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The Journal of Medicine

EDITORIAL

The price of HIV treatment

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ABSTRACT

The burden of HIV on society in the developing world and the price for treatment is enormous. The recent agreement between the Clinton Foundation and generic pharmaceutical companies is a major step forward. Also the cost-effectiveness is superior to treatment of for example hypercholesterolaemia. However, there are other prices to be paid, since drugs may induce unfavourable and unwanted side effects.
For the future the challenge remains to keep all prices for HIV treatment as low as possible. Only an affordable and harmless HIV treatment might bring the progress of the HIV pandemic to a halt.

In the developing world, the burden of HIV on society is increasing rapidly. In 2002, the World Health Organisation (WHO) estimated that more than 42 million people were infected, of whom 3.2 million under the age of 15 years and every day this number increases by 14,000 or 10 per minute. In consequence, 3.1 million of these patients died that year, 8500 per day, 6 per minute (HIV/AIDS - facts and figures: http://www.who.int/hiv/facts/en). There is no doubt that effective HIV treatment of these patients can turn the tide and might restore social and economic infrastructures. To address this emergency, the WHO is therefore fully committed to achieving the '3 by 5' target – getting three million people on this treatment by the end of 2005. Apart from logistic and political challenges, the cost of treatment on this scale would be enormous. Since there is no generic competition in the Netherlands, the price for one year of antiretroviral medication can vary between € 8500 and € 10,000.[1] An important step forwards, therefore, was the recent agreement between the Clinton Foundation and four generic pharmaceutical companies to dramatically lower the price of HIV/AIDS treatment to as low as US$140 per person per year (UNAIDS: http://www.unaids.org).

In the developed world, the cost of the medication is not an issue in getting HIV-infected patients on treatment. Although costly, with a cost-effectiveness ratio for three-drug therapy ranging from $13,000 to $23,000 per quality-adjusted life year (QALY), antiretroviral therapy is even more cost-effective than many therapies for non-HIV diseases, such as radiation therapy for early-stage breast cancer ($30,000/QALY), treatment of hypercholesterolaemia ($47,000/QALY), and dialysis in patients expected to live for less than six months ($150,000/QALY).[2,3] As a result of the treatment, the life expectancy of HIV-infected patients has increased to almost normal levels,[4,5] possibly enabling these patients to obtain normal life insurance in the future.[6]
However, for HIV-infected patients on treatment, there are more prices to be paid than cost alone. In this issue of the journal, two negative aspects of HIV treatment are described, which cause substantial morbidity and even increased mortality to patients, mainly after an initially successful treatment response.
In the article by De Boer *et al.*, the risk of rapid immune recovery after the start of effective antiretroviral therapy is described: immune restoration disease (IRD). This syndrome is the result of an exuberant inflammatory response towards indolent pathogens such as *Mycobacteria*,[7] *Pneumocystis carinii*[8] and *Toxoplasma gondii*,[9] as described

in this paper, but also *Cryptococcus neoformans*[10,11] and cytomegalovirus (CMV).[12] Early recognition is important, since symptoms can easily be confused with allergic reaction to HIV medication or other therapy. Those therapies need to be continued and anti-inflammatory drugs such as steroids have to be administered temporarily.[7] Although it induces serious morbidity in the patient, IRD might be seen as a positive phenomenon with a favourable outcome. Unfavourable effects of HIV treatment are described in an extensive review by Ter Hofstede *et al*. They summarise the increasing amount of literature concerning the adverse reactions of antiretroviral drugs. Some of these adverse reactions, such as the lipoatrophy syndrome, are disfiguring and therefore stigmatising for patients, while others are potentially lethal, such as lactic acidosis due to mitochondrial toxicity. The insight into the pathophysiological mechanisms of these adverse effects is increasing and we are learning how to manage them. Fortunately, alternative HIV drugs are often available to enable full control over the virus without these side effects, but sometimes the toxic drugs need to be continued, enforcing the administration of comedication such as statins or insulin. This seriously jeopardises the adherence of the patients to HIV treatment, which can ultimately lead to re-emergence of (resistant) HIV virus and HIV-related diseases.

The challenge therefore remains to start an antiretroviral combination that is potent enough to fully suppress viral replication, but scarcely induces any side effects. Only then can long-term treatment be guaranteed, which is the only way to a successful outcome. Sometimes, patients refuse HIV treatment to avoid these side effects, but this is never an option, as is shown every minute by the devastating evolution of the HIV pandemic. That price is too high.

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Antiretroviral therapy in HIV patients: aspects of metabolic complications and mitochondrial toxicity

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INTRODUCTION

Since the introduction of highly active antiretroviral therapy (HAART) in HIV-infected patients, morbidity and mortality due to HIV infection have dramatically decreased.[1] Although resistance to antiretroviral therapy is an important issue, toxicity is becoming an even more important problem. In the ATHENA cohort (a cohort of around 3000 HIV patients in the Netherlands), the main reason to switch antiretroviral therapy is toxicity: this is 44 to 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; therefore the risk for adverse events may increase. The benefits of HAART have led to a great number of HIV patients receiving antiretroviral therapy.[3-5]

In this review we will discuss two major groups of antiretroviral therapy-related toxicity: the lipodystrophy/lipoatrophy syndrome with metabolic changes and mitochondrial toxicity.[6,7]

LIPODYSTROPHY SYNDROME

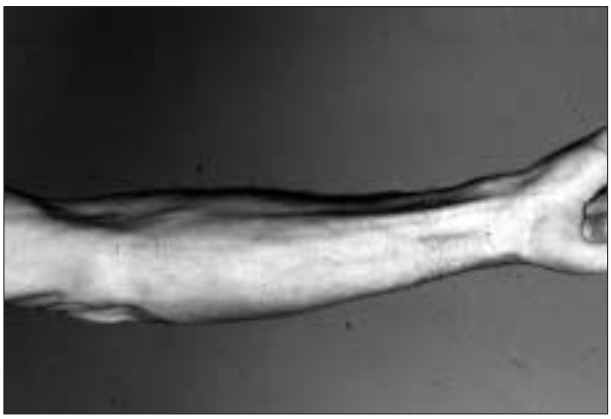
Symptoms

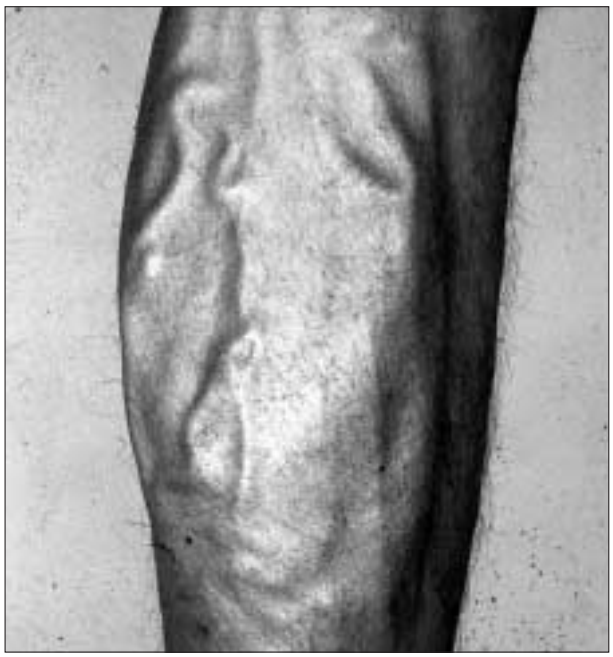
Exposure to antiretroviral drugs has been associated with the development of significant metabolic adverse effects, such as hyperlipidaemia, hyperglycaemia and insulin resistance with diabetes mellitus, peripheral fat wasting (lipoatrophy) and central adiposity. Fat loss on the extremities, buttocks 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 (*figures 1-6*).[8-13] This syndrome is called the lipodystrophy syndrome.

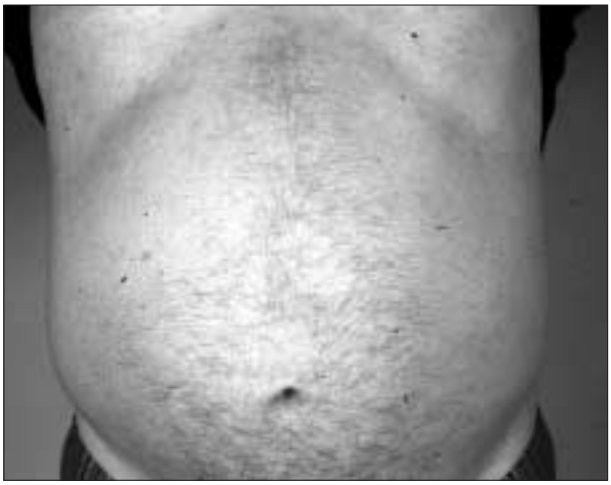
Epidemiology and predisposing factors

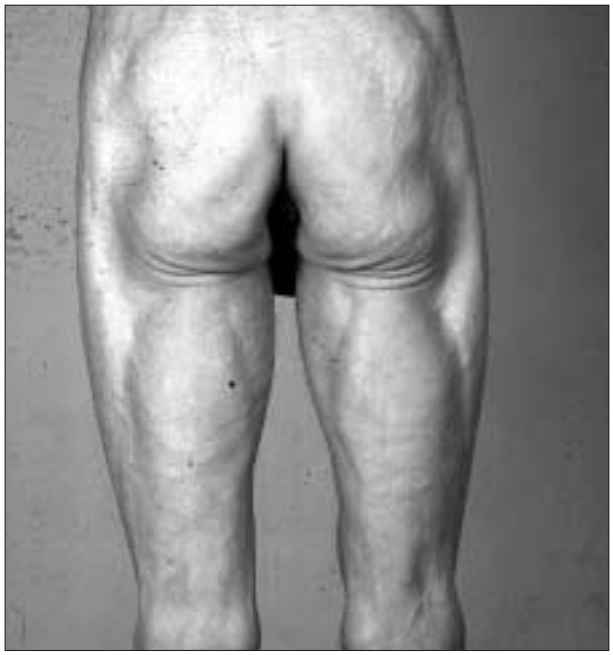
There are no exact data on the prevalence but in the literature it is estimated that it may occur in up to 80% of the patients on long-term therapy. A prospective cohort study from 1996 to 1999 found the following incidences: any lipodystrophy 11.7, lipodystrophy with subcutaneous lipoatrophy 9.2 and lipodystrophy with central obesity 7.7 per 100 patient years.[14] 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%.[15] However, most lipodystrophy studies included HIV-positive Caucasian men. The incidence of lipodystrophy in subjects other than Caucasians has not been studied well. One study showed that lipodystrophy was only seen in 3.5% of a cohort of Koreans.[16]

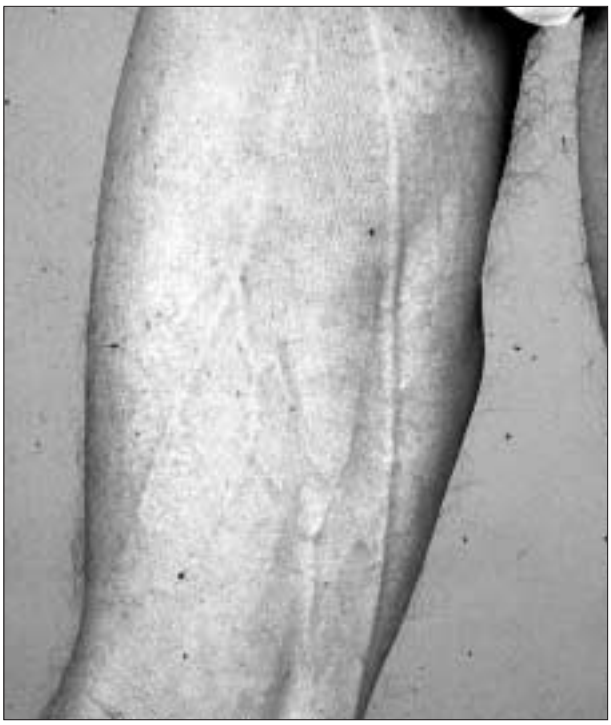
Three major patterns are distinguished: the lipoatrophy syndrome (fat depletion), a mixed or fat redistribution syndrome and a subcutaneous fat accumulation syndrome, often due to diet prescriptions when using some types of protease inhibitors (PIs).[17] Lipodystrophy with peripheral lipoatrophy is mostly seen in HIV-infected patients receiving HAART and therefore not likely to be associated with HIV infection itself. Furthermore, the occurrence and severity of the syndrome is independent of HIV load. Recent reports highlight the fact that the fat depletion component (lipoatrophy) is primarily linked to nucleoside reverse transcriptase inhibitor (NRTI) therapy, while fat accumulation with dyslipidaemia and insulin resistance

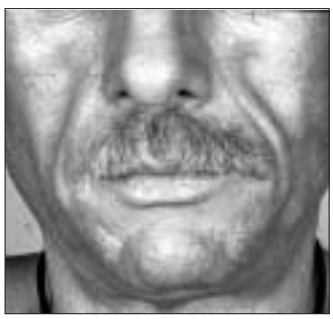












Figures 1- 6
Patients with lipodystrophy/atrophy

are more readily associated with PI therapy. Current studies often distinguish the syndromes of fat depletion (lipoatrophy) and fat accumulation, in contrast to previous studies which use the term lipodystrophy for both components of fat changes. It is often difficult to make a clear distinction between the two components because they often appear as a mixed syndrome and the majority of the patients are treated with a combination of NRTIs and PIs. Although risk factors for these fat redistribution syndromes are not exactly known, the following factors have been suggested to play a role: low body weight before the 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).[14,17-20] Lipoatrophy occurs frequently in regimens including NRTIs and is rare in NRTI-sparing regimens. Several studies confirmed the observation that the use of NRTIs contributes to the development of lipoatrophy, especially in patients on stavudine.[6,9,10,21,22] The risk of developing lipodystrophy was assessed in a study with 158 HIV-infected patients, 113 of whom 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.[18] This is 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]

Hypothesis on the pathogenesis

Changes in adipose tissue

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, which have partial homology to the catalytic site of HIV-1 protease, to which all PIs bind. PIs are suggested to inhibit cellular retinoic acid-binding protein (CRABP)-1-modified and cytochrome P450-3A-mediated synthesis of cis-9-retinoic acid and peroxisome proliferator-activated receptor type gamma (PPAR-γ) heterodimer.[23] 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.[6,18,24] 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.[25] 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 in 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-abdominally and subcutaneously, leading to central fat accumulation and peripheral fat loss as seen in the lipodystrophy/lipoatrophy syndrome in HIV-infected patients.[26]

Changes in adipocyte cell and glucose metabolism

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 with HIV patients without lipodystrophy. 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.[27] Also, an imbalance between peripheral lipolysis and lipogenesis, both regulated by cortisol and dehydro-epiandrosterone, has been found to play a role in lipodystrophy. Furthermore, subcutaneous adipocyte apoptosis has been found in lipoatrophic areas of HIV-patients treated with PIs.[28,29]

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 inhibition by PIs thereby leads to a decrease in insulin-stimulated glucose uptake.[30-32]

NRTI-associated mitochondrial toxicity has also been postulated to play a role in antiretroviral therapy-related

lipodystrophy.[21,22,33] The fat redistribution as seen in HIV patients treated with antiretroviral therapy resembles the body composition of patients with Madelung's disease. Mitochondrial impairment has been found in patients with benign or multiple symmetrical lipomatosis (Madelung's disease). Investigation of muscle mitochondria in HIV patients with lipodystrophy has shown abnormalities in mitochondrial respiratory chain complexes, mitochondrial DNA (mtDNA) and mitochondrial morphology suggestive of mitochondrial dysfunction.[34] 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.[35,36]

Cardiovascular risk of antiretroviral therapy

The occurrence of metabolic changes, such as hyperlipidaemia and insulin resistance, with the use of antiretroviral therapy may affect the risk of cardiovascular disease in HIV-infected patients. 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 already found after four weeks of drug use.[37]

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 traditional cardiovascular risk factors. A cohort of over 5000 HIV patients in the USA, followed between 1993 and 2002, showed an increase in myocardial infarctions after the introduction of protease inhibitors in 1996.[38] 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.[39] The DAD study assessed the risk of cardiovascular disease in HAART-treated patients in a prospective 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 CD4 counts and suppressed HIV replication.[40]

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, β_2-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 leucocyte 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 to 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.[41-44] 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).[38,45] 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 is being evaluated in large prospective trials with long-term follow-up. Until the results are available, monitoring the traditional cardiovascular risk factors and risk factors associated with HAART such as dyslipidaemia, glucose levels and visceral fat accumulation in HIV patients is justified (*table 1*).[38,39,44,46-48]

Table 1
Risk factors for atherosclerosis in HIV patients on 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

Diagnosis

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 the patient's report of a changed body com-

position. The HIV Lipodystrophy Case Definition Study Group evaluated a model to diagnose lipodystrophy. This model included a scoring 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 with scores using only clinical or metabolic variables.[49]

Lipoatrophy/dystrophy with lipid and glucose disturbances has been studied by means of blood measurements, CT 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.[50,51] 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 related to the lipoatrophy/lipodystrophy syndrome. Facial lipoatrophy, one of the most distressing features of the syndrome, cannot be measured in this way.[53] 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 for diagnosing abnormal regional fat distribution than that of intra-abdominal fat.[54]

Treatment

HAART-receiving patients with hyperlipidaemia and diabetes are currently treated with lipid-lowering agents and oral antidiabetic drugs or even subcutaneous insulin. Thiazolidinediones, such as 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 a decline in triglycerides and VLDL cholesterol.[55-57]

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. This is in contrast to the administration of a polyunsaturated fatty acid diet, which did not alleviate PI-induced metabolic abnormalities.[58] Patients with congenital lipodystrophy have been studied while on chronic leptin therapy. An improvement in insulin-stimulated hepatic and peripheral glucose metabolism in severe insulin resistance was demonstrated with marked reduction of hepatic and muscle triglyceride content.[59,60] However, the usefulness in HIV lipodystrophy has to be proven.

Lipid disorders are treated with statins (HMG-CoA reductase inhibitors) in case of elevated cholesterol, and fibrates are used in patients with elevated triglycerides. Up until now, pravastatin has been the statin of choice since it has no potential to cause rhabdomyolysis or to interact with the other medications often used in HAART regimens (especially the PIs).[61,62] Furthermore, traditional risk factors, such as age >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.[8,44,48]

Switching studies have shown that metabolic disturbances due to HAART appear to be partially reversible, but improvement in fat redistribution has not been clearly demonstrated. There have been a few studies switching PIs to abacavir, efavirenz or nevirapine showing improvement in insulin resistance. Furthermore, there seems to be a favourable change in lipid concentrations, particularly after switching to nevirapine and abacavir.[46,63] 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.[63-65] 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 in triglyceride levels.[66] Decrease in 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.[67-69]

Peripheral fat loss can sometimes be treated by implantation of the patient's subcutaneous fat or synthetic material (bio-alcamid, New Fill® and botulin toxin); unfortunately 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.[70]

MITOCHONDRIAL TOXICITY

Epidemiology, predisposing factors and pathogenesis

Side effects associated with NRTI therapy are (cardio)myopathy, neuropathy, pancreatitis, hepatic steatosis and lactic acidosis (*table 2*). These events generally 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.

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	+	-	+	+	+	-	-

*AZT = zidovudine, 3TC = lamivudine, d4T = stavudine, ddI = didanosine, ddC = zalcitabine, ABV = abacavir, TFV = tenofovir. *NtRTI.*

Therefore, it is supposed that the pathogenic pathway of these side effects can be explained by NRTI-induced mitochondrial damage. NRTIs interfere with an enzyme (polymerase γ), which is essential for the synthesis of mitochondrial DNA strands. The triphosphate compounds of the nucleoside analogues inhibit polymerase γ. By interference with this enzyme, NRTIs are built into a new mitochondrial DNA strand instead of the normal nucleosides, resulting in chain termination and thereby impaired mtDNA synthesis (*figure 7*). 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.[71-74]
Incidences are not well known and vary depending on the analysis, such as neuropathy 12 to 46%, myopathy 17% and pancreatitis 0.5 to 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, found retrospectively in a cohort of antiretroviral drug users.[5,71-81] 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.[82] 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.
Another striking feature of the NRTI toxicity is its apparent tissue specificity: myopathy can be caused by zidovudine, but rarely occurs with 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 (see also *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]
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 mtDNA mutations or deletions, with no symptoms yet, might be more susceptible to

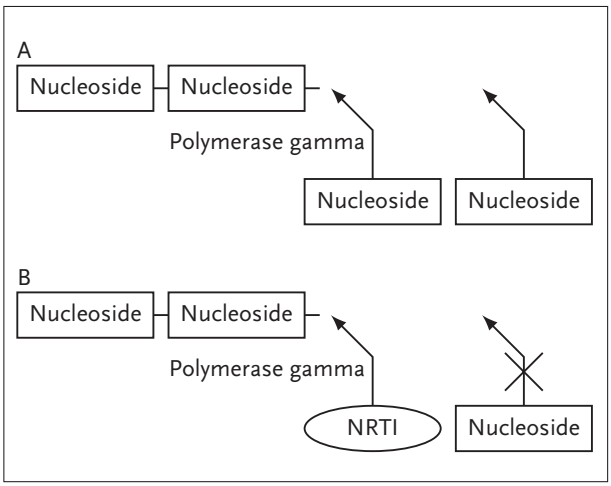


Figure 7
Synthesis of new mitochondrial DNA strand

A = Normal situation. B = Situation in NRTI use; blockage of the formation of a new strand by NRTI.

developing 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 with nuclear DNA replication. Older people might therefore be more susceptible than younger age groups. 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 range 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.[5,19,82]

In case of toxicity and discontinuation of medication, some tissues show a very slow recovery. Factors that determine this recuperation capacity are unknown but tissues with a low cell turnover, such as 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.

Diagnosis

Histology is the only method to demonstrate mitochondrial disorders conclusively. 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 of an acquired mitochondrial-related myopathy. Damaged mitochondria can be seen with electron microscopy, showing swollen mitochondria with disruption and fragmentation of cristae, cristalline inclusions and lipid droplets. However, tissue biopsies are not useful as screening tests and not every tissue can be sampled.[5,83]

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.[84-86]

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.[87-90]

Histochemical reaction to 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 shows a microvesicular pattern with necrosis. Additional histochemistry has to be performed to exclude infectious 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 the 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.[91,92] Although elevated lactate levels have been described in patients suspected of 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.[93-95] 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.[96-103] 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.[104] 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.[96,100,101,105-107] Routine lactate measurement is not recommended; a difference has to be made between symptomatic and asymptomatic hyperlactataemia and lactic acidosis. 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.[83,97,108,109]

Another option to study mitochondrial toxicity is to

measure 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, although 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, although 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 with 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.[110] 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 with HIV patients on HAART without lipodystrophy/lipoatrophy, 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 with HIV-infected patients without HAART. This suggests mitochondrial recovery during HAART. Apparently more mechanisms play a role in the mechanism of toxicity.[108,111-118]

Treatment

Besides the discontinuation of NRTIs, 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.[102,119-123]

CONCLUSION

Human immunodeficiency virus infection can be successfully treated with HAART. However, the long-term safety of the present drugs is a major concern. The pathogenesis of the major syndromes such as lipodystrophy and the so-called mitochondrial toxicity is still unclear, while treatment options are limited. Close observation and monitoring of side effects and potential risk factors as well as studies on pathogenesis and treatment remain warranted.

NOTE

A colour version of this article can be found on our website www.njmonline.nl.

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REVIEW

Pathophysiology of ANCA-associated vasculitides: are ANCA really pathogenic?

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ABSTRACT

The strong relation between antineutrophil cytoplasmic autoantibodies (ANCA) and primary vasculitic syndromes suggests a pathophysiological role for ANCA. Experimental evidence for the pathogenic potential of ANCA has been derived from *in vitro* studies that demonstrate that ANCA can activate tumour necrosis factor α primed neutrophils, monocytes and/or endothelial cells. The binding of ANCA to primed neutrophils results in activation of these cells by a process that is largely dependent on engagement of β-2 integrins and on the interaction of the Fc portion of ANCA. An Fc-independent mechanism is, however, also operative. In experimental animal models, it has been demonstrated that immunisation with myeloperoxidase (MPO) induces MPO-ANCA. The induction of ANCA, however, is not sufficient to induce vasculitis *in rats* since immune complexes first have to be deposited along the vessel wall before lesions develop. When MPO-deficient *mice* are, however, immunised with murine MPO, anti-MPO immunoglobulins are purified and subsequently injected into mice that are not deficient for MPO, systemic vasculitis and glomerulonephritis is induced. These experiments suggest that ANCA indeed induces vasculitis. Risk factors for breaking self-tolerance to ANCA antigens are genetic factors, drugs, chemical substances and/or infectious agents.

INTRODUCTION

Within the group of small-vessel vasculitides, Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), Churg Strauss Syndrome (CSS) and the form of these diseases that is limited to the kidneys, namely idiopathic necrotising crescentic glomerulonephritis (NCGN), are closely associated with antineutrophil cytoplasmic autoantibodies (ANCA). The lesions in those diseases, particularly demonstrated in the kidneys, are 'pauci-immune', meaning that no immune deposits are found in most cases. ANCA in these diseases are directed against proteinase 3 (PR3) or myeloperoxidase (MPO) (*table 1*). Besides being a helpful diagnostic tool, determination of ANCA levels can also be useful for monitoring disease activity, since relapses of disease are often preceded by rises in ANCA levels.[1,2] Furthermore, persisting high levels of ANCA are associated with a poor renal outcome.[3] The strong relation between ANCA and primary vasculitic syndromes suggests an important role for ANCA in the pathophysiology. Experimental evidence for the pathogenic potential of ANCA has been derived from both *in vitro* and *in vivo* studies and will be reviewed.

PATHOPHYSIOLOGY OF ANCA-ASSOCIATED VASCULITIS: *IN VITRO* DATA

In vitro, ANCA can activate neutrophils primed with tumour necrosis alpha (TNF-α) for the production of reactive oxygen intermediates (ROI), the release of lysosomal enzymes, and the secretion of interleukin-1β.[4,5] Furthermore, it has been demonstrated that ANCA are able to stimulate neutrophils to adhere to cultured human endothelial cells, a process that can be inhibited by anti-CD18 antibodies.[6] Johnson *et al*. have clarified the mechanism by which ANCA might

Table 1
Characteristics of ANCA-associated vasculitides

DISEASE	CLINICAL	MPO ANCA	PR3 ANCA
Churg-Strauss syndrome	Asthma, eosinophilia, neuropathy	>70% of patients	<10% of patients
Wegener's granulomatosis	Nose bleeds, nephritis, lung lesions	10-30% of patients	>70% of patients
Microscopic polyangiitis	Nephritis, purpura, haemoptysis	30-70% of patients	30-70% of patients
Idiopathic NCGN	Nephritis	>70% of patients	10-30% of patients

NCGN = necrotising crescentic glomerulonephritis.

stimulate neutrophil adherence by showing that ANCA stimulate the upregulation of CD11b on neutrophils *in vitro*.[7] Finally, it has been demonstrated that ANCA-stimulated primed neutrophils can lyse cytokine-pretreated cultured endothelial cells.[8,9] Apart from inducing the activation of neutrophils, ANCA also activate monocytes[10] and/or endothelial cells.[11,12]

The mechanisms involved in ANCA-mediated neutrophil activation are not completely understood. Upon priming with TNF-α, neutrophils express PR3 and MPO on the cell surface which then become accessible for interaction with ANCA.[4] It is thought that PR3 and MPO binding to the cell membrane is both through charge interactions and receptor mediated. Binding of ANCA to primed neutrophils may result in activation of neutrophils, a process that is largely dependent on engagement of β_2 integrins and the interaction of the Fc portion of ANCA.[13] A Fc-independent mechanism, however, has also been described to be operative *in vitro*.[4,14] Recently, Ben Smith *et al.* demonstrated that ligation of FcγRIIa and FcγRIIIb is necessary for ANCA-induced neutrophil activation. Since the signalling cascades that are used by ANCA are different from the signal pathways used by FcγR engagement only, it is suggested that apart from Fcγ engagement also other membrane co-factors are used by ANCA for neutrophil activation.[15] These other membrane co-factors have not been identified yet. The signals involved in neutrophil activation have been recently dissected and include p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) as well as phosphatidylinositol 3 kinase control systems.[16]

PATHOPHYSIOLOGY OF ANCA-ASSOCIATED VASCULITIS: ANIMAL STUDIES

Several types of pathophysiological events that may lead to vessel wall damage in vasculitis are currently recognised. These include pathogenic immune complex deposition or *in situ* formation, a 'Shwartzman-like' phenomenon in which intravascular activation and aggregation of neutrophils may be operative, antibody-dependent cell-mediated cytotoxicity, and cell-mediated immune responses.[17]

Pathogenic immune complexes (ICX) deposition-mediated vasculitis is best depicted in the serum sickness animal model. In this model, rabbits are injected with bovine serum albumin and about seven to ten days later immune complexes are found that may induce vasculitis and/or glomerulonephritis. In ANCA-associated vasculitis, however, immune complexes are generally not found in the lesions. Therefore, the classic renal lesion in ANCA glomerulonephritis is labelled 'pauci-immune'. In kidney biopsies of patients with ANCA-associated glomerulonephritis, we found no IgA or IgG deposits and only nonspecific IgM deposits in a minority of the patients.[18] Complement deposition, however, is often present (in about 50% of the cases).[18] This may point to prior ICX deposition, but there is no proof for this hypothesis.[19]

To test the hypothesis that ANCA themselves may induce vasculitis,[20] we immunised BN rats with human MPO which induced antibodies to human MPO which also cross-reacted with rat MPO.[21] Furthermore, in these rats a cellular response to MPO could be detected.[21] To our surprise, these rats appeared completely normal and no vasculitic lesions were found at autopsy. So, the induction of ANCA is not sufficient to induce vasculitis in rats. We hypothesised that there must first be ICX deposition at vessel walls. These ICX then attract neutrophils and these neutrophils then express MPO on their cell surface that may bind anti-MPO, which results in an overstimulation of the neutrophils resulting in vasculitis and also the rapid disappearance of ICX. To test this hypothesis, we injected MPO-immunised rats with an extract of neutrophils containing MPO, and hydrogen peroxide (H_2O_2). In this context we predicted that ICX deposition and subsequently vasculitis would occur. We observed vasculitis of the lungs and the gut in the rats that were immunised with MPO but not in the rats that were not MPO immunised and received the neutrophil extract only.[22] Unfortunately, no glomerulonephritis was found. However, after unilateral perfusion of the left kidney with the neutrophil extract and H_2O_2 we saw a severe form of necrotising crescentic glomerulonephritis in rats that had been immunised with

MPO and no lesions in nonimmunised rats.[21] More importantly, immediately after perfusion, ICX deposits were seen in the kidneys, but these disappeared very quickly and when the glomerulonephritis was at its maximum no further immune deposits were detected.[21] So, in the presence of ANCA, severe vasculitis and 'pauci-immune' glomerulonephritis can be induced in rats when immune complexes are first deposited along the vessel wall. The next question was what would happen if ICX other than ANCA/MPO ICX are deposited along the vessel wall. To study this, Heeringa *et al*. injected rats with an antibody to the rat glomerular basement membrane (GBM) and compared MPO-immunised rats with non-immunised rats. For these studies, a low dose of anti-GBM antibody was used that binds to the GBM but is not enough to induce a glomerulonephritis.[23] In rats with anti-MPO a severe glomerulonephritis developed whereas no lesions were found in the non-immunised rats.

In mice, ANCA have been identified in MRL-*lpr-/-lpr* and in SCG-/-Kj mice. In these models the role of ANCA is, however, difficult to tease out from the complex backgrounds of polyclonal B cell activation. Recently, however, convincing evidence was obtained that ANCA are sufficient to cause systemic 'pauci-immune' vasculitis and glomerulonephritis *in vivo*.[24] Two major strategies were used to demonstrate this. In the first, MPO-deficient mice were immunised with murine MPO and developed anti-MPO. Adoptive transfer of splenocytes from these mice into immune deficient *RAG2-/-* mice (lacking functioning B lymphocytes and T lymphocytes) resulted in anti-MPO and the development of glomerulonephritis and capillaritis. In contrast, transfer of splenocytes from mice that were immunised with BSA into *RAG2-/-* mice resulted in a mild form of immune complex glomerulonephritis without crescents. The nature of the background immune complex disease found in *RAG2-/-* mice that received either splenocytes from mice immunised with MPO or BSA is unclear. It was hypothesised that this relatively nonspecific response may represent a form of graft versus host disease. In the second strategy, purified anti-MPO was intravenously injected into *RAG2-/-* mice or wild type. 'Pauci-immune' necrotising and crescentic glomerulonephritis and systemic vasculitis, closely resembling the human disease, were observed.[24] These experiments indicate that ANCA can produce vasculitis without the further participation of T lymphocytes and/or B lymphocytes. This suggests that ANCA indeed induce vasculitis.

From these experiments, we come to our current working hypothesis. ANCA induce activation of neutrophils and monocytes resulting in ICX deposition in vessel walls. Other antigens, however, may also be involved in ICX formation. In the presence of ANCA this ICX deposition results in persistent activation of neutrophils and monocytes and subsequently severe glomerulonephritis or vasculitis.

INDUCTION OF AUTOIMMUNITY TO PR3 AND/OR MPO

The central mechanism in autoimmunity is the breaking of self-tolerance. It is now well established that autoreactive T and B cells exist in the blood of healthy individuals and that these cells can potentially induce autoimmunity if activated beyond a certain threshold.[25] A combination of risk factors may be present in patients who develop ANCA. Inherited determinants have been sought, i.e. associations with certain HLA class I or class II molecules and/or with the C3F component of complement, but have not been convincingly found in patients with ANCA-associated vasculitis and/or glomerulonephritis. Other genetic factors, however, were found to be involved in ANCA-associated vasculitis. These genes include the genes for PR3, MPO, FcγR, α_1 antitrypsin, CD18 and/or CTLA-4.[26] In addition, environmental factors are probably important modulators. Among these, drugs such as propylthiouracil and/or hydralazine[27] and/or exposure to chemical substances such as silicon[28] have been incriminated. Infectious agents are, however, the most likely candidates to cause autoimmunity. Several mechanisms by which infectious agents might induce autoimmunity have been postulated.[29] These include molecular mimicry, abnormal presentation of self-proteins, and/or abnormal stimulation of autoreactive T or B cells by agents such as superantigens. This latter mechanism has our special attention, since superantigens produced by staphylococci that may activate autoreactive B cells, in a T-cell dependent way, to produce ANCA depositions are often present in patients with ANCA-associated vasculitis.[30]

CONCLUSION

Vascular damage in ANCA-associated vasculitis and/or glomerulonephritis results predominantly from activation of neutrophils when these cells adhere to endothelial cells. These neutrophils may be initially attracted by immune complexes formed *in situ*. Once these adherent activated neutrophils are 'over-stimulated' by ANCA they may cause necrotising vasculitis and glomerulonephritis and, in addition, stimulate the rapid disappearance of immune complexes, thus explaining the absence of immune complexes in tissue biopsies.

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Immune restoration disease in HIV-infected individuals receiving highly active antiretroviral therapy: clinical and immunological characteristics

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ABSTRACT

Background: HIV-infected patients responding to HAART can show a diverse spectrum of symptoms caused by inflammatory reaction. The pathogenesis of this phenomenon, called immune restoration disease (IRD), is unclear. This study describes the spectrum of IRD and analyses the immunological and clinical parameters that could be related to its development.

Methods: In a retrospective, matched case-control study, 17 HIV-infected individuals who developed inflammatory symptoms <12 months after initiation of HAART were included. HIV-infected controls were matched for age, gender and CDC classification. Factors included in the analysis were: CD4+ and CD8+ cell counts, ΔCD4+ and ΔCD8+, CD4/CD8 ratios, HIV-1-RNA load (VL), ΔVL and the number of CDC events prior to HAART.

Results: The median time after initiation of HAART and developing IRD (n=17) was 72 days (range 2-319). In nine cases (53%) a mycobacterial infection was identified as the underlying cause. HAART was started at a mean CD4+ count (± SD) of 55 x 10^6 /l (± 59) and 85 x 10^6 /l (± 78.0) for cases and controls, respectively (p=0.13). After initiation of HAART, the CD4+ count showed a 10.6 fold increase at the onset of IRD in the cases and a 2.7 fold increase in the controls in an equal period of time (p=0.020). The other parameters analysed did not differ significantly between cases and controls.

Conclusion: We conclude that the risk of developing IRD is associated with a high-fold increase in CD4+ lymphocytes. In this study, mycobacteria are the pathogens most frequently associated with IRD.

INTRODUCTION

The future prospects for HIV-infected patients have changed dramatically because of the effectiveness of highly active antiretroviral therapy (HAART). In the majority of patients, plasma viral load decreases and CD4+ lymphocyte count starts rising directly following initiation of HAART, resulting in a lower morbidity and mortality.[1-4] However, over the past five years, the occurrence of opportunistic infections and newly developed inflammatory reactions shortly after initiating HAART was observed.[5,6] The cases reported in the literature include reactivation of hepatitis B virus, cytomegalovirus (CMV) infection and predominantly mycobacterial infection.[7-10] These episodes of opportunistic disease usually occurred in the first months after HAART was started.[7,11] It is hypothesised that the infective

pathogens are present before HAART is initiated. The recovery of the immune system enables the inducement of a local or systemic inflammatory response to these micro-organisms. This phenomenon is generally referred to as the immune restoration syndrome (IRS) or immune restoration disease (IRD).[12-14] Due to its atypical presentation, the immune restoration syndrome may well be overlooked. Here, we describe the spectrum of the immune restoration syndrome in 17 HIV-infected individuals treated with HAART, along with the immunological parameters, in order to detect factors that are associated with the development of IRD. To illustrate various aspects of IRD, we first describe a characteristic case history.

CASE REPORT

A 31-year-old woman recently diagnosed with an asymptomatic HIV infection (CD4+ lymphocyte count of 4 x 10^6/l, HIV-RNA of 267,000 copies/ml), was treated with cotrimoxazole (480 mg once daily) and HAART (consisting of zidovudine, lamivudine and indinavir). There was no history of prior infection with mycobacteria and a chest X-ray was normal. Four months after initiation of therapy she developed night sweats, malaise, fever, weight loss, an enlarged lymph node in her neck and a productive cough. Sputum showed acid-fast rods. The X-ray of the chest and a CT scan revealed extensive mediastinal lymphadenopathy but no intrapulmonary abnormalities (*figure 1*). Although infection with *M. tuberculosis* could not be excluded at that moment, the clinical presentation was compatible with a nontuberculous mycobacterial infection. Initial treatment with isoniazid (INH), clarithromycin, ethambutol and rifabutin was prescribed. Eventually *Mycobacterium avium* complex (MAC) was cultured from the sputum and INH was stopped. At the time of the manifestation of MAC the CD4+ lymphocyte count had increased to 71 x 10^6/l and no HIV-RNA could be detected in the plasma. The emergence of the mycobacterial infection, accompanied with signs of inflammation (clinical symptoms and lymphadenopathy), within months after initiation of HAART points to immune restoration disease (IRD). HAART and antimycobacterial therapy were continued all through this episode. The patient's symptoms subsided within three weeks; eventually the lymphadenopathy resolved. Anti-MAC therapy was continued up to six months after the CD4+ lymphocyte count exceeded 200 x 10^6/l.

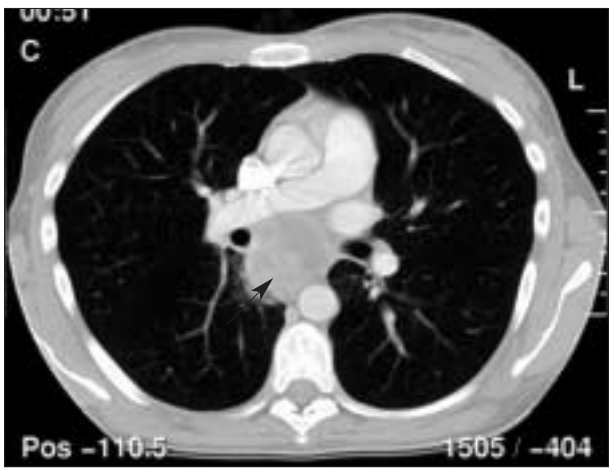

Figure 1
Slide of the thorax showing mediastinal lymphadenopathy (arrow)

PATIENTS AND METHODS

The study was conducted in the Leiden University Medical Centre (LUMC) and two large urban hospitals in The Hague, the Netherlands. In design, the research was a retrospective matched case-control study and the time frame covered the period between 1 January 1995 and 1 March 2001. For the purpose of this study, IRD was defined as the development of any inflammatory symptom, occurring within 12 months after initiation of HAART. As explained in the introduction of this article, it is hypothesised that underlying opportunistic infection causes these symptoms if combined with immune reconstitution. So, the presence of an opportunistic micro-organism also had to be confirmed or considered to be most likely.
Since the study was conducted in retrospect, the clinicians had to review their patient population carefully to detect each suspected case of IRD conform the definition mentioned above.
Exclusion criteria were the evidence of noncompliance to antiretroviral therapeutics or incomplete patient data. The control group was selected from the database of the LUMC of the HIV-infected individuals treated with HAART.
For each individual in the case group, one or two matched controls were included. Cases were matched for age, gender and CDC classification. The controls also received HAART. If two suitable controls were found, the mean outcome of the comparison was used.
Medical history, clinical data, CDC classification, gender, age, past and present treatment conditions were obtained from the patient files. To calculate the 'fold increase', i.e. relative increase in CD4+ lymphocytes for each individual, the number of CD4+ cells at a certain time point after initiation of HAART was divided by the number of CD4+ cells at the start of HAART. HAART was defined as combination antiretroviral therapy containing at least three antiretroviral drugs, which include at least a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI). The diagnosis 'infection' was made on

the basis of the clinical presentation and if the presence of the micro-organism was confirmed by culture, PCR, serology or direct microscopy. The time to onset of opportunistic infection is defined as the time from the moment of initiation of HAART to the first onset of clinical symptoms of IRD.
The CD4+ and CD8+ lymphocyte counts were determined by use of a direct immuno-fluorescence technique (Becton-Dickinson, San José, California, USA and flow cytometry (Facscan, Becton-Dickinson, Immunocytometry Systems). Quantification of HIV1-RNA load in plasma was performed using a quantitative polymerase chain reaction (RT-PCR) assay (AMPLICOR-HIV monitor test, Roche Molecular Systems, Branchburg, USA).
The immunological data are expressed as mean (± SD) and median values. Differences between both groups were statistically evaluated using the Student's t-test for paired samples, Levene's test for equality of variances and the Wilcoxon ranking test.

RESULTS

Seventeen cases out of a population of approximately 700 HIV-infected individuals receiving HAART were included. The main case characteristics are shown in *table 1*. Twenty patients were selected as matched controls by the conditions described above. The median time after initiation of HAART and IRD was 72 days. When HAART was started the mean CD4+ lymphocyte counts (± SD) were 55 x 10^6 /l (± 59) and 85 x 10^6 /l (± 78) for cases and controls respectively (p=0.13). At one month and two months after initiation of HAART there were no significant differences in absolute increase nor in fold increase in CD4+ lymphocytes.
The mean number of CD4+ lymphocytes at the onset of IRD in the case group and, in the comparable period of time, in the control group were 220 x 10^6 /l and 186 x 10^6 /l, respectively.
However, the CD4+ count after initiation of HAART to the onset of IRD showed a 10.6 fold increase (SE mean=3.28) in the cases and a 2.7 fold increase (SE mean=0.91) in the controls in matched period of time (p=0.020).
Neither the increase in CD8+ lymphocytes, the CD4/CD8 ratios nor the decrease in viral load was significantly different between cases and controls. The average number of CDC events prior to starting HAART was 3.2 for the cases and 1.6 for the controls (p=0.14).

DISCUSSION

This study demonstrates that IRD occurred predominantly within three months after initiation of HAART and, in our population of HIV-infected individuals, is most frequently (>50%) associated with mycobacterial infection.

Table 1

CASES	GENDER	AGE*	HAART	CDC#	CD4+ X 10^6 /L AT ONSET OF HAART	DAYS TO IRD	CD4+ X 10^6 /L AT ONSET OF IRD	PATHOGEN
1	M	44	3TC, AZT, indinavir	C2	110	132	210	*M. tuberculosis*€
2	M	42	3TC, d4T, indinavir	C3	30	185	360	*M. tuberculosis*€
3	F	28	d4T, 3TC, saquinavir, ritonavir	C3	21	215	518	*M. tuberculosis*€
4	M	36	3TC, d4T, nelfinavir	C3	2	20	50	*M. avium*€
5	M	33	d4T, 3TC, ritonavir, saquinavir	C3	10	34	180	*M. avium*
6	M	26	3TC + AZT, nelfinavir	C3	40	51	90	*M. avium*
7	M	31	3TC, d4T, ritonavir, saquinavir	C3	43	58	533	*M. avium*€
8	F	30	AZT, 3TC, indinavir	C2	43	150	54	*M. avium*
9	M	26	Ritonavir, amprenavir, 3TC, efavirenz, abacavir	C3	19	86	105	*M. xenopi*€
10	F	51	3TC, d4T, ddI, ritonavir	C3	104	8	141	*P. carinii*
11	M	57	3TC + AZT, nelfinavir	C3	220	17	250	*P. carinii*
12	M	36	AZT, 3TC, indinavir	C3	43	35	590	Toxoplasma
13	M	54	AZT, 3TC, indinavir	C3	6	137	59	HZV (disseminated)
14	M	62	3TC, AZT, ritonavir, saquinavir	C3	3	319	151	HZV (disseminated)
15	M	55	3TC, d4T, nelfinavir	C3	74	72	196	HBV
16	M	48	ddI, AZT, ritonavir	B3	20	82	96	HBV
17	M	32	3TC, d4T, ritonavir, saquinavir	C3	144	24	162	Viral meningitis‡

**Age at the moment of starting HAART, #CDC classification 1993, €extrapulmonary, ‡evident clinical picture, no pathogen identified, M = male, F = female, 3TC = lamivudine, AZT = zidovudine, ddI = didanosine, d4T = stavudine, 3TC + AZT = Combivir. HZV = herpes zoster virus, HBV = hepatitis B virus.*

Furthermore, the IRD cases demonstrated a significantly higher fold increase in CD4+ lymphocytes compared with controls. There was no association with baseline or absolute increase of CD4+ lymphocytes, CD8+ lymphocytes, or decrease in HIV1-RNA load.

The principal idea regarding the development of IRD is that the recovery of the immune system enables a response to a residing, but clinically silent, opportunistic infection. The significantly higher increase in CD4+ lymphocytes in the cases compared with the controls, observed in our study, suggests a relation with the occurrence of IRD. The exact nature of this relation now remains unclear. Restoration of pathogen-specific lymphoproliferative response (LPR) to recall antigens in the first months following HAART has been described earlier.[3,15-17] This increase in potency of an immunological response to recall antigens evidently correlated with the increase in CD4+ T lymphocytes.[3] However, it is also advocated that IRD reflects a pathogen-specific immune response but that a specific (Th2) cytokine environment is a critical factor in the development of IRD and a large redistribution of antigen specific T cells is not.[18-21]

The precise role of interleukin 6 and of associated HLA haplotypes are currently under investigation.[22,23]

Our results confirm the observation that mycobacterial infection is frequently associated with IRD.[8,11,24] *In vitro* experiments show that, in mycobacterial-related IRD, mycobacterial-specific lymphocyte reactivity can be induced by the presence of antigen.[14] In our study *M. avium*-related IRD occurred on average at a lower CD4+ cell count compared with *M. tuberculosis*-related IRD, 63 and 177 x 10^6/l respectively. In progressive HIV-1 infection with decreasing CD4+ T-cell numbers, *M. avium* is (on average) more frequently found at lower CD4+ T-cell counts than *M. tuberculosis*. The opposite is true for the situation where the CD4+ T-cell counts are rising, likely reflecting a difference between the complexity of the immune response to both pathogens.

The difference in the number of CDC events and the slightly lower mean number of CD4+ lymphocytes, prior to effective HAART in the IRD cases compared with the controls, probably merely reflects that a longer period of immunodeficency also predisposes to the development of IRD.

This finding could reflect a higher cumulative risk, or a higher antigen load, when the period of severe immune deficiency is longer.

Given the above and presuming that, in IRD, present asymptomatic opportunistic infection is unmasked by restoring immunity, both the restoration of immune reactivity (specific T-cell response and/or primary cytokine related) and antigen are the main interacting components. In future research the restoration of specific immune responses in combination with host-antigen interaction during and before starting HAART have to be studied in order to elucidate the exact pathogenesis of IRD.

ACKNOWLEDGEMENT

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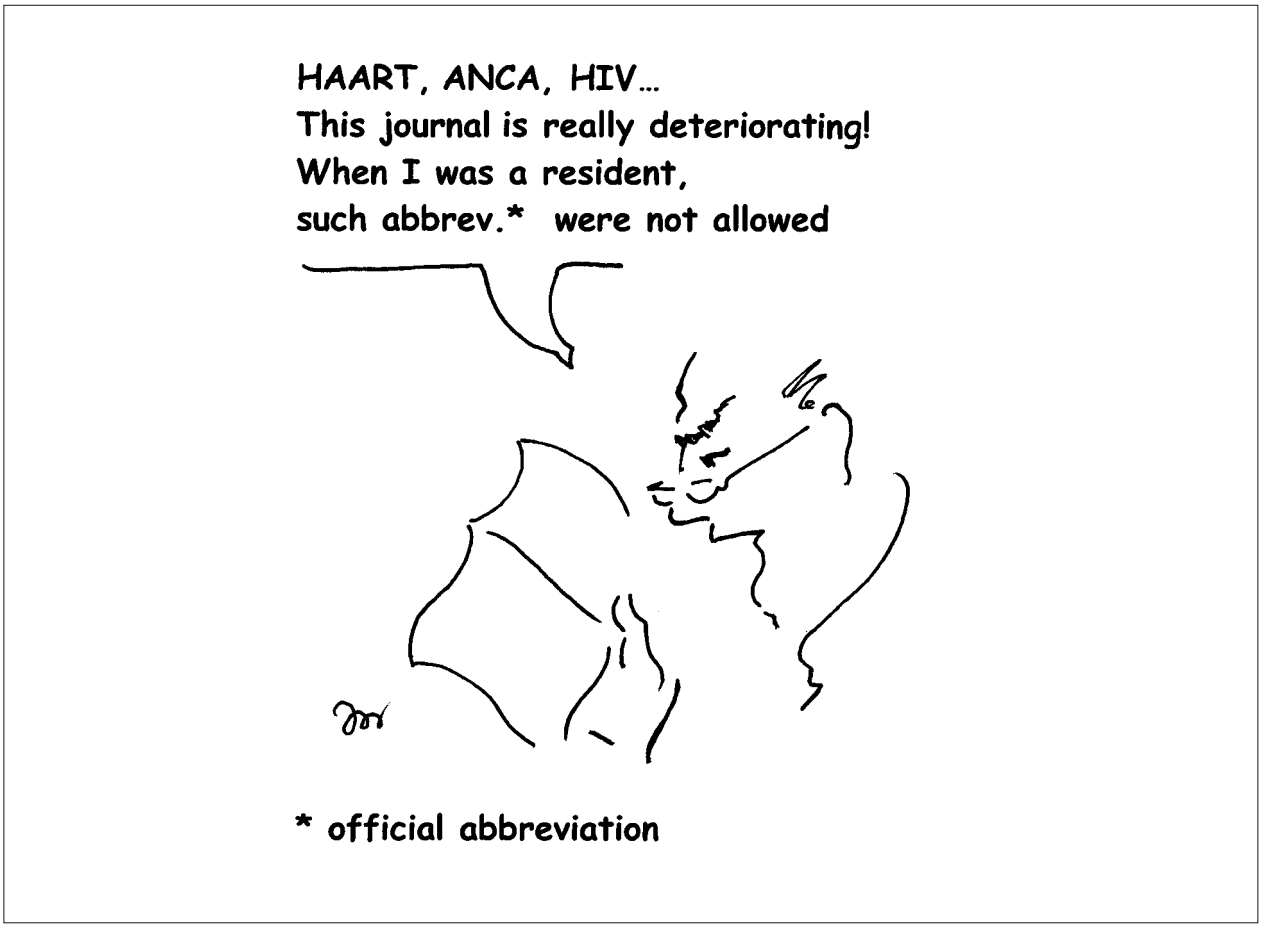

De Boer, et al. IRD in HIV-infected individuals receiving HAART.

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A man with a bulging mass in his left lumbar region

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CASE REPORT

An 87-year-old man with severe bilateral knee osteoarthritis was admitted to the hospital because of uncontrolled arthritic pain, generalised weakness, anaemia and inability to adequately take care of himself at home. Physical examination incidentally revealed a 6 x 4 cm bulge in the left costovertebral region (*figure 1*). The patient reported that he first noticed the painless mass about three month earlier, but had not sought medical care for it. An appendectomy and bilateral inguinal hernia repair had been performed many years ago. There had been no previous surgeries, instrumentations or trauma to the region of the bulge. The mass was nontender, disappeared with light manual pressure and reappeared if the patient coughed or strained. Bowel sounds were audible on the surface of the mass.

WHAT IS YOUR DIAGNOSIS?

See page 432 for the answer to this photo quiz.

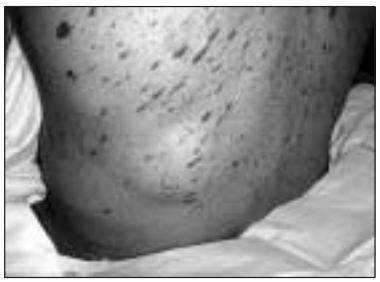

Figure 1
Bulging mass in patient's left lumbar region

A colour version of this photo quiz can be found on our website www.njmonline.nl.

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CASE REPORT

Successful treatment of metastatic esthesioneuroblastoma

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ABSTRACT

This case report describes a patient with a metastasised olfactorial esthesioneuroblastoma Hyams grade 4 who has been treated with debulking surgery and radiotherapy. After relapse in lymph node, lung and brain, he received additional irradiation and six cycles of carboplatin, vincristine and cyclophosphamide intravenously every three weeks. The patient has now been disease free for 7.8 years. Our data suggest that metastatic esthesioneuroblastoma is sensitive to platinum-based chemotherapy. This patient illustrates that this tumour is very sensitive to platinum-based chemotherapy and that durable complete response can be achieved, even in a metastatic ENB.

INTRODUCTION

Olfactorial neuroblastoma is a rare tumour, arising from the olfactory epithelium. It comprises about 3% of all nasal tumours and usually occurs in the third and fourth decade of life. The incidence in males and females seems to be similar. There are over 300 case reports of patients with localised disease to be found in the literature. The tumour frequently presents with unilateral nasal obstruction and epistaxis. Less frequent are proptosis and retrobulbar pain. At radiology, a unilateral intranasal soft tissue mass, opacity of the paranasal sinuses and destruction of the nasoantral and orbital walls, or extension to the skull base are usually observed. Pathological interpretation can be difficult due to the poor differentiation of tumour cells. Although a number of histochemical tumour markers are available such as neurofilament, neurospecific enolase, synaptophysine or S100, no specific marker has emerged. There are two kinds of classification systems:

1. The Hyams grading system, which is a pathologically based system with emphasis on the lobular architecture, mitotic activity and presence of necrosis.[1]
2. The Kadish staging system which, in the absence of a TNM classification, describes the local extent of the tumour and the possible presence of metastases necrosis.[1]

Metastases can be found in the cervical nodes, meninges, bone, parotid gland, lungs, spinal cord and prostate. In patients with haemotogenous metastases, several cytostatic agents, single and in combination, have been used. In this case report, we describe a patient who has been treated with combination chemotherapy because of distal metastases.

CASE REPORT

A 28-year-old man, presenting with a short history of proptosis, blurred vision of the right eye and diplopia, was found to have an esthesioneuroblastoma (ENB) of the lamina cribrosa in February 1992. Besides the presence of necrosis, histology showed a high mitotic index and the tumour was classified histologically as Hyams grade 4. Clinically it proved to be a Kadish stage C, due to involvement of the lamina cribrosa, as seen on the CT scan.[2]

Debulking surgery, comprising a right lateral rhinotomy and excentration of the right orbita, was followed by radiotherapy from March to May 1992: 50 Gy to a bi-directional field

with an additional booster dose of 16 Gy to the involved lamina cribrosa to a total of 66 Gy, in 33 fractions over 49 days, including the right orbita and right ethmoid in the target field. In May 1992, a CT scan of head and neck revealed a previously unknown enlargement of a right subdigastric lymph node and a bilateral supraomohyoidal cervical node dissection was performed. Histology showed one lymph node metastasis with extranodular tumour extension, and simultaneously headache pointed to a 2 cm diameter subdural metastasis laterally of the frontal lobe without a clear relationship to the primary site, as revealed by MRI. A chest X-ray in May 1992, before the supraomohyoidal cervical node dissection, showed no pathology. In July 1992, however, at the discovery of the subdural frontal metastasis, two lung metastases adjacent to the right hilus were found. From July to August 1992 radiotherapy (30 Gy in 10 fractions) was administered to the subdural frontal metastasis. After the radiation therapy, chemotherapy was initiated (carboplatin 300 mg/m^2, vincristine 1 mg/m^2, cyclophosphamide 750 mg/m^2 per cycle, COC) administrated intravenously every three weeks for six cycles. This resulted in a complete response of the lung metastases. In May 1995, he presented with pain between the scapulae. A bone scan and X-ray showed metastases in the corpus of the thoracic vertebrae Th4, Th5 and Th8. In June 1995 30 Gy radiation was given in ten fractions directed to Th3 - Th9. In November 1996 he experienced a seizure, but there were no new lesions found on the CT of the brain.[3]

Thereafter he received phenytoin 2 x 100 mg orally daily and no further seizures occurred. At this moment, the patient is 134 months post-diagnosis and is currently free of disease.

DISCUSSION

Primary treatment of an esthesioneuroblastoma consists of radical craniofacial resection, frequently combined with elective radiotherapy. This combination is based on whether or not the tumour is confined to the nasal cavity (Kadish stage A) or beyond (Kadish stage B and C). Standard radiotherapy consists of 50 Gy given as 25 fractions of 2 Gy.

A booster dose between 10 and 20 Gy can be given depending on the extent of invasion. Our patient relapsed within two months after radiotherapy. Based on a study of 13 patients this indicates a poor prognosis.[1] If invasion of the primary tumour site includes involvement of the cribriform plate, base of the skull, orbit or intracranial cavity (Kadish stage C) or if metastases to cervical lymph nodes or distant sites occur, chemotherapy has been added to surgery and radiotherapy. Active agents are: vincristine, cyclophosphamide, cisplatin, doxorubicin, etoposide and methotrexate.[4-7] The sequence of therapy modalities still needs to be clarified. Although two papers suggest a survival benefit with neo-adjuvant treatment, the patient population in both studies is too small to be conclusive.[4,7] A review of thirteen patients with metastatic ENB describes an objective response after cisplatin and etoposide in eight patients (61.5%).[8] These studies are based on the morphological, immunophenotypic and ultrastructural resemblance of ENB with the primitive peripheral neuroectodermal tumour-Ewing lineage (PNET). However, the typical absence of trisomy 8 and translocation t(11;22)(q24;q12) exclude ENB as a member of PNET.[9] The use of the Packer chemotherapy protocol, based on its efficacy in medulloblastoma as an example of another primitive small round cell tumour, is not supported by these specific chromosomal findings.[10,11] Eventually we preferred a combination of vincristine, carboplatin and cyclophosphamide because of less toxicity in the outpatient setting.

Recently, a 36-year-old woman presented with prolonged headache, loss of smell and vomiting and ENB was diagnosed in August 2002. She had an emergency bifrontal craniotomy and incomplete debulking surgery, followed by cranial irradiation. In April 2003 she became dyspnoeic and bedridden because she had developed large lymph node metastases in the right neck and multiple lung metastases (*figure 1*), but no liver or bone metastases. She was treated with the same chemotherapy regimen as the first patient (similar dosages of COC for six cycles). Even after three cycles of COC, the chest X-ray showed a complete remission (*figures 2* and *3*) and the masses in the neck had disappeared. She is now in follow-up.

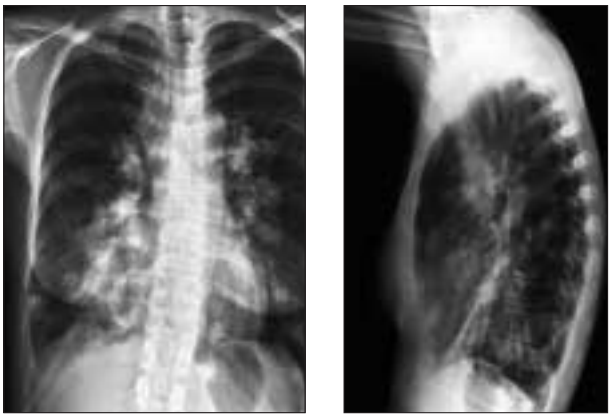

Figure 1
Lung metastases at diagnosis

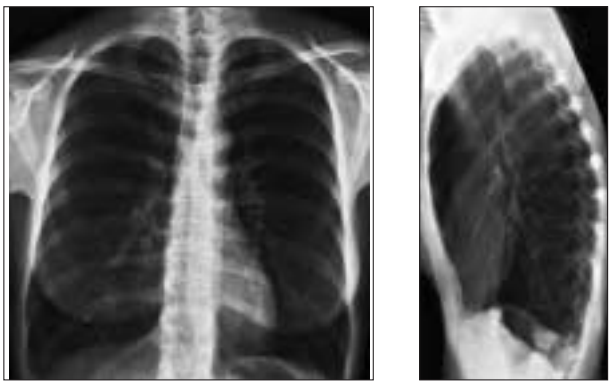

Figure 2
Complete response of lung metastases after three cycles of COC

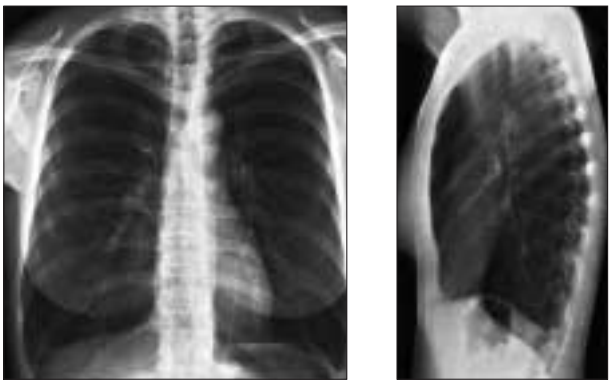

Figure 3
Complete response of lung metastases after discontinuation treatment

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Acute renal failure due to carnitine palmitoyltransferase II deficiency

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ABSTRACT

Carnitine palmitoyltransferase II (CPT-II) deficiency is the most common long-chain fatty acid oxidation defect, resulting in rhabdomyolysis and acute renal failure (ARF). There are three forms of CPT-II deficiency: the neonatal, infantile and adult form. We report an adult form of CPT-II deficiency in a patient who presented with attacks of exercise-induced rhabdomyolysis and ARF.

INTRODUCTION

Long-chain fatty acids (LCFA) are the main energy source of muscles during prolonged exercise.[1] LCFAs cannot diffuse into mitochondria passively; they must be activated by a long-chain fatty acyl-CoA synthetase on the outer mitochondrial membrane. Long-chain fatty acyl-CoAs are imported into the mitochondrial matrix by the carnitine palmitoyltransferase system. This system consists of two distinct enzymes: the outer membrane enzyme is carnitine palmitoyltransferase I and the inner membrane enzyme is carnitine palmitoyltransferase II (CPT-II). CPT-II deficiency is the most common long-chain fatty acid oxidation defect.[2] This deficiency results in energy depletion in myocytes during prolonged exercise leading to rhabdomyolysis. There are three different forms of CPT-II deficiency: the neonatal, infantile and adult form. The neonatal form is the most lethal and the adult form the most benign. In the adult form of CPT-II deficiency, episodic rhabdomyolysis attacks occur following prolonged exercise, infection or anaesthesia. When rhabdomyolysis occurs, acute renal failure (ARF) follows the cascade in proportion to the involved muscle mass and hydration status of the subject. We report a case of adult form CPT-II deficiency induced rhabdomyolysis resulting in ARF.

CASE REPORT

A 20-year-old man presented with nausea, muscle cramps and darkening of his urine. Seven years ago he first noticed that after strenuous exercise he had brown urine which resolved within three days. He was an amateur football player at that time. He then started to train hard to play competitively. At that time, again after an attack, he consulted a physician and was told that his blood urea was high and he was advised not to take prolonged exercise. He kept on doing light exercise from time to time and found out that if he sweated too much during exercise, his urine turned brown. One year before, while he was asymptomatic, he was hospitalised in the Neurology Department of our faculty for aetiological investigations. At that time electromyelographic studies and muscle biopsy were nondiagnostic. One week before he was admitted to our unit, he again engaged in prolonged exercise and he noticed discoloration of his urine. During that week he also noticed a decrease in his urine volume. We hospitalised the patient in our Nephrology Department with the diagnosis acute renal failure. He was in good condition. His blood pressure was 120/80 mmHg, pulse was 80 beats/min, axillary temperature was 36.7°C, breathing frequency was 16. His lung sounds and heart sounds were normal, without friction. There was no peripheral oedema. He had no neurological deficits and his muscle strength was normal.

He had diuresis, but he did have a history of an oliguric period before he was hospitalised. His blood chemistry was consistent with rhabdomyolysis (*table 1*). Urine examination by dipstick revealed blood in the urine, but microscopic examination of urine revealed two to three erythrocytes per high power field. This was consistent with myoglobinuria.

Table 1
Blood chemistry on admission

	VALUE	NORMAL RANGE
Urea	37.4 mmol/l	1.7-8.3
Creatinine	530 μmol/l	44-123
Creatine kinase	18.760 U/l	0-190
AST	2415 U/l	5-37
ALT	406 U/l	5-37
Myoglobin	356 nmol/l	11-41
Lactate dehydrogenase (LDH)	7685 U/l	150-350
Sodium	133 mmol/l	135-145
Potassium	4.2 mmol/l	3.5-5

AST = asparate aminotransferase, ALT = alanine aminotransferase.

The patient had a 6000 cc diuresis. The patient was in the polyuric phase of ARF, and was only treated with oral hydration. We performed a muscle biopsy and sent it to Buffalo University, NY, USA for examination of muscle enzyme levels. The phosphorylase and citrate synthase activity were in the normal range, but carnitine palmitoyl-transferase (CPT) II activity was 12.0 nmol/min/g (normal 77.8±13.3). Ratio of CPT-II/ citrate syntase was 0.85 (normal 5.13±1.62). The diagnosis of CPT-II deficiency induced rhabdomyolysis was made. After five days his urine volume fell to 1700 cc, and his blood urea and creatinine levels normalised. He was discharged from hospital and started on a carbohydrate-rich diet. He was told not to do any exercise. Six months later, he again played in a football match on a hot day, which again resulted in rhabdomyolysis induced acute renal failure. He recovered from the last attack as well. During his follow-up CK levels remained within normal ranges.

Discussion

In normal metabolic conditions, the main energy source for muscles is glucose. To support the contraction of skeletal muscles, physical exercise increases total body metabolism to 5 to 15 times the resting rate. LCFAs become the major source of energy after 30 minutes of exercise, and thus they must be transported into mitochondria by specific enzymes, including CPT-II. In CPT-II deficiency the spectrum of the clinical presentation mainly depends on the remaining enzyme activity. The least activity results in the most energy depletion. The unique pathway leading to rhabdomyolysis starts after depletion of energy within muscle cells (*figure 1*).

There are several factors that lead to myoglobin induced acute renal failure. The most important factor is dehydration.

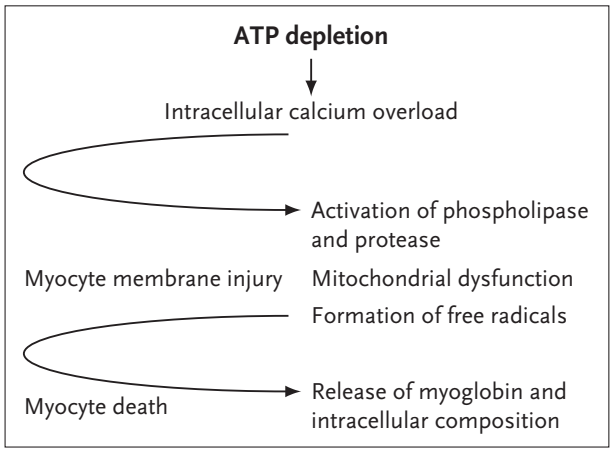

Figure 1
ATP depletion and myocyte death
ATP depletion results in intracellular calcium overload. Calcium activates phospholipase and protease enzymes, which lyse cellular membrane and alter the function of mitochondria. These enzymes also form free radicals that lead to myocyte death. Cellular ingredients including myoglobin, phosphorus and uric acid leak to outside of the membrane.[3]

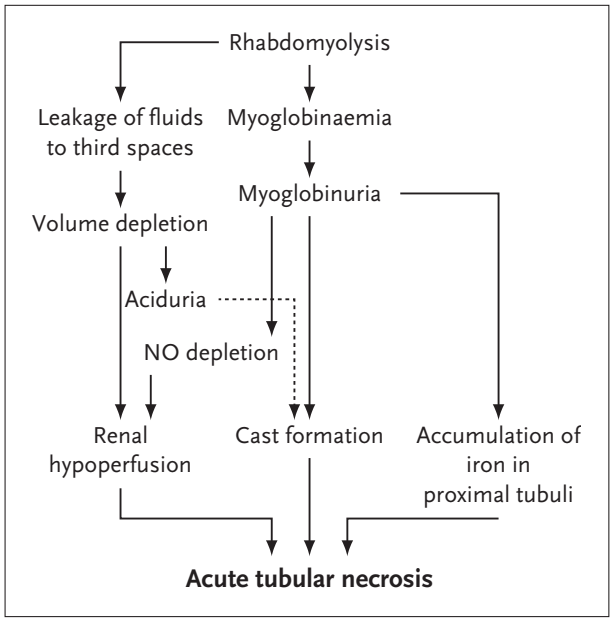

Figure 2
Rhabdomyolysis and tubular necrosis
Rhabdomyolysis results in myoglobinaemia. Myoglobinuria occurs when myoglobin exceeds 250 μg /ml (normal 5 ng/ml) and causes cast formation and accumulation of iron in proximal tubules. Sequestration of fluids in injured muscles results volume depletion, aciduria, nitric oxide depletion and renal hypoperfusion that altogether contribute to acute tubular necrosis.[5]

During physical exercise, especially in hot weather, the sweating rate is 1.0 to 2.5 l/h.[4] This dehydration can be augmented to 5% of the body weight depending on the intensity of the exercise, clothing worn and how hot the weather is. In our patient's history, his urine only became discoloured if he sweated too much during exercise. An intervention, rehydration, at this point may potentially prevent the renal dysfunction.

The causes of rhabdomyolysis differ from country to country. In Turkey, after the major Marmara earthquake in 1999, the most common cause of rhabdomyolysis became crush syndrome.[5] In USA, most cases of rhabdomyolysis are due to trauma, and in Poland alcohol abuse.[6,7] Whatever the cause, the rhabdomyolysis may progress to myoglobin induced acute renal failure (*figure 2*). The incidence of myoglobin induced ARF has been reported to be 16 to 33%.

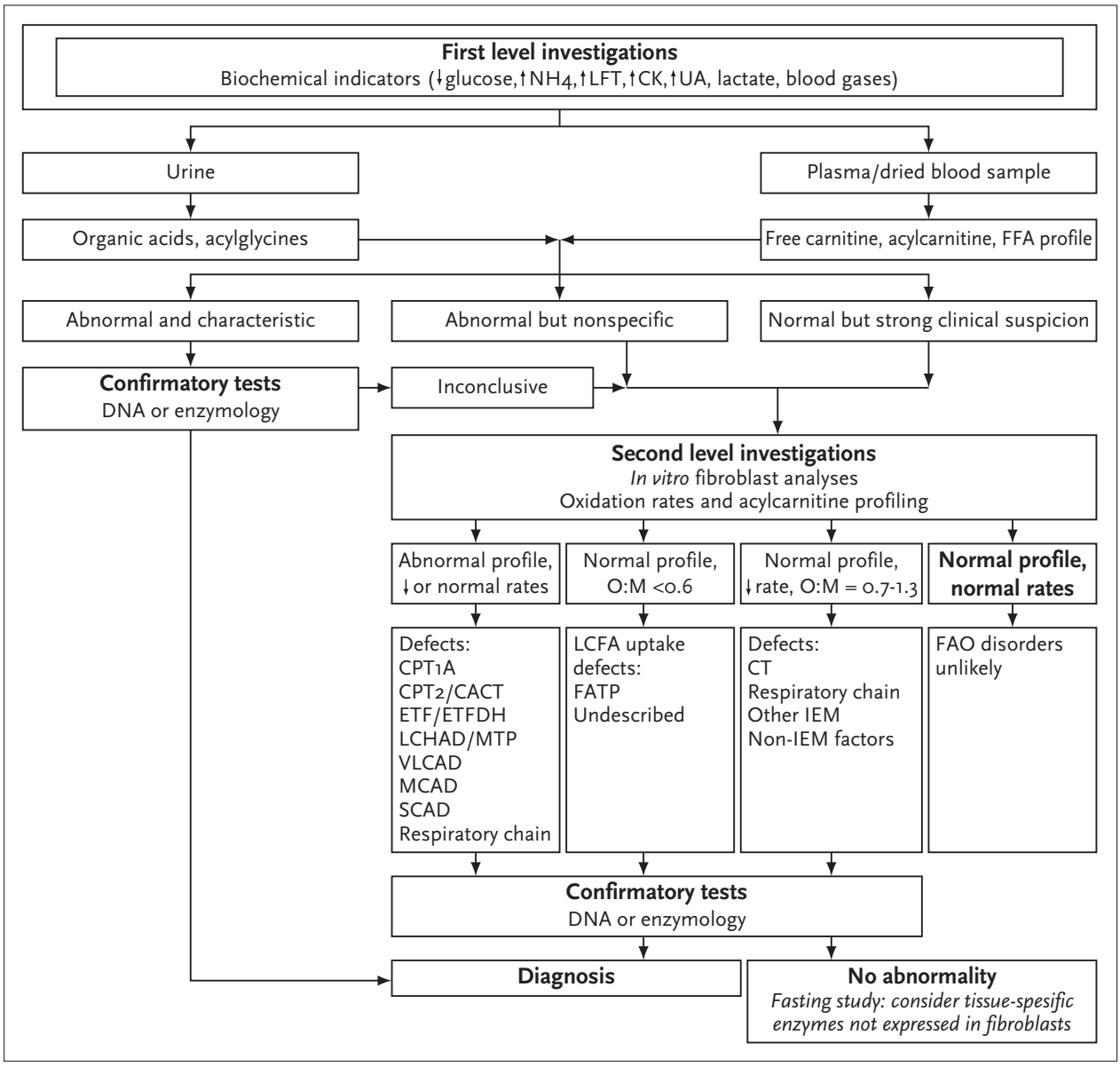

Figure 3
Diagnostic steps in suspected FAOD patients

NH4 = ammonium, LFT = liver function tests, CK = creatine kinase, U = uric acid, O = oxidation rate of [9,10(n)-3H] Oleate, M = oxidation rate of [9,10(n)-3H] Myristate, CPT1A = hepatic carnitine palmitoyltransferase I, CPT2 = carnitine palmitoyltransferase II, CACT = carnitine acylcarnitine translocase, ETF = electron transfer flavoprotein, ETFDH = electron transfer flavoprotein dehydrogenase, LCHAD = longchain L-3-hydroxyacyl-CoA dehydrogenase, MTP = mitochondrial trifunctional protein, VLCAD = very long chain acyl-CoA dehydrogenase, MCAD = medium-chain acyl-CoA dehydrogenase, SCAD = short-chain acyl-CoA dehydrogenase, FATP = long-chain fatty acid transporter protein, CT = plasma membrane carnitine transporter, IEM = inborn errors of metabolism, FAO = fatty acid oxidation.[8]

Recurrent rhabdomyolysis is characteristic of the adult form of CPT-II deficiency, and is often associated with deficiency of L-3-hydroxyacyl-CoA dehydrogenase (LCHAD), mitochondrial trifunctional protein (MTP), very long-chain acyl-CoA dehydrogenase (VLCAD) and short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD).[1]

Diagnosis of fatty acid oxidation disorder (FAOD) in a clinical case first requires the awareness and recognition of the disease. The introduction of tandem mass spectrometry (MS/MS) has substantially improved the ability to detect FAOD by acylcarnitine profiling. The diagnostic steps in a suspected FAOD include: routine blood chemistry, blood gases, acylcarnitine profiling by MS/MS, *in vitro* cellular-based screening assays (oxidation rate and quantitative acylcarnitine profiling), DNA analyses or biochemical measurement of the enzyme (*figure 3*). In CPT-II deficiency, urine organic acid profile, urine acylglycine and plasma C6-16 free fatty acids are normal, but free carnitine and acylcarnitine profiles of plasma or dried blood sample show an increase in C16, C16:1, C18:1 and C18:1.[8] What is lacking in our case is that we were unable to perform an acylcarnitine profile.

The value of muscle biopsy in unexplained rhabdomyolysis, or its marker as elevated levels of creatine kinase, has been investigated by some authors. Certain diagnosis can be reached in 18.4 to 45% for persistent hyper-creatine kinase-aemia (hyperCKaemia).[9] In recurrent rhabdomyolysis, underlying disease can be found in 15 to 47% of patients.[10] CPT-II deficiency was found to be the main aetiological factor in the majority of diagnoses. Normal histological or immunohistochemistry findings do not rule out inherited diseases. Prelle *et al.* reported normal histology or histo-chemistry in muscle biopsies of patients with persistent hyperCKaemia, but reached a specific diagnosis in 12% of them by biochemical, immunhistochemical or genetic investigation (dystrophinopathy, partial CPT deficiency, malignant hyperthermia).[11] In our patient, we could not reach the diagnosis by histology and histochemistry. We identified CPT-II enzyme defect by biochemical investiga-tion of the muscle tissue.

An intervention or preventive measures to avoid energy depletion can potentially prevent rhabdomyolysis. Preventions are either not doing exercise, or not augmenting the body metabolism (fever, anaesthesia). In our patient, all the rhabdomyolysis attacks occurred after prolonged exercise in hot and humid weather. Although he was asked not to engage in strenuous exercise, he kept on doing it on occasions. Intervention before energy depletion can consist of giving the necessary energy source with carbohydrates. This can be maintained by eating before exercise, but only intravenous glucose has been reported to be beneficial.[12]

In conclusion, patients with recurrent rhabdomyolysis should be further evaluated by means of muscle biopsies. Normal histology or histochemistry can be found in CPT-II deficient patients' muscle biopsies, hence the diagnostic steps, including acylcarnitine profile, need to be performed in suspected cases. Prolonged exercise must be avoided. If not, the patients must be fully rehydrated during exercise, or other conditions that elevate body metabolism, to prevent myoglobinuric acute renal failure.

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Optimisation of the antibiotic guidelines in the Netherlands VII. SWAB guidelines for antimicrobial therapy in adult patients with infectious endocarditis

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ABSTRACT

The Working Party on Antibiotic Policy (Dutch acronym is SWAB) is a Dutch organisation that develops guidelines for in-hospital antimicrobial therapy of bacterial infectious diseases. This present guideline describes the antimicrobial treatment for adult patients with infective endocarditis. The choice and duration of antimicrobial therapy is determined by the infecting micro-organism, sensitivity of this micro-organism for antimicrobial therapy, location of the endocarditis, left-sided or right-sided, and presence of intracardial prosthetic material. In this guideline, the empirical therapy for endocarditis is discussed as well as the therapy for the most frequent causative organisms: streptococci, enterococci, staphylococci and HACEK micro-organisms

The Stichting Werkgroep Antibioticabeleid (the Working Party on Antibiotic Policy) or SWAB develops guidelines for intramural use of antibiotics, the aim being to optimise antibiotic therapy to contribute to preventing resistance and to achieving better management of the costs and the use of antibiotics in the Netherlands. The guidelines apply for adult patients in hospital. For guidelines for children, see 'Blueprint for paediatric antimicrobial therapy'.

The guidelines are based on the following important criteria for the use of antibiotics: the indications for the prescription are correct, therapy is directed against the presumed causative agent or, preferably, the demonstrated causative agent, the drug is administered at the proper time and therapy is not unnecessarily prolonged because a drug with the smallest possible spectrum, which is as safe and inexpensive as possible, is administered via the preferred route.

The guideline for endocarditis has been written according to the principles of 'evidence-based medicine' (M. Offringa, W.J.J. Assendelft, R.J.P.M. Scholten, Inleiding in evidence-based medicine. Bohn Stafleu van Loghum 2000). The various levels of proof are defined in *table 1*. Due to the low incidence of endocarditis, large randomised clinical studies on the effect of the various therapies are scarce. This and other guidelines are, therefore, partly based on the experience of experts obtained with relatively small numbers of patients as well as on experimental animal studies. A shorter report in Dutch will be published in the Nederlands Tijdschrift voor Geneeskunde.

Table 1
Explanation of the levels of proof according to the principles of evidence-based medicine

A1	Meta-analyses which cover at least several studies on the A2 level, whereby the results of the separate studies were consistent
A2	Randomised comparative clinical study of good quality (randomised, double-blind controlled trials) of sufficient scope and consistency
B	Randomised clinical trials of moderate quality or of insufficient scope or other comparative studies (nonrandomised, cohort studies, case-control studies)
C	Noncomparative studies
D	Expert opinion, for example members of work groups

Classification of literature according to the degree of proof (CBO Handleiding voor werkgroepleden, 2000. www.cbo.nl).

EPIDEMIOLOGY

In the Netherlands, there are at least 250 cases of infective endocarditis (IE) a year.[2,3] The largest proportion of these patients has a community-acquired infection. The number of nosocomial cases of endocarditis is unknown but endocarditis as the result of an intravascular catheter-associated bacteraemia is not uncommon.[4-6] The disease occurs twice as often among men than women and the incidence clearly increases with age. Approximately half of adult patients are previously known to have a cardiac abnormality which predisposes to endocarditis. In decreasing order of frequency, the most common predisposing abnormalities are mitral valve prolapse with mitral insufficiency, degenerative abnormalities of the aorta and mitral valve, congenital abnormalities of the heart and rheumatic valve abnormalities. Before the introduction of antibiotics, mortality for endocarditis was 100%. Even today, endocarditis is a severe condition which is characterised by a high morbidity and mortality: approximately 20% of patients die during hospital admission.

CLASSIFICATION AND CAUSATIVE MICRO-ORGANISMS

For infective endocarditis a distinction is made between an acute and a subacute course and between IE of a native cardiac valve and a prosthetic valve. Acute IE is a fulminating disease which is often accompanied by rapid destruction of the valve and perivalvular and/or metastatic abscesses. It is caused by virulent micro-organisms, such as *Staphylococcus aureus*, and it often develops on what was a normal cardiac valve. Subacute endocarditis usually develops on a previously damaged cardiac valve due to relatively avirulent micro-organisms such as viridans streptococci. The course is slow and metastatic abscesses are rare. Endocarditis on a native cardiac valve in individuals who are not intravenous drug-users is almost always left-sided and is usually caused by Gram-positive cocci, such as viridans streptococci (60%), *S. aureus* (20%) or enterococci (10%).[7] In patients over 60 years of age endocarditis is caused by *S. bovis* in 10% of cases. Endocarditis caused by *S. bovis* is accompanied in about 45% of cases by abnormalities of the digestive tract, in particular colon carcinoma and villous adenoma.[8,9]

In intravenous drug-users endocarditis usually develops on the right side of the heart and is then caused by *S. aureus* in two-thirds of the cases.[10-12] Other causative agents among drug addicts are *Pseudomonas aeruginosa*, enterococci and *Candida* species. Polymicrobial infections occur regularly in drug-users.[13-15]

Prosthetic valve endocarditis (PVE) is classified according to the time since implantation of the valve. When the infection develops within two to three months of surgery, it is called early PVE. The infection is then usually the result of contamination during the operation or a central venous catheter infection. The most important causative agents of early PVE are *S. epidermidis* and to a lesser extent *S. aureus* and Gram-negative aerobic micro-organisms. After the period of two to three months, the condition is called late PVE; in this case the causative agents are similar to those found for endocarditis of a native cardiac valve.

Gram-negative micro-organisms can be isolated in about 5% of cases of endocarditis. In the past they were mainly *Salmonella* species; with modern culture techniques, mainly slow-growing bacteria such as *Haemophilus* species, *Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella* species (HACEK) are seen.[16]

MICROBIOLOGICAL DIAGNOSTICS

For the specific aetiological diagnosis of IE, the causative micro-organism must be demonstrated. Although in IE the number of bacteria in blood is often low, the bacteraemia is usually continuous so that often all blood cultures will be positive. For differentiation between contamination and endocarditis, it is recommended that separate samples be taken from peripheral vessels at intervals of at least 15 minutes, three blood cultures being obtained in the first 24 hours and eventually again on the second day. If the patient has already received antibiotic therapy beforehand, incubation of the blood cultures should last longer. Also for isolation of HACEK bacteria, longer incubation of the blood culture is needed. In some cases of endocarditis the bacteria can be cultured from septic embolisms or from material sampled during surgery or autopsy.[17] Serological

and molecular biological studies can help establish the diagnosis of, for example, *Coxiella* species, *Brucella* species, *Chlamydia* species, *Bartonella* species and *Tropheryma whippelii*.[18-24]
As a result of modern microbiological techniques, the percentage of patients with culture-negative endocarditis has decreased to less than 5%. Culture-negative endocarditis is the result of antimicrobial therapy before the blood samples were collected in more than 50% of cases.[17,25] Finally the differential diagnosis for culture-negative endocarditis must also include noninfectious conditions, such as systemic lupus erythematosus, Loeffler's endocarditis and myxoma.[26,27]

THERAPY

In these guidelines, we will limit ourselves to the antimicrobial treatment of IE and we will not consider the indications for surgery. In all patients with IE, the cardiothoracic surgeon must be consulted at an early stage, especially in patients with complications or with a prosthetic valve.
In these guidelines antimicrobial therapy is presented for the most common causative agents of endocarditis: veridans streptococci, *S. bovis*, enterococci, *S. aureus*, *S. epidermidis* and the HACEK group. For the treatment of less common causative agents, there is insufficient data available to provide a basis for guidelines.
The empirical therapy is presented in *table 2*. For acute endocarditis, immediate empirical therapy is always indicated, but never before three blood samples have been collected at 15-minute intervals. For the subacute form, one can wait in most cases for determination of the bacteria, after which specific therapy can be initiated.

Table 2
Empirical therapy for endocarditis

ANTIBIOTIC	DOSE
Native valve	
Subacute onset and long-term course	
Penicillin *and*	2.10^6 IU iv every 4 h
Gentamicin	3 mg/kg iv once daily
Penicillin allergy	
Vancomycin *and*	15 mg/kg iv every 12 h (max. 1 g every 12 h)
Gentamicin	3 mg/kg iv once daily
Acute onset and fulminating course or iv drug-user	
Flucloxacillin *and*	2 g iv every 4 h
Gentamicin	3 mg/kg iv once daily
Penicillin allergy:	
Vancomycin *and*	15 mg/kg iv every 12 h (max. 1 g every 12 h)
Gentamicin	3 mg/kg iv once daily
Prosthetic valve	
Vancomycin *and*	15 mg/kg iv every 12 h (max. 1 g every 12 h)
Gentamicin	3 mg/kg iv once daily

PRINCIPLES OF TREATMENT

Within the vegetation, micro-organisms are often present in high concentrations (109 CFU/gram),[28] in a metabolically inactive growth phase and surrounded by thrombocytes and fibrin. As a result access is difficult for phagocytising cells and the bacteria are relatively insensitive to antimicrobial therapy. Antibiotics must therefore be bactericidal and have to be administered in high doses intravenously for prolonged periods. The choice and duration of antimicrobial therapy are determined by the type and sensitivity of the isolated micro-organism, the presence of a prosthetic valve, localisation of the infection (right-sided or left-sided) and the occurrence of complications such as intracardial abscesses. In practice the sensitivity is given by the minimal inhibitory concentration (MIC), which is the minimum concentration of antibiotic that inhibits growth *in vitro*.
Several studies have described successful oral treatment of endocarditis. These studies involved such small groups of patients with a short follow-up and the selection of patients who were eligible was so specific that we have excluded this form of therapy from our considerations.[29-32]
Another new development is intravenous home treatment; however in this case, too, the conditions for both the patient and the required situation at home were so specific that we will not discuss this form of therapy in this paper.[33-35]
On the basis of theoretical pharmacokinetic principles and the results of experimental animal studies, some physicians prefer continuous over intermittent administration of β-lactam antibiotics for endocarditis.

EVALUATION OF THE THERAPEUTIC EFFECT

Frequent and careful clinical observation is the best way to evaluate the effect of treatment. The patient must be examined at least once a day for signs of cardiac decompensation or metastatic infections. In addition, the initial microbiological response, in particular of *S. aureus* infections, must be monitored by taking a blood culture 72 hours after initiation of treatment. Most patients with IE become free of fever within three to five days, for those with IE due to *S. aureus*, fever can last somewhat longer. If fever persists for more than a week, this can be attributed to perivalvular infection in most cases.[36,37] When the fever initially disappears and later recurs, then this is usually attributable to hypersensitivity to the antibiotics used. Other causes of persisting or recurrent fever are septic embolisms and an infection of the intravenous entry route.

STREPTOCOCCI

Viridans streptococci and *S. bovis* cause 40 to 60% of all cases of community-acquired endocarditis of the native valve.[1] The viridans streptococci are a heterogeneous group of micro-organisms that form the normal flora of the oropharyngeal cavity. The most important clinical representatives are *S. oralis (mitis), S. sanguis, S. mutans, S. milleri* and *S. salivarius. S. bovis* is a group D streptococcus; endocarditis due to this micro-organism is often associated with colorectal growths.[8,9]

The antimicrobial treatment of IE due to viridans streptococci and *S. bovis* is determined by the sensitivity of the micro-organism for penicillin. A distinction is made between 1) MIC ≤0.1 mg/l, 2) MIC >0.1 but <0.5 mg/l and 3) MIC >0.5 mg/l. Most of the viridans streptococci and *S. bovis* have an MIC of <0.1 mg/l. For these micro-organisms there are three different therapeutic regimens, all of which have advantages and disadvantages (*table 3*).[38-44] The oldest therapy is the four-week course of penicillin only, which has the advantage that the use of aminoglycosides with their possible ototoxic and nephrotoxic effects is avoided. The combination of a four-week regimen of penicillin and two weeks of gentamicin is used if the IE has existed for more than three months, if the infection has recurred or if the IE is accompanied by complications. Several studies have demonstrated that in uncomplicated endocarditis a two-week regimen can also be adequate.[42,43] There are, however, a number of prerequisites that must be satisfied: (*table 3*) (level of proof C).[42,44] For the treatment of viridans and bovis strains that exhibit a high resistance against aminoglycosides (MIC >500 mg/l), see the section on enterococci.

Viridans streptococci and *S. bovis* with a relative lack of sensitivity to penicillin (MIC >0.1 but <0.5 mg/l) must be treated with combination therapy consisting of four weeks of penicillin and two weeks of gentamicin (*table 3*) (level of proof D).[38,40,44,45] Strains with an MIC ≥0.5 mg/l must be treated as enterococci (*table 4*).

Table 3
Treatment of endocarditis caused by viridans streptococci and S. bovis with MIC ≤0.1 mg/l of MIC >0.1 and <0.5 mg/l

ANTIBIOTIC	DOSE	DURATION
Native valve MIC <0.1 mg/l		
Penicillin *or*	2.10^6 IU iv every 4 h	4 weeks
Penicillin *and*	2.10^6 IU iv every 4 h	4 weeks
Gentamicin *or*	3 mg/kg iv once daily	2 weeks
Penicillin *and*	2.10^6 IU iv every 4 h	2 weeks*
Gentamicin	3 mg/kg iv once daily	2 weeks*
Penicillin allergy		
Ceftriaxone** *or*	1 dd 2 g iv or IM	4 weeks
Vancomycin	15 mg/kg iv every 12 h (max. 1 g every 12 h)	4 weeks
Native valve MIC ≥0.1 and <0.5 mg/l		
Penicillin *and*	2.10^6 IU iv every 4 h	4 weeks
Gentamicin	3 mg/kg iv once daily	2 weeks
Penicillin allergy	See above but plus two weeks gentamicin 3 mg/kg iv once daily	
Prosthetic valve MIC <0.1 mg/l and MIC ≥0.1 and <0.5 mg/l		
Penicillin *and*	6 dd 2.10^6 IU iv	6 weeks
Gentamicin	3 mg/kg iv once daily	2 weeks

**Requirements for two-week treatment: 1. MIC penicillin <0.1 mg/l, 2. no contraindications or high resistance against aminoglycosides, 3. no cardiovascular risk factors such as heart failure, aortal insufficiency or disturbed conductance, 4. no thromboembolitic complications, 5. native valve, 6. no vegetations >5 mm, 7. clinical response within seven days, 8. duration of clinical phenomena <3 months, 9. no relapse of the endocarditis.*
***Cephalosporins only in the event of a mild (see text) penicillin allergy (cross reactivity).*

Table 4
Treatment of endocarditis due to enterococci or viridans streptococci and S. bovis with an MIC >0.5 mg/l

ANTIBIOTIC	DOSE	DURATION
Native valve		
Penicillin *and*	2.10^6 IU iv every 4 h	4-6 weeks*
Gentamicin *or*	3 mg/kg iv once daily	4-6 weeks**
Amoxicillin (1st choice with enterococci) *and*	2 g iv every 4 h	4-6 weeks*
Gentamicin	3 mg/kg iv once daily	4-6 weeks**
Penicillin allergy		
Vancomycin *and*	15 mg/kg iv every 12 h (max. 1 g every 12 h)	4-6 weeks*
Gentamicin	3 mg/kg iv once daily	4-6 weeks**
High gentamicin resistance (MIC >500 mg/l)		
Amoxicillin *or*	2 g iv every 4 h	8-12 weeks
Vancomycin	15 mg/kg iv every 12 h (max. 1 g every 12 h)	8-12 weeks
Prosthetic valve		
Amoxicillin	2 g iv every 4 h 6 dd 2 g iv	6-8 weeks
Gentamicin	3 mg/kg iv once daily	6 weeks**
β-*lactamase formation*		
Amoxicillin-clavulanate acid *and*	2000/200 mg iv every 4 h	6-8 weeks
Gentamicin	3 mg/kg iv once daily	6 weeks**
High gentamicin resistance (MIC >500 mg/l)		
Amoxicillin	2 g iv every 4 h	8-12 weeks

**Six weeks of treatment when the infection exists for more than three months, in the event of complications (e.g. septic embolisms) and relapse of infection.*
***There are indications that the period of administration of aminoglycosides can be shortened.[54]*

For the treatment of patients with endocarditis due to streptococci with a prosthetic valve or a known allergy to penicillin, see *table 3*.
Less common streptococci that also cause endocarditis are *S. pneumoniae, S. pyogenes* and group B, C, and G streptococci. *S. pneumoniae* has become a rare cause of endocarditis since the introduction of penicillin (less than 2%).[2,46] The course is highly fulminating and is accompanied by meningitis in 60% of cases. Most of the pneumococci in the Netherlands are sensitive to penicillin.[47] Endocarditis due to β-haemolytic streptococci is not very common but is an acute disease with a high morbidity and mortality. Group A streptococci (*S. pyogenes*) are quite sensitive to penicillin and the preferred treatment therefore consists of penicillin (6 million U/4 hours iv) for four to six weeks. Alternatives to penicillin are first-generation cephalosporins, vancomycin or teicoplanin. No data are available on the value of including clindamycin in the treatment of endocarditis, in contrast to the streptococcal toxic shock syndrome. Group B, C and G streptococci are in general not as sensitive to penicillin as group A streptococci. Some experts recommend, therefore, that gentamicin should also be administered during the first two weeks of treatment of these causative agents (level of proof D).

ENTEROCOCCI

Enterococci were formerly included in the genus *Streptococcus* but are now classified separately under the genus *Enterococcus*. There are at least 12 species, *E. faecalis* being the most important clinically followed by *E. faecium*. In a Dutch study, 35 of 40 (87.5%) patients with endocarditis caused by enterococci had *E. faecalis* and three had *E. faecium*.[7] This is in agreement with the findings of an American study of enterococcal endocarditis[48] and is approximately equal to the species distribution in nosocomial enterococcal bacteraemias.[49] Enterococci are part of the normal flora of the digestive tract and the proximal part of the urethra. They cause about 10% of cases of IE, especially among men over 60 and women who have recently delivered a child or undergone abortion.[49] Both normal and damaged cardiac valves can be affected by this group of bacteria.[48,50] The mortality for endocarditis due to enterococci is 25%, which is much higher than that for endocarditis due to viridans streptococci (6%).[7] Intrinsically, enterococci are relatively resistant to penicillin with a median MIC of 2 mg/l, sensitive to amoxicillin and totally resistant to cephalosporins. The β-lactam antibiotics only have a bacteriostatic effect on enterococci so that treatment in the form of monotherapy usually fails (*table 4*). All enterococci are resistant to the standard dose of aminoglycosides.[50] However, gentamicin and streptomycin do have a clear synergistic effect when added to a treatment regimen with penicillin, amoxicillin or vancomycin so that *in vitro* the combination has a bactericidal effect.[40,50,52-57] Streptomycin and gentamicin cannot simply be replaced by other aminoglycosides. Tobramycin, for example, does not have a synergistic effect when administered in combination with penicillin.[53]

In general, it is recommended that the aminoglycosides are added to the antimicrobial regimen during the entire period of treatment. Because enterococcal endocarditis occurs more frequently among elderly patients, prolonged administration of aminoglycosides can sometimes lead to problems related to nephrotoxic and ototoxic complications.

A recently published observational Swedish study indicated that a proportion of the patients can also be cured with a short-term supplementary course of aminoglycosides.[54] Resistance against aminoglycosides is variable, an MIC of 500 mg/l is usually taken as the cut-off point between low and high resistance. In a Dutch investigation, approximately 10% (4/40) of enterococci isolated from patients with endocarditis exhibited high resistance against gentamicin and amikacin, whereas only one of the 40 isolates showed high resistance against streptomycin.[7] For these high-resistant bacteria it is not worthwhile to add an aminoglycoside because the synergetic effect no longer occurs.

STAPHYLOCOCCI

Endocarditis can be caused by coagulase-positive (*S. aureus*) and by coagulase-negative (including *S. epidermidis*) staphylococci. Endocarditis caused by coagulase-negative staphylococci (CNS) occurs particularly in patients with a prosthetic valve and to a much lesser extent in patients with a native valve. The treatment of staphylococcal endocarditis differs according to the location of the infection (right-sided or left-sided) and the presence or absence of artificial material. Endocarditis caused by *Staphylococcus aureus* differs in the negative sense from endocarditis due to other causative agents in the high mortality and the frequent occurrence of complications in and outside the heart.[59,60] The treatment of staphylococcal endocarditis of the native valve is given in *table 5*. As a result of β-lactam formation, approximately 90% of the *S. aureus* strains isolated in the Netherlands are not susceptible to penicillin but they are sensitive to the semisynthetic penicillinase-resistant penicillins such as floxacillin. Combination of a β-lactam antibiotic with aminoglycosides is controversial because it does not decrease the morbidity and mortality of *S. aureus* infections, yet there is more nephrotoxicity (level of proof A2).[61-63] By adding an aminoglycoside the patient becomes free of fever much sooner and the blood

Table 5
Treatment of native valve endocarditis caused by staphylococci (coagulase-positive and coagulase-negative)

ANTIBIOTIC	DOSE	DURATION
Left-sided		
PSSA[*] of PS coagulase-negative staphylococci		
Penicillin *and*	2.10^6 IU iv every 4 h	4-6 weeks[$]
Gentamicin	3 mg/kg iv once daily	3-5 days
MSSA[#] of MS coagulase-negative staphylococci		
Flucloxacillin *and*	2 g iv every 4 h	4-6 weeks[$]
Gentamicin	3 mg/kg iv once daily	3-5 days
MRSA[‡] of MR coagulase-negative staphylococci or penicillin hypersensitivity		
Vancomycin *and*	15 mg/kg iv every 12 h (max. 1 g every 12 h)	4-6 weeks[$]
Gentamicin	3 mg/kg iv once daily	3-5 days
Right-sided		
PSSA[*]		
Penicillin *and*	2.10^6 IU iv every 4 h	2-4 weeks[&]
(possibly with gentamicin)	3 mg/kg iv once daily	2 weeks
MSSA[#]		
Flucloxacillin	2 g iv every 4 h	2-4 weeks[&]
(possibly with gentamicin)	3 mg/kg iv once daily	2 weeks[&]
MRSA[‡] or penicillin hypersensitivity		
Vancomycin *and*	15 mg/kg iv every 12 h (max. 1 g every 12 h)	4 weeks
Gentamicin	3 mg/kg iv once daily	3-5 days

[*] PSSA = penicillin sensitive S. aureus, [#] MSSA = meticillin sensitive S. aureus, [‡] MRSA = meticillin resistant S. aureus, [$] in case of metastatic infections and poor clinical reaction to initial therapy: 6 weeks, [&] prerequisite for two-week treatment: no septic embolisms outside of the lungs, no severe pulmonary embolisms, no combination of right-sided and left-sided IE, no high aminoglycoside resistance.

cultures are negative sooner.[64] For this reason, it is usually recommended that an aminoglycoside be included in the therapeutic regimen for the first three to five days (level of proof D). In the event of contraindications for aminoglycosides or resistance, some authors recommend including fusidic acid in the treatment protocol; data on the effectiveness of this combination are, however, limited.[65,66] In the event of a light or mild penicillin allergy (gastrointestinal complaints or exanthema after use of amoxicillin/ ampicillin), a cephalosporin can be chosen.[67] Cephazolin, for instance, is effective as antistaphylococcal agent. In the event of a severe penicillin allergy (IgE-mediated, type 1 allergy or other types of allergy involving the organs or with fever) vancomycin is to be preferred above teicoplanin because it is more effective.[68-70] Coagulase-negative staphylococci and *S. aureus* are usually quite sensitive to rifampicin but in the case of monotherapy, resistance develops quickly.[68] Rifampicin penetrates well into tissues and abscesses and also penetrates into the biofilm on artificial materials. When administered in combination with other antibiotics, synergism, antagonism and a lack of any extra effect have all been described. In certain cases, especially for patients who react poorly to the therapy instituted, rifampicin is sometimes added to the therapeutic regimen. The duration of treatment for left-sided endocarditis caused by staphylococci on a native valve is four weeks; in the case of metastatic infections or a poor reaction to initial therapy this must be prolonged to six weeks (level of proof D). The choice of the antibiotic is dependent on the sensitivity of the micro-organism (see *table 5*). In principle the same rules apply to the treatment of coagulase-positive and coagulase-negative strains.

Right-sided staphylococcal endocarditis of the native valve, encountered mainly among drug-users, is a milder disease with a much lower mortality (5-10%) than left-sided endocarditis (25-40%). Patients with right-sided endocarditis are often young and usually do not exhibit severe comorbidity; usually there are no metastatic infections outside of the lungs and there is rarely a problem of heart failure so that surgical intervention is, in general, not necessary. Various studies have been carried out to investigate the possibility of shorter and/or oral treatment of right-sided endocarditis.[31,32,63,72-74] Intravenous treatment for 14 days with only a penicillinase-resistant penicillin, such as floxacillin, appears to be an effective therapy for this form of endocarditis (*table 5*) (level of proof B). Prerequisites for this two-week therapeutic course are that there are no septic embolisms outside of the lung, there are no severe pulmonary problems (such as empyema) and the endocarditis is localised only in the right side of the heart. To satisfy the first prerequisite, history-taking and the physical examination of these patients must be directed specifically toward the discovery of eventual signs of metastatic infections and – when necessary – supplementary imaging techniques must be carried out. In the event of allergy to penicillin, vancomycin or teicoplanin can be used as alternative. Both drugs are, however, less effective against *S. aureus* so that in this case treatment must last for four weeks (level of proof B).[68,69,72,75] A study of patients with IE caused by methicillin-resistant *S. aureus* treated with vancomycin showed a clearly delayed response with a longer period of fever (median 7 days) and bacteraemia (median 9 days).[75,76] Addition of rifampicin did not improve the response.

Prosthetic valve endocarditis caused by staphylococci can, as previously mentioned, be subdivided into early and late PVE. In early PVE due to staphylococci, perivalvular abscesses and valve dysfunction often develop and then both antimicrobial and surgical therapy are needed.

The therapies recommended for PVE are presented in *table 6*.[77-79]

The mortality for prosthetic valve IE due to *S. aureus* is high. For this reason, treatment during the first two weeks is usually combined with gentamicin to be on the safe side. Data on this aspect are lacking.

Table 6
Treatment of prosthetic valve endocarditis caused by staphylococci

ANTIBIOTIC	DOSE	DURATION
S. aureus		
Flucloxacillin *and*	2 g iv every 4 h	6 weeks
Gentamicin *and*	3 mg/kg iv once daily	2 weeks
Rifampicin	600 mg orally every 12 h	6 weeks
Coagulase-negative staphylococci		
Vancomycin *and*	15 mg/kg iv every 12 h (max. 1 g every 12 h)	6 weeks
Gentamicin *and*	3 mg/kg iv once daily	2 weeks
Rifampicin	600 mg orally every 12 h	6 weeks
MRSA		
Vancomycin *and*	15 mg/kg iv every 12 h (max. 1 g every 12 h)	6-8 weeks
Gentamicin *and*	3 mg/kg iv once daily	2 weeks
Rifampicin	600 mg orally every 12 h	6-8 weeks

HACEK MICRO-ORGANISMS

HACEK micro-organisms (*Haemophilus* species, *Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella* species) are slow-growing Gram-negative bacteria. As a result it often takes a long time for the blood culture to become positive. When the primary source is unknown, the growth of these bacteria from blood is highly suggestive of the diagnosis of endocarditis, even when clinical signs of endocarditis are absent.[16] Because β-lactam-producing strains have recently been identified and it is sometimes difficult to determine the resistance of these micro-organisms, some authors recommend empirical treatment with third-generation cephalosporins (such as cefotaxime or ceftriaxone) with adjustment of the therapy based on the antibiogram (level of proof D).[40] When amoxicillin is used, gentamicin must be added for the same reason (see *table 7*). In the event of hypersensitivity to penicillin, the choice of antibiotic is difficult since only a few case reports on alternative therapies have been published. *In vitro* the HACEK micro-organisms are susceptible to cotrimoxazole and fluorchinolone.

Table 7
Treatment of endocarditis caused by HACEK micro-organisms

ANTIBIOTIC	DOSE	DURATION
Native valve		
Ceftriaxone *or*	2 g iv once daily	4 weeks
Cefotaxine *or*	2 g iv every 8 h	4 weeks
Amoxicillin *and*	2 g iv every 4 h	4 weeks
Gentamicin*	3 mg/kg iv once daily	4 weeks
Artificial valve		
Ceftriaxone *or*	2 g iv once daily	6 weeks
Cefotaxine	2 g iv every 8 h	6 weeks

**Gentamicin need not be administered if it is known that the strain does not produce β-lactamase.*

NOTE

A brief Dutch version of this paper will appear in Nederlands Tijdschrift voor Geneeskunde.

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LETTER TO THE EDITOR

Renal replacement therapy for acute renal failure on the ICU: coming of age?

With interest I read the review article by Van Bommel about renal replacement therapy for acute renal failure (ARF) on the intensive care unit, in the Netherlands Journal of Medicine no. 8.[1] Because most intensive care patients with ARF are treated in non-academic ICUs without fulltime intensivist staffing, the article may be useful for many doctors who treat critically ill patients, especially those without specific nephrology or intensive care training. Although it is impossible to review and include all the published papers concerning the various aspects of continuous renal replacement therapy (CRRT), I have some comments.
Firstly, the author states that 'ongoing anticoagulation is needed with CRRT to prevent clotting of the extracorporeal circuit' and 'contrary to CRRT, intermittent haemodialysis can be safely and adequately performed with or without anticoagulation'. Several authors have investigated the safety and operative efficacy of continuous venovenous haemofiltration (CVVH) without anticoagulation in patients at high risk of bleeding. In a prospective cohort study in patients with ARF undergoing CVVH at 2000 ml/h, Tan et al. compared 40 haemofiltration circuits (in 12 patients) without anticoagulation and 40 control circuits (in 14 patients) treated with low-dose pre-filter heparin infusion.[2] In patients receiving CVVH without anticoagulation mean circuit life was 32 h (95% CI: 20-44.4) and 43% of the filters lasted longer than 30 h. Circuit lifespan did not correlate with international normalised ratio (INR), activated partial thromboplastin time (aPTT) or platelet count. There were no bleeding complications and azotaemic control was not compromised by lack of circuit anticoagulation during treatment. A control group of consecutive similarly ill patients not at high risk of bleeding received low-dose pre-filter heparin (mean dose 716 IU; 95% CI: 647-785). Their mean filter life was 19.5 h (95% CI: 14.2-23.8), significantly shorter than in the study patients (p=0.017). In 1993, Bellomo et al. found similar results: in patients assessed to be at high risk of bleeding, CVVHD without anticoagulation provided a mean filter survival of 40.9 h (95% CI 27-54.8 h).[3] Therefore, critically ill patients at high risk of bleeding who require CVVH(D) can be safely managed without circuit anticoagulation. This strategy minimises bleeding risks and is associated with acceptable filter survival.
Likewise, another elegant technique of anticoagulation during CVVH is not mentioned: the use of prostacyclin. Although this technique is not widely used today, positive results were already reported in the 1980s.[4] Prostacyclin, either alone or in combination with heparin, resulted in enhanced filter survival without an increased risk of bleeding complications.[5,6] Prostacyclin, combined with low-dose heparin, inhibits platelet reactivity and preserves haemofilter life dose-dependently.[7] A recent study shows that prostacyclin as the sole antihaemostatic agent during CVVH entails a low risk of haemorrhagic complications, while maintaining the patency of the circuit long enough to allow the delivery of an adequate dose of renal replacement therapy.[8]

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REACTION FROM THE AUTHOR

Van de Klooster comments on the suggested need for ongoing anticoagulation with CRRT (continuous renal replacement therapy) to prevent clotting of the extracorporeal system and on the use of intermittent haemodialysis (IHD) as a safe and adequate alternative for CRRT in patients at high risk of bleeding in the absence of a citrate anticoagulation protocol.[1] He also provides some data on alternative anticoagulation methods for use in patients at high risk of bleeding. I did not intend to provide a comprehensive review of all the various aspects of CRRT on the ICU. Instead, I focused on the prescription of adequate dialysis in terms of its timing and intensity. Therefore, pros and cons of the different anticoagulation regimes fell beyond the scope of my article and the interested reader was referred to some recent reviews concerning this aspect.[1] In general, filter clotting occurs unacceptably frequent unless ongoing and adequate anticoagulation is used with CRRT.[2,3] In selected patients (i.e., those with a bleeding tendency), CRRT can sometimes be performed without anticoagulation with acceptable filter life without increasing the haemorrhagic risk.[2,3]
As far as I know, the most frequently used anticoagulation methods in the Netherlands are (low-dose) heparin, followed by (fixed-dose) LMW heparin and citrate. Many centres do not have an alternative anticoagulation regimen other then 'heparin-free' CRRT. One should recognise the increased workload and need for strict adherence to existing protocols for the ICU or dialysis nurse with this method: hourly saline flushes, repeated venous chambre (air trap) and filter inspection, adequate and prompt handling of changes in TMP, arterial and venous pressures. In addition, impending signs of clotting may require (repeated) pre-emptive replacement of filter and lines because with clotting, blood loss may be up to 250 cc. Those familiar with IHD will immediately recognise its use without anticoagulation in patients at high risk of bleeding, whether performed on the ICU or on the dialysis unit. As such (and when available), it may be an alternative for CRRT in the patient who is at high risk of bleeding and where there is no readily available alternative anticoagulation regimen for heparin (e.g., following repeated filter clotting with nonanticoagulated CRRT). Because even tightly controlled systemic heparin contributes to an increased haemorrhagic risk,[2,3] its routine use as the first choice for anticoagulation with CRRT may no longer be defensible. In this regard, it is noteworthy that an increasing number of Dutch centres are replacing heparin by citrate anticoagulation as the first-line anticoagulation method with CRRT. *Van de Klooster* also refers to the use of prostacyclin (PGI_2), alone or in combination with heparin, as an alternative and effective method of anticoagulation with CRRT. However, adverse haemodynamic effects of the use of PGI_2 with CRRT have been reported.[4,5] Therefore, some have used PGI_2 analogues to avoid these potentially adverse effects.[6] Nevertheless, although first positive results stem from almost two decades ago, data for both PGI_2 or its analogues are still limited. Moreover, the method is complicated, requires close monitoring of platelet function and is very expensive.[7] Therefore, this method can not yet be recommended for routine use. While recognising the availability of alternative and safer methods for anticoagulation with CRRT in patients at high risk of bleeding, as long as (LMW) heparin is the most frequently used method, one should recognise that heparin-free IHD is a safe and adequate alternative in such patients.

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ANSWER TO PHOTO QUIZ (ON PAGE 413)

A MAN WITH A BULGING MASS IN HIS LEFT LUMBAR REGION

DIAGNOSIS

The clinical diagnosis of a freely reducible spontaneously acquired lumbar hernia was made and subsequently confirmed by ultrasound (*figure 2*). In accordance with the patient's wishes, no surgical intervention was performed.
Lumbar (or dorsal) hernias are anatomically divided into Petit's hernias (through the inferior lumbar triangle), Grynfeltt's hernias (through the superior lumbar triangle) or diffuse hernias.[1] Based on their aetiology they are either congenital, post-traumatic, iatrogenic or spontaneous.[2] The diagnosis can be made clinically and confirmed by ultrasound or computed tomography.[2-4] These hernias usually present with a flank mass, abdominal or back pain, as well as abdominal distension and vomiting.[4] Because the neck of the hernia is usually wide, strangulation is uncommon and is reported to occur in about 10% of cases.[2,4]

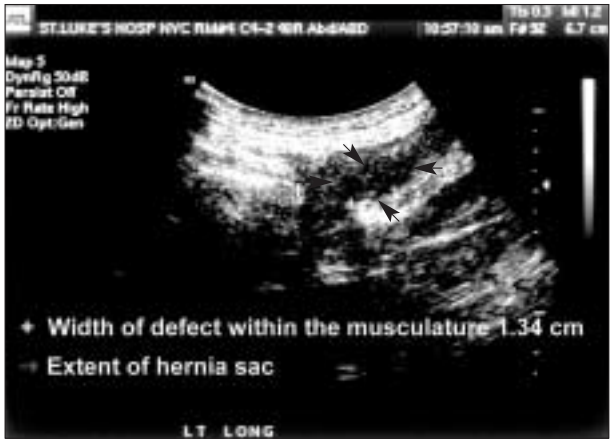

Figure 2
Longitudinal image of the clinically evident mass with the patient in prone position

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ABOUT THE COVER

Summertime Jazz

Tim Hinterding

Tim Hinterding (1952) is the artist of the cover of this December issue of the Netherlands Journal of Medicine. He lives and works in Vorden. Tim studied at the Academy of Art in Arnhem. After his study he attended a workshop 'Heliogravure' by Johan de Zoete. Besides making graphic art, Hinterding is a succesfull painter whose work has been exposed in many exhibitions. On occassion he publishes his graphic work in combination with poetry and literary texts through his own publisher Killa Kossa.

His work can be seen in several galleries; such as Clement in Amsterdam, Kunst+ in Vlaardingen, PARC in Lent, Demedici in Putten, Von Oberbach in Arnhem, Jansen and Kooy in Wanrnsveld, Business Art Service in Raamsdonksveer and Galerie Albion Putti in Groningen.
An original print of this months' cover is available at a price of € 225, at Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl.

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Examples:

[1.] Smilde TJ, Wissen S van, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med 2001;59:184-95.

[2.] Kaplan NM. Clinical Hypertension. 7th Edition. Baltimore: Williams & Wilkins; 1998.

[3.] Powell LW, Isselbacher KJ. Hemochromatosis. In: Harrison's Principles of Internal Medicine, 15th Edition, Braunwald E, Fauci AS, Kasper DL, et al. (eds). New York: McGraw-Hill; 2001. p. 2257-61.

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