therapy. **AIDS** 2001; 15(1):129-131.

8 Hugen PW, Burger DM, ter Hofstede HJM, Koopmans PP, Stek M, Hekster YA, Reiss P, Lange JM. Dose-finding study of a once-daily indinavir/ritonavir regimen. **J Acquir Immune Defic Syndr** 2000; 25(3):236-245.

9 ter Hofstede HJM, de Marie S, Foudraine NA, Danner SA, Brinkman K. Clinical features and risk factors of lactic acidosis following long-term antiretroviral therapy: 4 fatal cases. **Int J STD AIDS** 2000; 11(9):611-616.

10 Hugen PW, Burger DM, de Graaff M, ter Hofstede HJM, Hoetelmans RM, Brinkman K, Meenhorst PL, Mulder JW, Koopmans PP, Hekster YA. Saliva as a specimen for monitoring compliance but not for predicting plasma concentrations in patients with HIV treated with indinavir. **Ther Drug Monit** 2000; 22(4):437-445.

11 Hugen PW, Burger DM, ter Hofstede HJM, Koopmans PP, Hekster YA. Development of an indinavir oral liquid for children. **Am J Health Syst Pharm** 2000; 57(14):1332-1339.

12 Hugen PW, Burger DM, Brinkman K, ter Hofstede HJM, Schuurman R, Koopmans PP, Hekster YA. Carbamazepine--indinavir interaction causes antiretroviral therapy failure. **Ann Pharmacother** 2000; 34(4):465-470.

13 ter Hofstede HJM, van der Ven AJ, Koopmans PP. Drug reactions to contrimoxazole in HIV infection: possibly not due to the hydroxylamine metabolites of sulphamethoxazole. **Br J Clin Pharmacol** 1999; 47(5):571-573.

14 Brinkman K, ter Hofstede HJM, Willems J, Burger DM, Koopmans PP. Glucose tolerance test for tracing of mitochondrial toxicity from nucleoside analogs. **Ned Tijdschr Geneeskd** 1998; 142(40):2231-2232.

15 ter Hofstede HJM and Brinkman K. Mitochondrial toxicity owing to nucleoside reverse transcriptase inhibitors: importance of clinical features and early diagnosis. **International Antiviral News** 1999; 7(10):148-151.

16 Brinkman K, and ter Hofstede HJM. Mitochondrial toxicity of nucleoside reverse transcriptase inhibitors: Lactic acidosis, risk factors and therapeutic options. **AIDS Reviews** 1999; 1(3):140-146.

17 Brinkman K, ter Hofstede HJM, Burger DM, Smeitink JA, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. **AIDS** 1998; 12(14):1735-1744.

18 ter Hofstede HJM, Koopmans PP, van Haelst UJ. Hepatic steatosis during treatment with zidovudine and lamivudine in an HIV-positive patient. **Ned Tijdschr Geneeskd** 1998; 142(8):415-419.

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Dankwoord

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Curriculum Vitae

Curriculum Vitae

Hadewych ter Hofstede werd geboren op 16 februari 1969. Na het behalen van haar VWO diploma (eindexamen in 8 vakken) ging zij in 1987 geneeskunde studeren in Nijmegen. In 1995 behaalde zij haar artsdiploma en werkte tot 1997 op de afdeling inwendige geneeskunde in het Rijnstate ziekenhuis in Arnhem. In 1997 ging zij werken in het UMC St Radboud in Nijmegen als onderzoeksarts en als klinisch beoordelaar voor het College ter Beoordeling van Geneesmiddelen. In 1998 startte zij haar opleiding tot internist onder leiding van Professor J.W.M. van der Meer. In 2004 ging zij onder leiding van Professor B.J. Kullberg verder met haar subspecialisatie infectieziekten. In 2006 rondde zij haar internistenopleiding af en in 2007 haar subspecialisatie als infectioloog. Zij is thans werkzaam als internist-infectioloog in het UMC St Radboud en als senior klinisch beoordelaar bij het Agentschap College ter Beoordeling van Geneesmiddelen.
Zij is getrouwd met Marco en samen zorgen zij voor hun kinderen David, Mathilde en Julian.

Alles is anders

Ineens draait alles
en ziet alles er anders uit

verbaasd draai je mee
maar de richting is weg;
de weg is anders

je volgt
maar begrijpt het niet;
het uitzicht is anders

je past je aan
aan iets dat er eerst niet was
en je doet;
maar wat

Alles is anders

Stellingen

1. Het routinematig meten van lactaat is niet aan te bevelen bij mensen die antiretrovirale therapie gebruiken. In specifieke gevallen kan het echter additionele waarde hebben om bijwerkingen op te sporen.

2. De huidige morbiditeit en mortaliteit veroorzaakt door HIV is meestal niet de infectie zelf maar behandeling ervan.

3. In tegenstelling tot de begindagen van de antiretrovirale combinatietherapie, zijn lipiden- en glucose-veranderingen nu een van de belangrijkste problemen.

4. Het meten van geneesmiddelenconcentraties in het bloed (Therapeutic Drug Monitoring) draagt bij aan het opsporen en vervolgen van bijwerkingen veroorzaakt door antiretrovirale therapie.

5. De behandeling van HIV-patiënten is continu balanceren tussen goed en kwaad doen.

6. Het ontkennen van HIV-infectie in sommige landen maakt dit gezondheids-probleem alleen maar groter.

7. Een organisatie steunt vaak het meest op de mensen die de minste waardering krijgen.

8. Het snijden in een organisatie in het kader van een bezuinigingsoperatie is als het amputeren van een arm en denken dat je afgevallen bent.

9. Als we dit jaar eens geen kerstbomen verlichten, dan verlichten we de problemen van de pinguïns.

10. Life can be understood backwards; but it must be lived forwards.