Microalbuminuria and Hypertension in the HIV-Positive Population of Oslo (MAHO Study)

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2009

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Series of dissertations submitted to the
Faculty of Medicine, University of Oslo
No. 865

ISBN 978-82-8072-575-2

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Cover: Inger Sandved Anfinsen.
Printed in Norway: AiT e-dit AS, Oslo, 2009.

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CONTENTS

Abstract

Background: The survival of human immunodeficiency virus (HIV) -infected patients has increased significantly since the introduction of combination antiretroviral therapy, leading to the development of important long-term complications including cardiovascular and renal disease. Microalbuminuria, an indicator of early glomerular injury, and hypertension is associated with an increased risk of progressive renal deterioration, cardiovascular disease and mortality. There is a scarcity of data regarding prevalence of microalbuminuria and hypertension in HIV-infected individuals. Although causal blood pressure is linked to cardiovascular complications, ambulatory blood pressure and in particular nondipping pattern is more closely associated with hypertensive target organ damage and mortality in hypertensive individuals without HIV-infection. Furthermore, there are no data available with regard to ambulatory blood pressure in HIV-infected individuals.

Aims of the thesis: To investigate the prevalence of microalbuminuria in HIV-infected individuals, compare the prevalence in Caucasians in the HIV-infected population with the general population, and identify possible predictors of microalbuminuria.

To investigate the prevalence of hypertension in HIV-infected individuals, compare the prevalence in Caucasians in the HIV-infected population with the general population, and indentify possible predictors of hypertension.

To describe ambulatory blood pressure in hypertensive, but untreated HIV-infected individuals, and compare the circadian blood pressure rhythm with that of HIV-uninfected controls and identify possible predictors of circadian blood pressure pattern.

Methods: Patients were enrolled in an unselected manner from the outpatient clinic of the department of infectious diseases, Ullevål University Hospital, Oslo, Norway between March 2004 and November 2005 (n = 597). General data was obtained from the study questionnaire, local HIV database and patient record. When the database was closed in January 2007, 542 individuals had completed the study.

Based on two or three urine samples, collected at separated clinical visits, microalbuminuria was defined according to albumin creatinine ratio between 2.5 and 30 mg/mmol. The nonhypertensive, nondiabetic HIV-positive cohort (n = 495) constituted the study sample

for paper I, and the Caucasian share was compared with a population-based nonhypertensive, nondiabetic control group (n = 2091).

Blood pressure was measured under standardized circumstances in duplicate at three consecutive clinical visits. Hypertension was defined according to the guidelines of the European Society of Hypertension. The study sample was compared with an age-, sex- and body mass index matched population-based control group (n = 24968, paper II).

Based on the casual blood pressure measurements, ambulatory blood pressure over 24h was carried out in 77 newly diagnosed untreated hypertensive HIV-positive individuals and compared with 76 HIV-uninfected untreated hypertensive controls (paper III).

Results: The prevalence of microalbuminuria was 8.7% in the nonhypertensive, nondiabetic HIV-infected cohort, which is for the Caucasian fraction, depending on different age groups, 2–5 times higher than in the general population. Combination antiretroviral treatment did not affect the prevalence of microalbuminuria. In multivariate analysis, systolic blood pressure ($P < 0.0001$), serum beta 2-microglobulin ($P = 0.001$) and duration of HIV infection ($P = 0.019$) were found to be independent predictors of microalbuminuria.

The prevalence of hypertension among the Caucasian HIV-infected subjects was 36.5%, which was not significantly different from the general population. The mean diastolic blood pressure was significantly higher in both HIV-infected men and women (only for those with BMI < 25 kg/m^2) compared to control subjects. The highest prevalence of hypertension in entire cohort was found in those who were treated by combination antiretroviral therapy for more than five years (44.4%). Multivariate analysis revealed age ($P < 0.0001$), gender ($P = 0.002$), body mass index ($P < 0.0001$), cholesterol ($P = 0.045$), duration of combination antiretroviral therapy ($P = 0.02$) and microalbuminuria ($P = 0.003$) as independent predictors of hypertension.

Hypertensive, HIV-infected subjects had more frequently an attenuated ambulatory blood pressure rhythm with a reduced nocturnal blood pressure fall than HIV-negative control subjects (60% vs. 33%, respectively; $P = 0.001$). Multiple logistic regression analysis with dipping pattern, i.e. normal nocturnal fall in blood pressure as dependent variable showed that HIV status, was an independent predictor of nondipping blood pressure ($P = 0.002$), while combination antiretroviral therapy had no predictive value.

Conclusions: Our findings indicate that inflammatory activity induced by HIV *per se* may be involved in the development of microalbuminuria, high blood pressure levels and attenuated blood pressure rhythm. Cardiovascular risk in the HIV-infected population seems to be influenced by a composite of the duration of both cART treatment and HIV infection. With respect to the increasing risk of developing cardiovascular, renal diseases and mortality, the high prevalence of microalbuminuria and attenuated blood pressure rhythm in HIV-infected individuals warrants special attention.

Preface and Acknowledgements

Out of an unpretentious afternoon 'chatter on ward 112' of the infectious diseases at Ullevål University Hospital in spring 2003 emerged what later would become the MAHO study. Nevertheless do politicians still speculate about what we are unnecessarily debating about…

After introduction of combination antiretroviral therapy, the promising prospects for HIV-infected individuals were disturbed by evidence of enhanced cardiovascular and although less striking even renal diseases. Metabolic adverse events by the antiretroviral therapy itself were primarily suspected, and even intrinsic long-term complications analogous to diabetic patients were postulated.

The term 'microalbuminuria', a well-documented risk factor for cardiovascular and renal diseases was launched into a general afternoon discussion among infectious disease physician. In this premature phase, Olav Øktedalen supported the theory that elevated levels of microalbuminuria might be present in HIV infected individuals and motivated me to elaborate a study protocol. Finally, Oddbjørn Brubakk, the former chief of the department was confident to the idea and provided the necessary 'backup' to run this prospective cohort study at the outpatient clinic.

First of all, I would like to thank the participants of the MAHO study who have been outstanding patient and generous in their collaboration. Their sense for responsibility was the most necessary perquisite to execute the study. Furthermore, the nurses of the outpatient clinic have constituted the major pillar of the study, not only to carry out the blood pressure measurements and logistic challenges but also to motivate the study participants. Thank you Astrid Moe Rudi, Kjersti Selnes, Lise Sørsvang, Jorun Almark, Linda Skeie and Heidi Bertheussen; you did a fantastic job!

I am very grateful to my daily mentor and main supervisor, Olav Øktedalen who had to absorb my frustrations and impatience in the critical phases. His gentle advices and guidance throughout the study have been irreplaceable and without his faith the study would not have been possible.

Without a shadow of a doubt I stand in immeasurable gratitude to professor Ingrid Os. Her scientific creativity, never ending enthusiasm and imminent professional and sympathetic

support in the progress of this thesis has been phenomenal and will be essential for the MAHO study in the near future.

In this special moment, I could not forget the person who actually ‘shepherded’ me to the world of research – Prof. Ivar Eide, my first ‘boss’ at Ullevål definitely sparked off the unknown scientific curiosity in me.

This study was carried out at the Department of Infectious Disease and I want to thank profoundly all my colleagues of the outpatient clinic for the inclusion of study participants and meticulous completion of the questionnaire.

Furthermore, I want to express my appreciation to the following for their contribution to the MAHO study:

- Professor Leiv Sandvik for his always-entertaining statistical and scientific support to the papers.
- Sigrun Elden of the Department of Nephrology, for her professional assistance with ambulatory blood pressure measurement.
- Aud Stenehjem for the valuable provision of the control group data and collaboration in paper III.
- Professor Jostein Holmen for the kindly provision of the data from the population survey in the Health Study of Nord-Trøndelag County, Norway (paper I and II).
- The department of clinical chemistry for the promptly analysis of the urine samples.
- Professor Johan Bruun for the establishment of the HIV database.
- Oslo University Hospital, Ullevål for the opportunity to work under perfect research conditions in this fantastic environment.

In spite of everything, my sincere and deepest thoughts go to all of them who today do not benefit from the most basic scientific medical advances.

Abbreviations and glossary

ABP	ambulatory blood pressure
ACE	angiotensin-converting enzyme
ACR	albumin/creatinine ratio
AIDS	acquired immunodeficiency syndrome
ARA	angiotensin-receptor antagonist
BMI	body mass index
BP	blood pressure
cART	combination antiretroviral therapy
CVD	cardiovascular disease
CKD	chronic kidney disease
CRP	C-reactive protein
DAD study	Data Collection on Adverse events of Anti-HIV Drugs
DBP	diastolic blood pressure
ESRD	end-stage renal disease
GFR	glomerular filtration rate
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
HIVAN	HIV associated nephropathy
HT	hypertension
MA	microalbuminuria
MDRD	Modification of Diet in Renal Disease
HUNT study	Health Study of Nord-Trøndelag County, Norway
NRTI	nucleoside reverse transcriptase inhibitor
NNRTI	non-nucleoside transcriptase inhibitor
PI	protease inhibitor
SBP	systolic blood pressure

List of papers

I. Baekken Morten, Os Ingrid, Sandvik Leiv, Oektedalen Olav.
Microalbuminuria associated with indicators of inflammatory activity in an HIV-positive population.
Nephrology Dialysis Transplantation. 2008;23:3130-3137.

II. Baekken Morten, Os Ingrid, Sandvik Leiv, Oektedalen Olav.
Hypertension in an urban HIV-positive population compared with the general population: influence of combination antiretroviral therapy.
Journal of Hypertension. 2008;26:2126-2133.

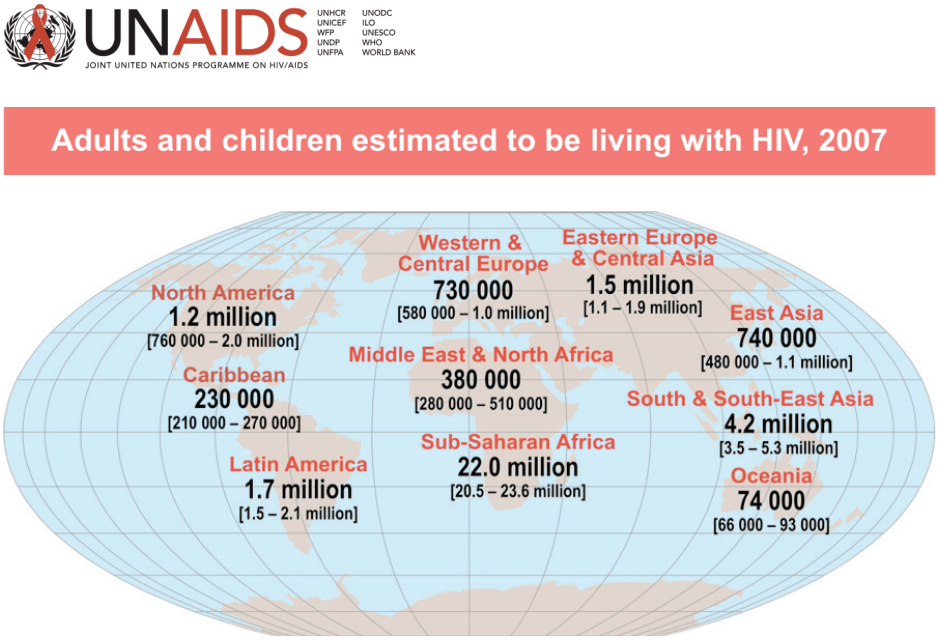
III. Baekken Morten, Os Ingrid, Stenehjem Aud, Sandvik Leiv, Oektedalen Olav.
Association between HIV infection and attenuated diurnal blood pressure rhythm in untreated hypertensive individuals.
HIV Medicine. 2009;10:44-52.

1. Background

1.1 Human Immunodeficiency Virus Infection

In 1981 the first cases of acquired immune deficiency syndrome (AIDS) were described.[1] Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate or breast milk. The four major routes of transmission are unsafe sex, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth (Vertical transmission). HIV has been found at low concentrations in the saliva, tears and urine of infected individuals, but there are no recorded cases of infection by these secretions and the potential risk of transmission is negligible. Furthermore, the infectiosity of HIV is strongly related to the viral load in the blood of the patients. There are two strains of HIV known to exist: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered; it is more virulent, relatively easily transmitted, and is the cause of the majority of HIV infections globally. HIV-2 is less transmittable and is largely confined to West Africa. Shortly after, the HIV was identified as the cause of AIDS, and preventive campaigns emerged all over the industrialised world in order to condition our sexual behaviour. Africa, the epicentre of the epidemic did not respond to it before the 90s, and even in recent years irresponsible political behaviour and unethical economic interests do still appear on the agenda. Despite an unprecedented global commitment, the number of people living with HIV is increasing and less than 1 of 4 who is in need of treatment does receive appropriate therapy, and this affects especially children. Today approximately 33 million individuals (Fig. 1) are infected and due to high viral load are many of them highly contagious, leading to a prevalence that have reached up to 25% among the population between 15-49 years in certain localities.[2] In the countries most heavily affected, HIV has reduced life expectancy by more than 20 years, slowed economic growth, and deepened household poverty. In sub-Saharan Africa alone, the epidemic has orphaned nearly 12 million children aged less than 18 years. According to the United Nations Development Programme, HIV has inflicted the “single greatest reversal in human development” in modern history. Globally transmission modes remain predominantly heterosexual contact, sex work and vertical transmission during and shortly after pregnancy, while in the industrialised world men who have sex with men and intravenous drug abuse account for the majority of new cases of HIV infection.

Fig. 1



Until 1987, when the first antiretroviral therapy trials were initiated, patients with HIV infection died after few years, predominantly by opportunistic infections. The mono-therapy with zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI), and later duo-therapy was finally followed in 1996 by the combination of at least three different drugs, phrased 'highly active antiretroviral therapy' (HAART) combining two NRTI's with either a protease inhibitor (PI) or a non nucleoside transcriptase inhibitor (NNRTI). Recently two new groups, the fusion- and integrase inhibitors have been added to the repertoire to meet the increasing challenge of therapy resistance. Where available, these medications have led to eminent mortality reduction in HIV cohorts all over the world,[3] reaching even mortality rates comparable to the general population.[4] However, since there is no medication without side effect, combination antiretroviral therapy (cART) has shown to induce metabolic abnormalities, such as increased triglyceride and cholesterol levels, insulin resistance and lipodystrophy,[5] in particular with PI. Consequently, HIV infection became a chronic, manageable and controllable disease. Other non-AIDS defined maladies, like malignancies,

cardiovascular, liver and renal diseases have gained increasing importance, as a result of the increased life expectancy, increased duration of HIV-infection *per se* and/or the inevitable adverse events by the antiretroviral therapy.

1.1.1 Immunological implications

HIV infection is characterized by the continuous loss of CD4+ T cells and an increase in viral load, leading to immunodeficiency, opportunistic infections, and death. The stage of infection can be determined by measuring the patient's CD4+ T cell count, and the viral load of HIV in the blood.

HIV infection has basically four stages: incubation period, acute infection, latency stage and AIDS. The initial incubation period upon infection is asymptomatic and usually lasts between two and four weeks. The second stage is an acute infection, which lasts on average 28 days and can include symptoms like fever, lymphadenopathy, pharyngitis, rash, myalgia, malaise, and mouth and oesophageal sores. The latency stage shows few or no symptoms and can last anywhere from two weeks to twenty years and beyond, before reaching AIDS, the fourth and final stage of HIV infection characterized by complicating opportunistic infections or tumors.

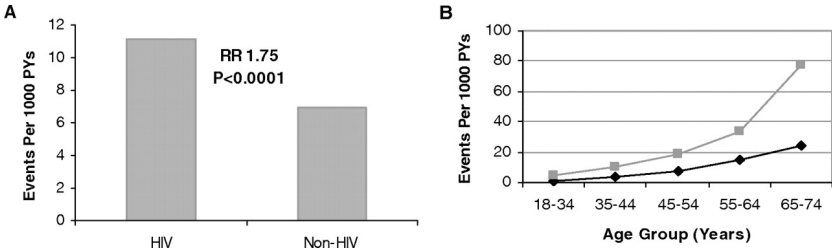
HIV primarily infects vital cells in the human immune system such as helper T cells (specifically CD4+ T cells), macrophages, and dendritic cells. The main cell target is the CCR5+CD4+ T cell (Th 17+), predominantly found in the mucosal lymphoid tissues, such as the gastrointestinal tract, but direct infection of other organs, such as central nervous system, heart and kidneys have been reported. Additionally, HIV infection leads to low serum levels of CD4+ T cells through three main mechanisms in the lymph nodes and thymus: firstly, direct viral killing of infected cells; secondly, increased rates of apoptosis in infected cells; and thirdly, killing of infected CD4+ T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+ T cell numbers decline below a critical level ($<200/mm^3$), cell-mediated immunity is severely decreased, and the body becomes progressively more susceptible to opportunistic infections. Thus, in asymptomatic individuals with low viral load (<100,000 copies/ml), cART is initiated in the industrialized world at CD4 $<350/mm^3$.[6] CART increases the number of CD4 cells and suppresses viral load. The initial phase of 3-6 month after starting treatment is characterized by a rapid increase of CD4 cell count through release of memory cells trapped in the lymphoid system,

while in the second slower phase additional naïve cells from the thymus contribute to the reconstitution of the immune system. Recovery can be overwhelming, and lead to the immune reconstitution inflammatory syndrome (IRIS), an inflammatory response towards either an occult infection or paradoxical symptomatic relapse of a prior treated infection. The CD4 cell range reached by treated patients strongly depends on CD4 cell values at baseline, age, duration of HIV infection and viral suppression, and one-third will not reach a normal CD4 cell counts >500/mm^3 after 5 years of treatment despite continuous viral suppression.

1.1.2 Cardiovascular risk/disease in the era of cART

Immediately after the introduction of combination antiretroviral therapy (cART), case reports of myocardial infarction in young patients infected with HIV sparked interests in the relationship between HIV infection and cardiovascular disease.[7] The first to report an association between cART/PI exposure and myocardial infraction was based on the French hospital HIV database.[8] The same study group confirmed later the increased risk of myocardial infarction in HIV-positive individuals compared to the general population (Fig.2).[9]

FIG. 2. *A, Myocardial infarction rates and corresponding adjusted RR. Bars indicate crude rates of AMI events per 1000 PY as determined by ICD coding. RR and associated P value are shown above the bars. RR was determined from Poisson regression analysis adjusting for age, gender, race, hypertension, diabetes, and dyslipidemia. Associated 95% CIs for RR shown are 1.51–2.02. B, Myocardial infarction rates by age group. Light line indicates patients diagnosed with HIV disease. Dark line indicates patients not diagnosed with HIV disease. Data shown include both genders. Rates represent number of events per 1000 PY as determined by ICD coding.*



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After AIDS defining events, hepatitis B or C induced liver disease and non HIV-related malignancies, cardiovascular disease (CVD) has become the fourth cause of death in well treated HIV infected cohorts.[10] Patients with HIV infection share cardiovascular risk factors

with the general population,[11] and some of them can be induced by the different components of cART.[12] Increased total cholesterol, reduced high-density lipoprotein (HDL) -cholesterol, increased triglyceride levels and a diagnosis of hypertension (HT) and diabetes are all associated with cART. Especially PI-containing cART induces dyslipidemia, insulin resistance and altered fat distribution or lipodystrophy,[5,13] while the mitochondrial dysfunction caused by the thymidine analogues stavudine and zidovudine within the NRTI's results in insulin resistance and increased incidence of diabetes.[14,15] However, besides the treatment-associated metabolic side effects, prolonged cART exposure *itself* was identified as an independent risk factor, with a 26% relative increase in the rate of myocardial infarction per year during the first four to six years of use.[16] The researchers conducting that study described later that increased exposure to PI's[11] and recent use of certain NRTI's[17] were associated with an increased risk of myocardial infarction, while no such evidence was found for NNRTI's. Interestingly, PI's have in small studies been associated with impaired vasodilatation,[18] vasomotor dysfunction[19] and increased aortic stiffness.[20] Thus, endothelial dysfunction has emerged as a putative mechanism of the enhanced arteriosclerosis in HIV-infected individuals.

The traditional cardiovascular risk factors, such as hypercholesterolemia, elevated BP and insulin resistance nowadays are seen as factors associated with increased inflammation. Moreover, several inflammatory molecules are expressed in the early stages of arteriosclerosis suggesting that it is mainly an inflammatory process. Therefore, it is not surprising to propose that chronic HIV infection itself, low-grade inflammation and immunological status are also involved in the development of CVD.[21] Unfavourable lipid changes can be induced by high HIV replication and lower CD4 count. In addition, dilated cardiomyopathy has frequently been found in even naïve HIV-positive cohorts[22,23], and up to 57% of asymptomatic individuals have ECG-abnormalities.[24] Although not in abundance, HIV infects directly the myocytes,[25] and persists within macrophages between myocardial cells even after the effective initiation of cART. These reservoir cells may exhibit HIV on their surfaces and generate progressive tissue damage through virus-induced chronic release of cytotoxic cytokines.[26]

Overall, most likely both cART and HIV contribute to the pathogenesis of CVD by mechanisms that may affect lipids, insulin sensitivity and the structural and functional vasculature. These mechanisms are not well understood and conflicting results are often

based on short-term studies, while the development of CVD is undoubtedly a long-term consequence of multifactorial aetiology.

1.1.3 Renal diseases in HIV-infected individuals

Chronic kidney disease (CKD) in its different stages is in the general population independently related to higher risk of death, development of cardiovascular diseases, and higher hospitalization rates.[27] HIV-associated nephropathy (HIVAN), first described in 1984,[28] the most common or "classical", type of HIV-associated renal diseases is characterized as a collapsing focal segmental glomerulosclerosis. HIVAN occurs almost exclusively in black individuals with an advanced stage of the HIV infection, i.e. low CD4 cell count <200 and high viremia presents with nephrotic syndrome and rapid progressive renal failure. In a large CKD cohort, HIV-infected African American individuals had a adjusted incidence of end-stage renal disease (ESRD) approximately 6.3 times greater than Caucasians and a rate similar to African American individuals with diabetes.[29] However, with prolonged survival and aging of the HIV-infected population in the industrialized world with access to cART, the spectrum of kidney disease in individuals with HIV also reflects the growing burden of co-morbid diabetes and HT,[30,31] and kidney function has been estimated to be abnormal in up to 30% of HIV-infected individuals.[32] Furthermore, different HIV-related histological forms, beside HIVAN, such as immune complex glomerulonephritides and thrombotic microangiopathy have gained attention, and are also present in non-black ethnicities. Moreover, several drugs used in the treatment of HIV, including antiretrovirals, antibacterials used to treat opportunistic infections and medications used for associated co-morbid conditions have nephrotoxic potential.[33] Among antiretroviral agents, especially indinavir and tenofovir have caused tubular disorders and interstitial nephritis,[34] and found to be associated with increased risk of chronic renal failure.[35] Regardless of the underlying histology, renal diseases in HIV-positive patients are associated with an increased risk of death.[36]

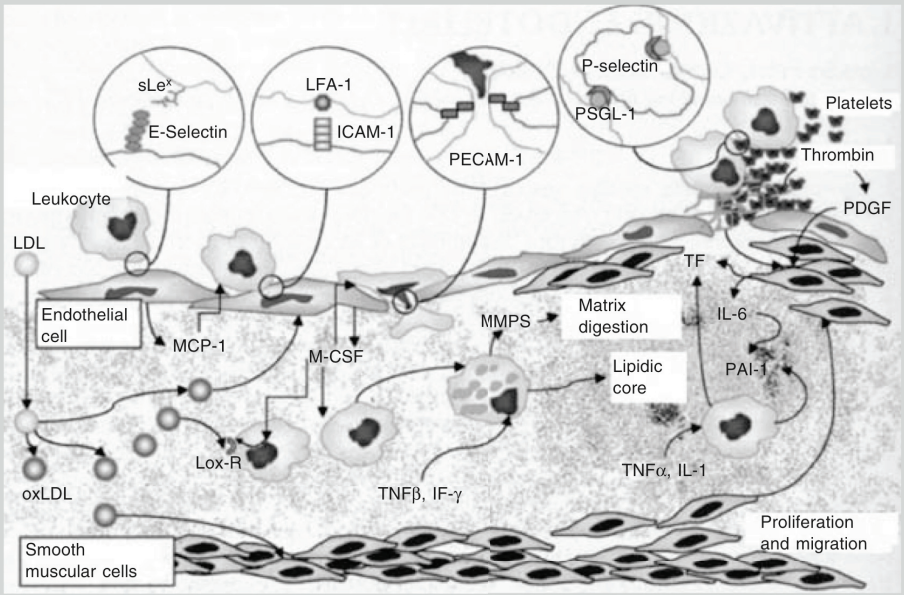
Although the increasing prevalence of chronic kidney disease in HIV-infected cohorts can be largely attributed to traditional risk factors,[37] they might in addition be caused by direct HIV infection of the renal cells, with resulting renal damage of both glomerular and tubular cells through the viral gene products.[38] Other chronic inflammatory pathways induced by HIV infection also seem to be plausible but further prospective investigations have to be

undertaken. Microalbuminuria (MA), an early marker of progressive renal diseases in the general population, is associated with inflammatory markers in patients with malignancies,[39] chronic inflammatory diseases,[40] as well as diabetes.[41] Whether MA is associated to any inflammatory process in HIV-infected individuals has previously never been observed. Nevertheless, MA was found to be a predictor of renal diseases in a selected South African HIV cohort.[42] Despite that, there is agreement that cART[43] in combination with angiotensin-converting enzyme (ACE) inhibitors[44] is the indispensable therapy of advanced HIV related renal diseases and might even be prevented.[45]

1.2 Inflammation and endothelial dysfunction

Recent research indicates that not only traditional risk factors such as lipid abnormalities, insulin resistance and high blood pressure, but also inflammatory processes are involved in the pathogenesis (Fig. 3) and progression of cardiovascular and renal diseases.[46,47] Proinflammatory cytokines can cause endothelial injury and dysfunction that leads to a less negatively charged glomerular capillary wall and subsequently to MA. In diabetic patients with MA, biomarkers of endothelial dysfunction and inflammation may predict the progression of nephropathy.[48] In addition, systemic inflammations may enhance atherogenesis, e.g. the increased incidence of CVD in patients with systemic lupus erythematosus,[49] rheumatoid arthritis, inflammatory bowel disease, HIV[7] and even periodontitis.[50] Inflammation and endothelial dysfunction might be important factors for the increasing incidence of CVD among HIV-infected patients.[51] Carotid intima media thickness, a validated marker of preclinical atherosclerosis, has been found to be increased in HIV-infected compared to uninfected individuals.[52] In that study an inflammatory mechanism associated to cytomegalovirus specific T-cell responses was identified. Maggi et al. found an astonishing similarity in endothelial plaques of the carotid arteries between patients with arthritis and HIV infection, suggesting a similar inflammatory component in HIV-infected individuals.[53] Furthermore, systemic inflammation can induce endothelial dysfunction, through a reduction of the vasodilator nitric oxide (NO), a mechanism also attributed to adverse events by certain protease inhibitors even in healthy volunteers.[19,54] In contrast, HIV causes excessive production of NO, which enhances the production of peroxynitrite[55] another contributor to endothelial dysfunction.[55]

Fig. 3 *The role of inflammation in the pathogenesis of atherosclerosis. 1) Inflammation of the endothelium stimulates the production of adhesion molecules (E-Selectin, ICAM-1, PECAM-1). 2) Chemotactic cytokines (MCP-1 and M-CSF) lead the migration of monocytes and macrophages. 3) Leukocytes turn into macrophages and activate the receptor for the oxidized LDL cholesterol. 4) TNFβ and TNFα are produced by lymphocytes. Metalloproteases are responsible for local necrosis. 5) IL-6 induces the production of tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1), that activate the coagulation and inhibit the fibrolysis.*



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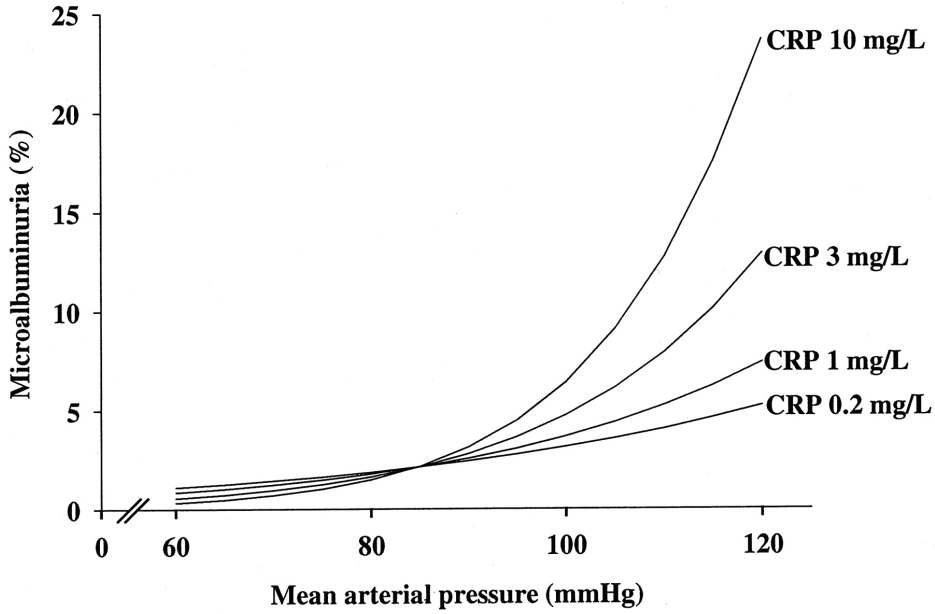
Several inflammatory markers have been identified as a putative cause for CVD in the general population, and some of them have also been found to be increased in HIV-infected individuals.[51] C-reactive protein (CRP), a marker of low-grade inflammation has been associated with CVD, hypertension and end-stage renal disease in the general population,[56,57] but this could until now not be confirmed in HIV-infected individuals. Nevertheless, CRP positively increases the association between MA and BP (Figure 4).[58] Although the level of CRP has been shown to be relatively low in various studies for a populations with ongoing HIV infection, CRP is associated with inverse CD4 cell count, progression to AIDS and mortality.[59] Thus, it has even been proposed in follow-up of HIV-infected patients in regions where antiretroviral therapies are becoming available but where frequent monitoring with viral loads and CD4 counts is still cost-prohibitive.[60] Furthermore, increased levels of marker of endothelial activation and proinflammatory cytokines, have

Fig. 4 *Graphical depiction of the interaction between CRP and mean arterial blood pressure on the estimated prevalence of microalbuminuria (multivariate design-based logistic regression analyses). Thus, this figure indicates the regression lines of a never-smoking male with an average age (48.4 years), BMI (25.9 kg/m^2), waist circumference (93 cm), glucose (4.7 mmol/L), cholesterol (5.6 mmol/L) and not using antihypertensive or lipid-lowering drugs. Predicted prevalence of microalbuminuria (%) according to levels of C-reactive protein and mean arterial blood pressure.*



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been associated with progression and severity of HIV-infection.[21,61] HIV in synergy with tumour necrosis factor-α, can bind to the endothelial cells, causing proliferation and through adhesion molecules, migration of monocytes into endothelium responsible for inflammatory cascade.[62] The SMART study, demonstrated that an increased risk of myocardial infarction was associated with IL-6 and D-dimer, in the CD4 guided treatment interruption arm compared with matched patients receiving continuous cART. Although, it might be expected that cART attenuates inflammation by reducing viral load, it is plausible that HIV *per se*, as well as the immune reconstitution process, promote premature endothelial lesions by a massive activation of inflammation molecules and cells.[63]

1.3 Microalbuminuria

The term 'microalbuminuria' first appeared in 1981, when Viberti et al. observed the presence of proteinuria below the detection limit of standard dipstick in diabetic patients and

it's predictive value for nephropathy.[64] MA occurs when the kidney leaks small amounts of albumin into the urine, secondary to glomerular endothelial injury, i.e. increased permeability for albumin in the renal glomerular barrier. The increased permeability may be a consequence of the loss of negative charged surface of the glomerular capillary wall that normally repels the negative charged proteins, intraglomerular hypertension and podocyte injuries, caused by oxidative radicals, proinflammatory cytokines and endothelial dysfunction.

Only small amounts of albumin are normally present in the urine, because it is retained in the bloodstream or reabsorbed in the tubuli of the kidneys. MA diagnosed from a 24-hour urine collection (20 to 200 μg/min), is a cumbersome and often unreliable method, while measurements of albumin concentration (MA range 30 to 300 mg/L) in spot urine is more commonly used. As there is a wide day-to-day variation, and MA is influenced by activity, fever and nutrition, MA should be measured on at least two occasions weeks to months apart. To compensate for variations in albumin urine concentration in a spot-urine, albumin is compared against the concentration of creatinine. This is termed the albumin/creatinine ratio (ACR) and MA is defined as ACR ≥2.8 mg/mmol (female) or ≥2.0 mg/mmol (male) and below 30 mg/mmol for both sexes.[65] These cut-offs are arbitrary, and cardiovascular and renal risk is increasing even using lower cut-off levels.[66,67]

MA is observed in a number of clinical conditions, i.e. diabetes, hypertension, malignant diseases,[39] symptomatic urinary tract infections[68] and other inflammations,[69] intake of protein rich food,[70] exercise[71] and longstanding upright position[72]. Notwithstanding, MA is an important:

- Indicator of subclinical cardiovascular disease in the general population[73,74]
- Predictor of mortality in the general population[75,76]
- Marker of vascular endothelial dysfunction[77]
- Prognostic marker for progressive kidney disease
    - In the general population[78]
    - In diabetes mellitus[79]
    - In hypertension[80]

MA has therefore gained increasing recognition as a simple marker of an atherogenic propensity.

1.3.1 Microalbuminuria in the HIV-infected population

The association between HIV infection and MA has previously been observed in selected study populations in the pre-cART era, with a prevalence ranging from 19% to 34%.[81-83] Apart from a recently published study, from which a fivefold higher rate of MA was reported in HIV infected patients than in a matched, but not population-based, control group,[84] most studies have investigated proteinuria related to HIV associated nephropathy.[42,85,86] Prospective studies have shown that individuals with proteinuria after the initiation of cART, have higher mortality, higher risk of hospitalisation and increased risk of cardiovascular disease compared to those without proteinuria.[36,87] In a South African study of cART naïve individuals, MA was an early marker of biopsy confirmed HIVAN.[42] Moreover, in a recent autopsy study from New York, where chronic kidney disease was present in the majority it was presumed that several individuals could have identified by pre-mortem MA testing.[88] Today, the Infectious Diseases Society of America (IDSA) guidelines on the management and detection of chronic kidney disease in HIV recommend use of a conventional urine dipstick, with a threshold for detection of renal disease at 1+ proteinuria.[32]

1.4 Hypertension

Persistent HT is the most important risk factor for strokes, heart attacks, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure.[89,90] The World Health Organization attributes HT as the leading cause of cardiovascular mortality, and even moderate elevation of arterial blood pressure leads to shortened life expectancy. Beginning already at 115/75 mmHg, CVD risk doubles approximately for each increment of 20/10 mmHg. The hypertensive target organ damages can primarily be detected in the retina, heart and kidneys. It is estimated that 50% of the hypertensive population worldwide is unaware of their condition, and of those treated, less than half have their blood pressure controlled to a level of <140/90 mm Hg – the threshold for HT.[91]

Even though age remains the most important risk factor, other variables like sedentary lifestyle, obesity, high alcohol intake and increased salt sensitivity have been identified to be associated with increased BP. The pathogenesis of essential HT is a complex, and with no single cause, but rather an interaction between genes, environmental factors, and activation of pressor systems, e.g. autonomic nervous system or renin-angiotensin-aldosteron system

and local contributor to functional alteration in the vasculature eventually leading to structural changes and endothelial dysfunction.[92]

Antihypertensive drugs were first developed in the 60s. Despite the development of an armamentarium of medications, the magnitude of BP reduction is more important predictor for cardiovascular benefit than the choice of drug. Unfortunately, even if generic products are available for less than one cent per person per day, the reality is that most people, who need antihypertensive treatment, receive no treatment whatsoever.

1.4.1 Hypertension in the HIV-infected population

Before cART, HT has been a rather neglected research field among HIV cohorts. With increased life expectancy and occurrence of premature CVD in HIV-infected individuals, hypertension has gained interest. However, the prevalence of HT in different selected HIV-infected cohorts ranges from 8% to 34%.[93-100] This may reflect both differences in age, sex, race, immunological status and cART medication regimes and treatment duration in the cohorts. Only a few studies have investigated the prevalence of HT[93,94,96,99,100] together with BP levels[93,96,101] in HIV-infected populations compared with HIV-negative controls and these have shown diverging results. As in the general population, HT is strongly influenced by risk factors such as age, male gender, BMI, cholesterol and probably kidney diseases in HIV-infected individuals. An increase in BP has been found in HIV-infected patients during cART.[93,94,98,100,102-105] However, most studies, including the The Data Collection on Adverse events of Anti-HIV Drugs (DAD) study, do attribute this not to cART therapy or one particular agent,[100,102,103] but rather to the metabolic side effects of cART.[94,98,104,105] Another interesting observation has been made by Crane et al. They found increased SBP or HT not previously recognized in HIV-infected individuals with CD4 cell count below 200/mm^3 at treatment start, after a mean cART exposure of 13.5 months compared to those with higher CD4 cell count.[104] To complete the inconsistency in findings, even reduced BP has been observed, mainly when selected cART naïve patients (e.g. young age, unadjusted for BMI or small samples) were compared with HIV-negative controls.[93,96,100,101]

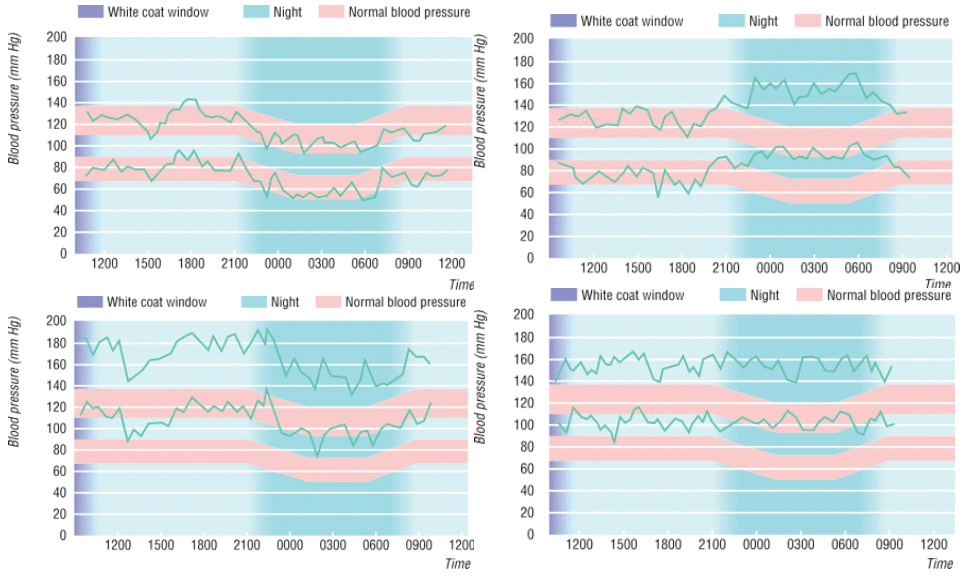
1.5 Ambulatory blood pressure

Initially reserved for research purposes, ambulatory blood pressure (ABP) monitoring has gradually entered standard medical practice and has now become a widely used clinical tool,

both for diagnostic purposes and for assessment of treatment efficacy. Indications for use of ambulatory blood pressure monitoring include white-coat hypertension, borderline hypertension, and resistant hypertension. Twenty-four hour daytime or nighttime average BP values correlate with subclinical organ damage more closely, and are more predictive for cardiovascular risk than office values.[106,107] A small study, has even suggested that elevated ABP is more strongly correlated with progression to ESRD than casual BP.[108]

Ambulatory BP values are usually lower than the corresponding office values, as a consequence of the missing white-coat effect. Thus, the 24h BP should be lower than 125/80 mmHg, while daytime BP should be below 130-135/85 mmHg, more closely corresponding to casual BP values of 140/90 mmHg.[109] The predictive consequences of white coat hypertension might be linked to an increased risk of developing HT and strokes.[110,111] Masked hypertension, i.e. normal office BP but elevated ABP, has emerged as an independent predictor of cardiovascular morbidity.[112] ABP varies between day- and night-time (Fig.5).

Fig. 5 *Examples of ambulatory blood pressure graph in normotensive (upper-left), nocturnal hypertensive (upper-right), hypertensive with nocturnal dip (lower-left) and hypertensive without nocturnal dip (lower-right) individual.*



Reproduced with permission from BMJ; O'Brien, E. et al. BMJ 2001;322:1110-1114

The average nocturnal fall in BP is approximately 15% from daytime values in both normo- and hypertensive individulals.[113] Patients have been categorized into dippers and nondippers according to the nocturnal SBP fall compared daytime SBP of greater or less than 10%, respectively.[109] Nondippers have the highest risk of cardiovascular complications.[114] The blunted fall in BP is associated with left ventricular hypertrophy,[115-117] increased albumin excretion,[115,118] and mortality.[119-121] The underlying mechanisms are unknown, but intrinsic renal defects may contribute.[122] Nevertheless, a positive association between markers of inflammation (i.e. C-reactive protein and tumor necrosis factor) and ABP variability has been described for healthy, normotensive individuals.[123] Others have observed that the nondipping BP pattern is related to inflammatory markers and endothelial dysfunction.[124] However, whether reversal of nondipping is possible or beneficial is yet not clarified. No former published investigations of ABP monitoring in HIV-infected individuals are available.

2. Objectives of the thesis

Given this background, there is clearly a need for more data with regard to microalbuminuria, hypertension and ambulatory blood pressure in HIV-infected individuals. Thus, the specific objectives of the study were as follows:

1 Identify the prevalence of microalbuminuria in a population-based, nonhypertensive and nondiabetic HIV-positive cohort, compare it to the general population and detect possible independent predictors.

2 Identify the prevalence of hypertension in an entire urban HIV-cohort, compare it to the general population and detect possible independent predictors.

3 To analyse the ambulatory blood pressure pattern in untreated hypertensive HIV-positive individuals and compare it to an untreated hypertensive HIV-negative control group.

3. Materials

3.1 Study sample

The individuals included in the MAHO study cohort were recruited from the outpatient clinic of the Department of Infectious Diseases, Ullevål University Hospital, the main HIV clinic in Oslo. In the hospital server a HIV database was early established in the 90' and record meticulously general characteristics, blood sample results and antiretroviral therapy regimes. All HIV-infected patients attending the clinic between March 2004 and November 2005 were invited to participate in the study, and 597, all aged >20 years were enrolled in the MAHO

Table 1
Demographic and clinical data of the 542 individuals who completed the MAHO-study.

Characteristics or laboratory value	All n = 542	Men n = 392	Women n = 150
Age groups, years			
< 30	60 (11.1%)	24 (6.1%)	36 (24.0%)
30–39	170 (31.4%)	114 (29.1%)	56 (37.3%)
40–49	179 (33.0%)	131 (33.4%)	48 (32.0%)
> 50	133 (24.5%)	123 (31.4%)	10 (6.7%)
Ethnicity			
White	382 (70.5%)	322 (82.1%)	60 (40.0%)
Black	125 (23.1%)	54 (13.8%)	71 (47.3%)
Asian	35 (6.5%)	16 (4.1%)	19 (12.7%)
Smokers	227 (41.9%)	177 (45.2%)	50 (33.3%)
Cholesterol (mmol/L)	5.1 ± 1.1	5.1 ± 1.2	5.0 ± 1.1
BMI (kg/m^2)	24.1 ± 3.6	24.1 ± 3.3	24.0 ± 4.4
SBP mmHg	130.2 ± 17.8	134.2 ± 17.0	119.8 ± 15.5
DBP mmHg	81.0 ± 10.7	82.7 ± 10.7	76.3 ± 9.3
HT (diagnosed before inclusion)	26 (4.8%)	25 (6.4%)	1 (0.7%)
Hepatitis C positive	50 (9.2%)	31 (7.9%)	19 (12.7%)
Duration since HIV test (years)	7.4 ± 5.9	7.5 ± 6.0	7.1 ± 5.9
cART			
Naïve throughout the study	158 (29.2%)	117 (29.8%)	41 (27.3%)
Naïve at study inclusion	25 (4.6%)	19 (4.8%)	6 (4.0%)
< 2 years	169 (31.2%)	121 (30.9%)	48 (32.0%)
2–5 years	100 (18.5%)	67 (17.1%)	33 (22.0%)
> 5 years	90 (16.6%)	68 (17.3%)	22 (14.7%)
HIV RNA (copies/mL)	200 (0–31,000)	200 (0–35,000)	65 (0–19,000)
CD4 (10^9 cells/L)	0.38 (0.25–0.56)	0.38 (0.26–0.58)	0.38 (0.22–0.53)
Creatinine (µmol/L)	70.8 ± 15.1	75.3 ± 13.2	59.1 ± 13.6
Microalbuminuria	51 (9.4%)	40 (10.2%)	11 (7.3%)

study. No exclusion criteria were used, although inability to understand and sign the written information constituted a limitation to participate. Based on estimates from the Norwegian Surveillance System of Communicable Diseases, these patients constitute about 75% of the entire HIV-positive population in Oslo. Thus, they can be considered an unselected population of HIV-infected individuals from the whole city. Subjects were followed for up to 34 months, and 542 completed study criteria of three clinical visits when the database was closed in January 2007 (Table 1).

3.1.1 Paper I

From the 597 patients enrolled in the MAHO study, 55 patients were excluded because of dropout after the initial visit (n = 26), i.e. impossibility to establish contact, death (n = 12), moving abroad or to other clinics in Norway (n = 11) or unwillingness to continue the study after inclusion (n = 6), leaving 542 patients that completed the study. In addition, patients with previously diagnosed diabetes mellitus (n = 14), HT (n = 25), macroalbuminuria (n = 5) or insufficient number of urine samples (n = 3) were excluded, remaining 495 individuals to be analysed. For the comparison with the control group, only Caucasians (n = 348) were included.

3.1.2 Paper II

All 542 individuals that completed the MAHO-study were analysed. For the comparison with the control group, only Caucasians (n = 236) were included.

3.1.3 Paper III

All individuals (n=147), categorized in the MAHO-study as hypertensive, based on the casual BP, and did not receive antihypertensive treatment were invited to undergo an ambulatory blood pressure monitoring. Of these invitees, 70 had either commenced antihypertensive treatment in the interim period or did not reply to the invitation; the remainder (n = 77) were eligible for inclusion in the study.

3.2 Ethics

All patients were given oral and written information by an infectious disease physician about the purpose of the study at the clinical consultation. Written consent to participate was given at the same session (Appendix, Pasientinformsjon). Additional written information

was given to those invited to the ambulatory blood pressure monitoring. Patients were recorded unidentified in the database with the identical code from the HIV database. The protocol of the MAHO-study was approved by the Regional Committee for Medical Research and the Norwegian Data Inspectorate. The study has been conducted in accordance with the World Medical Association Declaration of Helsinki.

3.3 Control groups

3.3.1 Paper I/II

For both papers the large population-based second Nord-Trøndelag Health Study (HUNT-2), 1995-1997, Norway (n=66140) served as comparative data. The HUNT-2 study covered the entire country's population and is one of the most reliable health studies ever performed.

All residents of Nord-Trøndelag County (n=92702), aged 20 years and older were invited to the health survey. An invitation letter was sent by mail attached to a questionnaire that had to be completed and returned at the attendance to the screening site, where a second was handed out. A wide range of topics, like previous diseases, personal environment, habits and family medical history was addressed. In total 66.7% of the invited men and 75.5% of the invited women participated.[125]

Five per cent of a randomly selected sample of the total population (n=2091), who did not answer 'yes' to one or both of the questions: 'Do you have diabetes?' and 'Do you take antihypertensive medication now?' constituted the MA control group (paper I).

In contrast, the control group for paper II was based on the entire HUNT-2 study population.

3.3.2 Paper III

The control group (n=76) consisted of untreated and newly diagnosed hypertensive subjects referred to the outpatient HT clinic at Ullevål University Hospital for ABP measurement. They had entered a study on the reproducibility and characteristics of ABP in a hypertensive population.[126] To be eligible for that study, DBP measured at the HT clinic using a mercury sphygmomanometer had to be between 95 and 105 mmHg after 5 minutes rest in a sitting position and on two different occasions weeks apart.

4. Methods

4.1 Study design

The MAHO study was an unselected population-based, prospective, epidemiological cohort screening study of MA and blood pressure.

4.1.1 Data collection

General data was electronically extracted from the local HIV database to SPSS®. The basic questionnaire (Appendix; Spørreskjema) with information about race, smoking habits, drug abuse, malignancies, hepatitis C, diabetes, use of antihypertensive drugs and/or NSAID was entered into a epinfo® database based on the hospital server. Data were taken from the date nearest to the inclusion in the study. Further information, i.e. date of visit, BP data, height and weight was copied from the backside of that questionnaire. Urinary albumin and creatinine, glycosylated hemoglobin HbA1c, cholesterol and high-density lipoprotein were manually copied from the data patient record. Finally, data from the HIV database, questionnaire and patients record were joint for further statistical analysis in a SPSS® database.

4.1.1.1 Microalbuminuria screening

A urine sample was collected at each research visit, i.e. at the same time as BP measurements, for determination of the ACR, a measure for the urinary excretion rate of albumin.[127] At the first visit the patient was asked to deliver a urine sample 'straight away'. An additional urine container was handed out, in order to collect a morning midstream sample at the next visit. Patients, who underwent an antibiotic treatment for urinary tract infection or had ongoing urinary tract infection symptoms, where asked to deliver a urine sample at the next visit. Urine samples were sent and analysed promptly at the Department of Clinical Chemistry, Ullevål University Hospital.

4.1.1.2 Blood pressure screening

Highly trained physicians undertook three clinical visits, days to months apart. After the clinician consultation, BP was measured using a validated semiautomatic oscillometric device (Omron M4, Matsusaka Co. Ltd., Matsusaka, Japan). Cuff size was adjusted according to the arm circumference. Well-trained nurses performed two consecutive BP

measurements 2 min apart using an appropriate cuff size after the patient had rested in a relaxed, sitting position for 5 min in a quiet room. BP together with date was registered on the backside of the questionnaire. In addition, height and weight were measured at the first clinical visit. The BP in the control group of the first and second paper was measured with a validated semiautomatic device (Dynamap 845XT, Créteil, France).[125]

4.1.1.3 Ambulatory blood pressure measurement

Based on the casual BP measurements, untreated, hypertensive individuals were invited by letter to participate in an ABP monitoring. General information about the purpose of the investigation was enclosed to the letter (Appendix; MAHO – Pasientinformasjon – 24 t Blodtrykksregistrering). Furthermore, at attendance a basic questionnaire (Appendix; Dagbok) was handed out. This included information about the sleeping habits and experiences of discomfort during the ABP monitoring.

ABP monitoring was performed using an oscillometric device (model 090207; SpaceLabs Medical Inc., Redmond, WA, USA). The 24-h BP measurements always began on a working day between 8:00 and 10:00. BP was measured at 20-min intervals during the day (07:00–22.00) and at 30-min intervals during the night (22.00–07.00). Cuff size was selected according to measured arm circumference, and the nondominant arm was always used. Furthermore, the patients were instructed to report any unexpected or unusual events during the registration period and whether sleep was markedly interrupted. The patients were instructed to keep their arm still during BP measurements to ensure good quality recordings. While in the control group ABP monitoring was performed using an auscultatory device; Tycos Quiet Trak (Welch/Tycos Instruments Inc., Skaneateles Falls, NY, USA) in the same outpatient clinic, definitions of HT were identically.

4.1.1.4 HIV database

Professor Johan Bruun established the HIV database in the 90s. It has been used for research purposes and for several scientific articles in highly esteemed and peer-reviewed journals[128,129] and has been adequately evaluated. Age, sex, date of the patient's first HIV-positive test, transmission mode, CD4, HIV RNA, serum beta 2-microglobulin, serum creatinine, starting date of cART, hepatitis B status and death date were obtained from the local HIV database, updated continuously from the patients records.[130]

4.1.1.5 Questionnaire

A basic questionnaire (Appendix; Spørreskjema) was completed in presence of a physician during the clinical consultation, comprising Yes/No questions of race, smoking habits, drug abuse, malignancies, hepatitis C, diabetes, hypertension and NSAID use. The questionnaire was primarily designed to evaluate possible causes of MA and has not been evaluated.

4.1.1.6 Definitions

MA was defined according to the ACR. Based on at least two urine samples, patients were categorized as having normoalbuminuria (< 2.5 mg/mmol), microalbuminuria (2.5–30 mg/mmol) or macroalbuminuria (> 30 mg/mmol). The same criterion for the definition of microalbuminuria was used in Paper I for the HUNT-2 study control group.[131]

Glomerular filtration rate (GFR), a measure of kidney function, was calculated based on the Modification of Diet in Renal Diseases (MDRD) equation, i.e. eGFR (ml/min) = 32788 × serum Creatinine ($\mu mol/L)^{-1.154}$ × $Age^{-0.203}$ × [1.21 if black] × [0.742 if Female], which takes into account serum creatinine, age, sex and race.[132]

For statistical analyses, the mean of all three BP measurements taken in duplicate was calculated. HT was defined, according to guidelines by the European Society of Hypertension as systolic BP (SBP) ≥ 140 and/or diastolic BP (DBP) ≥ 90 mmHg, or reported diagnosis of HT with use of antihypertensive medications. Isolated systolic HT was defined as SBP ≥ 140 mmHg and DBP < 90 mmHg, isolated diastolic HT as SBP < 140 mmHg and DBP ≥ 90 mmHg and combined systolic and diastolic HT as SBP ≥ 140 mmHg and DBP ≥ 90 mmHg.[109] BP in the control group Paper II (HUNT-2) was taken once in triplicate using the mean of the two last readings for statistical analyses.

Ambulatory blood pressure daytime and nighttime were defined as 07:00-22:00 and 22:00-7:00, respectively. Nocturnal fall was defined as the percentage decrease in average SBP and DBP at night relative to daytime values. The patients were classified, according to the European guidelines[133] as dippers or nondippers based on a nocturnal reduction in SBP of more or less than 10%, respectively.

Duration of HIV was calculated from the date of the first positive HIV test to the date of the first clinical visit. Duration of cART treatment was calculated from the cART starting date

to the date of the first clinical visit. CART was defined as a combination of at least three different drugs including NRTI's and at least one PI or NNRTI.

4.1.2 Laboratory analyses

Most of the study participants had their routine blood samples drawn between 8 and 11 a.m. after an overnight fasting. Urine albumin and creatinine were measured using an immunoturbimetric method (antihuman serum albumin antibody from Roche, Basel, Switzerland) and an enzymatic method (Roche), respectively. HIV RNA in EDTA plasma was quantified using polymerase chain reaction amplification with a COBAS Amplicor HIV-1 Monitor Test (Roche Diagnostics, Branchburg, NJ, USA). CD4 cell count was determined by routine flow cytometry using TriTest CD4/CD8 with TruCount Tubes (Becton Dickinson Biosciences, San Jose, CA, USA). All laboratory analyses were performed at the Department of Clinical Chemistry at Ullevål University Hospital.

4.1.3 Statistical analyses

All statistical analyses were worked out in tight collaboration with Prof. Leiv Sandvik, Oslo University. The software package SPSS® (SPSS Inc, version 14.0, Chicago, Ill, USA) was used to perform the statistical analyses. Differences/associations with a *p*-value <0.05 were considered statistically significant in all papers. Data were first tested for normality of distribution. Data are expressed either as the mean ± standard deviation (SD), as a number and percentage, or as the median with the interquartile range for skewed data. While for the comparison of two continuous variables a Student's *t*-test was used, the Mann–Whitney *U* test was chosen for skewed data. Dichotomous variables were compared with the Fisher's exact test. Logistic regression analyses was used to describe the relationship of a dichotomous dependent variable and related independent variables, using a backward stepwise procedure, with P-to-enter < 0.1 and P-to-remove > 0.1. Odds ratio (OR) was calculated by logistic regression analysis or cross tables. Linear regression analysis (paper II) was used to assess associations between continuous dependent variables and selected independent variables, expressed as the unstandardized coefficient (B) and standard error (SE). Independent variables correlating with r > 0.7 were not included simultaneously in a regression analysis. For comparison between multiple groups, one-way analysis of variance and Pearson's χ^2 linear-by-linear association to test categorical variables was used.

5. Summary of the individual papers

5.1 Paper I

Microalbuminuria associated with indicators of inflammatory activity in an HIV-positive population.

Nephrology Dialysis Transplantation. 2008;23:3130-3137.

The aim of this study was to identify the prevalence of microalbuminuria in a HIV-cohort, furthermore to compare the findings in the Caucasian HIV-infected individuals to a matched general population.

The HIV study cohort consisted of 495 nonhypertensive and nondiabetic patients. Microalbuminuria was determined on the basis of three urine samples.

The prevalence of microalbuminuria was 8.7% in the HIV-infected cohort, which is 3–5 times higher than in the general population. Systolic blood pressure, serum beta 2-microglobulin and duration of HIV infection emerged as independent predictors of microalbuminuria.

It was concluded that microalbuminuria was markedly increased in HIV-infected individuals compared to the general population, due to hemodynamic effects and possibly to increased inflammatory activity.

5.2 Paper II

Hypertension in an Urban HIV-Positive Population Compared with the General Population: Influence of Combination Antiretroviral Therapy.

Journal of Hypertension. 2008;26:2126-2133.

The aim of this study was to identify the prevalence of hypertension in an entire HIV-cohort and compare the findings in the Caucasian HIV-infected individuals to an age, gender, and body mass index matched general population.

The HIV study cohort consisted of 542 unselected individuals. Hypertension, defined as ≥140/90 mmHg, was based three measurements taken in duplicate under standardized procedures.

The prevalence of HT among the Caucasian HIV-infected subjects was 36.5%, which was not significantly different from that of the general population although the diastolic BP was higher in HIV-cohort. The highest prevalence of HT was found in those who were treated by cART for more than five years, while the lowest was found in those treated <2 years and not in cART naïve individuals. However, beside traditional risk factors, cART duration emerged as an independent predictor for hypertension.

It was concluded that the prevalence of hypertension in Caucasian HIV-infected individuals did not differ from that of the general population. However, cART seemed to induce an inscrutable time dependent difference on the blood pressure.

5.3 Paper III

Association between HIV infection and attenuated diurnal blood pressure rhythm in untreated hypertensive individuals

HIV Medicine. 2009;10:44-52.

The aim of the study was to investigate the diurnal blood pressure rhythm in newly diagnosed hypertensive HIV-infected individuals and compare them to HIV-uninfected controls.

Ambulatory blood pressure was measured over 24 h in 77 unselected newly diagnosed hypertensive individuals from the study cohort.

A blunted nocturnal blood pressure fall was more frequent in the HIV-infected individuals compared to the hypertensive individuals without HIV infection (60% vs. 33%, respectively; $P = 0.001$). This finding was dependent on the HIV status, but seemed independent of the exposure to cART.

It was concluded that the prevalence of a nondipping BP pattern in HIV-infected subjects with hypertension was significantly higher than in hypertensive control subjects.

6. Discussion

6.1 Discussion of the results

The main findings of this thesis are the lucidly increased prevalence of microalbuminuria in HIV-infected individuals, which in the Caucasian fraction was 2-4.7 times higher compared to the general population, and secondly the pioneering finding of the attenuated nocturnal ambulatory blood pressure rhythm in the newly diagnosed hypertensive individuals. Thirdly, we observed an interesting 'oscillating' prevalence of hypertension in the MAHO cohort with reduced prevalence in the initial phase after initiating cART compared to naïve and cART exposure beyond two years.

6.1.1 Prevalence

Compared to the general population the prevalence of MA was mostly increased in HIV-infected men <50 years. Supported by the duration of HIV infection and beta 2-microglobulin as independent predictors for MA, one might speculate that in these young individuals the inflammatory component is most pronounced, whereas in the older age groups traditional risk factors become more prominent. Even in HIV-positive women <50 years the prevalence of MA was relatively high compared to uninfected controls (5.8% vs. 2.4%, $P = 0.18$), but the group was to small to reach statistical significance. Previously, and at the time of planning of our study no data were available. A multicenter study in which the prevalence of MA in HIV-infected individuals was investigated, was published while we were preparing paper I. That study was in contrast to ours, however, based on a selected population of the Fat redistribution and metabolism (FRAM) study with significant lipodystrophy as an entry criterion.[84] Therefore, we do consider that our findings not only confirm previous studies, but also are original in an unselected urban HIV cohort compared to a highly representative general population.

Paper II was the first study to investigate the prevalence of HT in an entire HIV cohort and compare it with an age, gender and BMI-matched general population. The finding that HT is not increased in HIV-infected individuals compared to the general population, is in line with the DAD study and others,[93,96,105] who attribute changes in BP rather to traditional risk factors for hypertension than to cART or HIV *it self*. The prevalence of hypertension (HT) in different selected HIV-infected cohorts ranges from 8% to 34%.[93-100] Thus, our results are

in the higher range. Only a few studies have investigated the prevalence of HT[93,94,96,99,100] together with blood pressure (BP)[93,96,101] in HIV-infected populations compared with HIV-negative controls and have showed diverging results. Unstandardized BP measurements, diversity in study populations and/or control groups and lack of adjustment for confounding factors may explain the nonuniform results. Another interesting finding of our study, especially considering the low number of treated hypertension, is that isolated systolic hypertension seems to be the prominent related subgroup of hypertension between naïve and cART subjects as well as within the different cART groups based on treatment duration. Isolated systolic hypertension is most common in the elderly subjects[134] and has been shown to be more closely correlated to cardiovascular risk than diastolic hypertension.[135] Moreover, the prevalence of isolated systolic hypertension in the HIV-infected cohort is comparable to that in a 5-15 years older general population.[136] Thus, it seems that the HIV population may develop isolated systolic hypertension earlier than the general population, indicating that vascular stiffness may occur at a younger age due to atherosclerosis and metabolic aberrations.

Paper III revealed a very high prevalence of nondipping pattern in the HIV-positive cohort compared to the HIV-negative control group. We do not have a reasonable explanation but wish to investigate this in the near future. Unfortunately only few cART naïve individuals entered the study, but cART did not seem to affect the ABP pattern. A postulated hypothesis might be among others, that inflammatory processes could influence the diurnal blood pressure rhythm in HIV-infected patients. Van Kanel *et al.* observed in a group of relatively young unmedicated HIV-uninfected individuals that nondipping BP pattern seems to be related to inflammatory markers and endothelial dysfunction.[124] Another attractive speculation could be cART-induced processes and even an unknown inherent kidney abnormality causing tubular sodium handling, could explain the abnormal circadian pattern.[137]

6.1.2 The impact of traditional risk factors

In paper I and II the main traditional risk factors for MA and HT, such as age (I and II), BP, creatinine, GFR (paper I), male sex, BMI, cholesterol and MA (paper II) were confirmed in our study. Others, such as gender, smoking, cholesterol (paper I) and ethnicity (paper I and II) did not reach statistical significance. It has to be emphasized that the MAHO cohort is

relatively young and too small to reveal the entire 'picture' of MA and HT. However, despite that, the shared traditional risk factors, with that of the general populations may express strength of validity in our cohort.

In paper III we did not find significant difference in MA between dipping and nondipping pattern (9.7% vs. 22.7%; $P = 0.14$), as it would have been expected, but this could be due to low statistical power.

6.1.3 The impact of HIV duration/inflammation

HIV duration and beta 2-microglobulin, a marker of immunoactivity emerged as independent predictors for urinary albumin excretion, suggesting an inflammatory component in the development of MA. Only Luke et al. have observed in a small selected cohort, higher levels of TNF in patients with MA before the era of cART.[82] To date, no study has investigated more specific biomarkers for endothelial dysfunction (vWf, sVCAM-1, sICAM-1, sE-selectin) or inflammation (hs-CRP, IL-6, fibrinogen) in HIV-infected individuals with and without MA. This would require fasting blood samples taken and stored under standardized procedures, which we unfortunately did not do for the present study.

There seems to be a gender difference in the predictors of MA. HIV duration was a predictor for MA in men, but not in women. In contrast, beta 2-microglobulin was a predictor for MA in women, but not in men (paper I). This could reflect a beneficial effect of female sex hormones on inflammation and endothelial dysfunction, and has been suggested to be the explanation for less cardiovascular disease in premenopausal women compared to men.[138,139]

HIV infection *per se* was the only independent predictor of attenuated BP rhythm among these hypertensive individuals. The explanation remains obscure, but inflammatory processes have to be considered and our findings need to be confirmed not only in other HIV-infected cohorts, but also in normotensive individuals with HIV infection.

We did not identify an association between CD4 or viral load and the primary outcome, i.e. MA, HT or dipping status. Decreasing CD4 cell count, and increasing viral load are known to correlate with disease progression and the duration of HIV infection. After the introduction of cART this continuum has been interrupted and even reversed. The immune

capacity in our cohort was well maintained as 85% had a CD4 cell count above $0.2x10^9$ cells/l and close to 50% had HIV RNA below 400 copies/ml. Furthermore, important traditional risk factors for MA and HT are influenced by the adverse events of the antiretroviral therapy. In a population where more than half of the patient are using cART an analysis would therefore be highly confounded. However, even in cART naïve patients, no association could be found between CD4 or HIV RNA and MA or HT. Crane et al. found increased SBP or new diagnosis of HT in HIV-infected individuals with CD4 cell count below $200/mm^3$ at treatment start with cART, after a mean cART exposure of 13.5 months.[104] Also increased odds of chronic renal failure has been observed in HIV-infected individuals with lower nadir CD4 cell count.[35] Therefore, it might be interesting to investigate the level of CD4 cell count at treatment start in HIV-infected individuals with MA or HT compared to those without in our cohort.

6.1.4 The impact of cART and its duration

The majority of individuals in our cohort received cART, and more than two third of them received a PI containing regime. In paper I and III, cART had no influence on MA or nondipping pattern, while cART duration was predictive for HT (paper II) in HIV-infected individuals. These findings must be interpreted with caution as the matched cohort became rather small to study especially low prevalence's such as MA (paper I). Moreover, HT varied substantially on the basis of cART duration. There was no difference in the prevalence of HT between cART naïve and cART individuals (paper II), but the number of cART naïve individuals in paper III was exceedingly low. Furthermore, in paper II, one might speculate that BP may decrease promptly after the initiation of cART, possibly due to an improved inflammatory state. However, in the long run, the enhancing effects of the chronic HIV inflammation on BP alongside with metabolic side effects induced by cART may prevail.[105] Anyways, the impact of cART adverse events will remain a highly controversial topic. We do believe, that the diverging results of previous studies on HT and even CVD in HIV cohorts might be due to the difficult interpretation of different cART regimes, as therapy frequently are altered in patients due to availability of newer drugs, adverse events or failure of the treatment. Thus, we have not specified cART regimes in our study participants. Finally, it is even plausible that cART might have a positive impact on MA as it has on a deteriorated renal function[43] and urinary albumin excretion in women.[140] This has to be elucidated in more detail in future prospective studies.

6.2 Methodological issues

Even though the MAHO-study was designed and conducted with the aim of reducing random and systematic errors the findings may have an alternative explanation.

6.2.1 Study sample

The role of chance may always affect the observed results, when we apply them from a sample to the entire population. At study start the HIV-cohort of Oslo consisted, based on estimations from the Norwegian Surveillance System for Communicable Diseases of approximately 800 individuals. Of those 700 followed-up at the outpatient clinic of Infectious Diseases, Ullevål University Hospital, 597 were enrolled. Although, the MAHO-study cohort, with approximately 75% of the possible participants, can be considered as highly representative of an unselected urban HIV population in a developed environment with almost unlimited free access to health care, one might expect minimal sampling errors, i.e. selection bias in the study subjects. Furthermore, this Norwegian cohort has from a European perspective a relatively low share of drug abusers, which is reflected by the low prevalence of hepatitis C co-infection (9.2%). The majority of newly diagnosed HIV cases are either imported from high-endemic countries or men having sex with men.

In paper I, diabetic and patients using antihypertensive treatment were excluded. We did so to minimize the effect of traditional risk factors of MA. Furthermore in the comparison to the general population, which is ethnically homogenous, with only 3% of non-Caucasian origin, non-Caucasian HIV-positive individuals were excluded (paper I and II). Nevertheless, ethnicity did neither affect the prevalence of MA nor of HT. The MAHO cohort became statistically to weak when reduced to subgroups by matching it to the general population. Also in the future our cohort will probably only produce reasonable sized subgroups >35 years for cART individuals and between 20 and 30 years for naïve individuals. Even more difficult will it be for the female gender.

Only slightly more than 50% of the newly diagnosed hypertensive HIV-infected individuals responded to the ABP monitoring invitation (paper III). This was mainly due the necessity to start promptly antihypertensive treatment after the diagnosis of HT. Thus, there was a difference in casual diastolic blood pressure of approximately 3 mmHg between those included in the ABP study and those who did not attend. We may only speculate what

implications this could have. However, it may indicate that we actually underestimate the true occurrence of blunted diurnal blood pressure rhythm, as one may expect on abnormal blood pressure rhythm with lower casual blood pressure levels. Furthermore, we had a control group of untreated hypertensive individuals, which unfortunately differed substantially in casual blood pressure. However, the percentage of dipping pattern in the HIV-negative group is comparable to other observations.[141]

6.2.2 Limitations of the study

In the collection of the data several physicians and nurses have been involved, this could potentially affect the data quality. The questionnaire regarding medical history, hypertension, diabetes, smoking, use of NSAID's, drug abuse and cancer was completed by the physician, based on information from the patients. Thus, information might be under- (e.g. smoking, drug abuse, presence of hypertension or urinary tract infection) or overestimated by the participants. In certain cases of doubt the medical record gave additional supportive information about the patients history.

Usually, possible misclassification of for example MA would be expected to occur randomly and dilute associations. However, this would be a problem if the misclassification was differential or non-random.

MA was repeatedly collected and analysed under standardized procedures, ideally taken as midstream morning sample. While the first urine sample was taken at the same time with the clinical visit between 9 am and 3.30 pm, the second and third were supposed to be taken at home as midstream morning sample. If forgotten, the sample was collected at the outpatient clinic at the time of the visit. The day-to-day variation in MA might have influenced the results. Likewise, the blood samples were supposed to be taken after an overnight fasting, between 8 and 11 am, however, we do not know that all patients followed the recommendation about fasting. None of the main blood parameters (CD4 cells, HIV-RNA, beta 2-microglobulin, creatinine) were dependent on fasting samples. BP measurement procedures were highly standardized, but time and interval differed according to the clinical visit.

The intervals between the three clinical visits varied between 1 to 34 months. Ideally the patients should attend clinical examination every 3rd month. Despite the variability in the time frame, only 25 individuals started cART between the first and third visit.

HIV duration was calculated on the basis of the first positive HIV test, and the correctness of this could be discussed. However, it may be difficult to ascertain the correct point of time at which the individuals became HIV-positive. This is especially the case for some of the HIV-infected immigrants, who had frequently more advanced disease before they actually underwent HIV testing.

In addition, we have 'thrown a line' between HIV infection/duration and inflammatory activity as a cause for the high prevalence of microalbuminuria and the abnormal blood pressure pattern, which remains speculative. Thus, investigations of inflammatory markers are dearly needed. This will require fasting blood samples taken and stored under standardized procedures, which we unfortunately did not do in the first MAHO study.

Because direct GFR measurements are expensive and inconvenient, estimated GFR was calculated by the MDRD equation. This is in line with the recommendations by the National Kidney Foundation[142] and has even been evaluated in our population-based control group.[143] However, the accuracy of the MDRD equation to estimate GFR might have some limitations, due to age and degree of renal disease and underestimates GFR in populations characterized by normal serum creatinine levels.[144] Nevertheless, MDRD equation has not been evaluated in HIV-infected individuals.

6.2.3 Representativeness and generalizability

An important question is whether the conclusions based on the MAHO cohort can be considered valid for the HIV-infected patients in Oslo. The MAHO-study enrolled approximately 75% of the HIV-infected individuals in Oslo, and of those >90% completed the study with three clinical visits. Non-participants have not been registered and analysed, but one might speculate if several immigrants with limited language knowledge and ongoing drug abusers have not been included in the study. However, neither ethnicity nor drug abuse did influence our findings. Although the MAHO cohort has a statistically satisfactory size as a whole, when splitting in gender and age decades for the comparison with the general population, certain groups became too small and lack statistical power

especially for analyses of low prevalence's such as MA. Furthermore, to increase the statistical power of our finding, we categorized BP to HT, ACR to MA and nocturnal BP fall to nondipping status, while the linear analysis might identify a more accurate picture of this young cohort.

We used the HUNT-2 data from the years 95-97 as control group (paper I and II). There might have been changes in treatment and follow-up of diabetic and/or hypertensive patients with MA, which could have affected the presence of MA, but as these patient groups were excluded for statistical analyses, treatment changes over time should not affect the prevalence of MA in the general population in paper I. It is furthermore uncertain, whether the prevalence of HT in the HUNT-cohort has changed in the last decade. Both the Norwegian HUNT[145] and Tromsø[146] cohorts observed an increase linked to BMI over the preceding decade. The definition of HT and MA were identical in our study and the HUNT study. However, the sampling procedures, laboratory facilities, BP device and the number of BP measurements differed substantially between the two studies.

Several variables were potential confounders, and not all could be included and adjusted for in the analyses. The choice of variables treated as confounders in the analyses were based on our knowledge of risk factors associated with MA or HT and were addressed in the questionnaire. Even so, alcohol, physical activity, dietary nutrient intake and other medications do all constitute potential independent, unmeasured factors that might be associated with exposure. There were no exact data on medications, unless NSAID's, antihypertensive medications and cART. Some study support the hypothesis that both aspirin and statins have anti-inflammatory effects and decrease CRP.[147,148] Neither the impact of lipodystrophy, nor the sub-group lipoatrophy were identified and analyzed in our study. While, it is reasonably to assume that like for cART the traditional risk factors for HT account for most of the burden in lipodystrophic individuals, lipoatrophy seems more likely to influence BP independently in HIV-infected individuals. It is uncertain how this might have inflicted our results and leads us to the lack in our study of more specified analyses of cART. As discussed previously, we do believe that the interpretation of the influence of different cART regimes in HIV cohorts might be difficult, as therapy frequently are altered due to availability of newer drugs, adverse events or failure of the treatment. Furthermore, cART subgroups would have reduced the statistical power. However, we analysed PI in paper II and could not identify any association to HT or difference in the prevalence of HT

between PI and non PI containing cART regimes. The study was performed in an urban setting and might hence only generalize to comparable locations. However, despite the high attendance, the MAHO-study cohort has a rather low external validity for a general urban HIV-populations. While age, gender, HIV duration and naïve/cART distribution might be fairly similar to other city cohorts in the industrialised world; this does not apply for ethnicity, transmission mode and socioeconomic status. However, it seems rather futile to reach this goal since the disease is partly dependent on cultural behaviour and there will always exist substantial differences between international cohorts. Furthermore, comparison of the prevalence of MA and HT were limited to the Caucasian population, as the HUNT-2 control group has a low percentage of non-Caucasian. The generalisation might therefore be limited at the most to Caucasian HIV-infected individuals in urban areas.

6.3 Implications

In terms of attention towards BP measurement, the MAHO-study has already had an impact on the daily clinical care of the patients in the ward and at the outpatient clinic of the infectious disease department. Clinicians have also become increasingly aware of the importance of BP follow-up and the usefulness of ABP monitoring.

There is clearly a need for further large, prospective studies on MA, HT and impact of intervention. Furthermore, the threshold of BP intervention and specific drug therapy has to be identified, if it should differ from the general population. The findings with regard to ABP should be confirmed in larger studies, also in normotensive HIV-infected individuals. The relationship between diurnal blood pressure rhythm in relation to cardiovascular and renal diseases in both naïve and cART cohorts should be explored. Even more it is imperative to carry out some of these studies also in HIV-infected individuals of African and Asian origin.

The up to four-fold increase in MA might have predictive value for cardiovascular and renal diseases as demonstrated for the general population. Studies, in large international cohorts need to be undertaken to prove this theory. The detection of MA might identify a subgroup of patients with several modifiable risk factors for CVD, like overweight, hyperlipidemia, hypertension, smoking and glucose intolerance. Correction of these abnormalities by lifestyle and/or pharmacological therapy might reduce the incidence of cardiovascular events, but needs to be further explored. Chronic kidney disease is today defined according

to the National Kidney Foundation and Infectious Disease Society of American guidelines as a GFR< 60 ml/min/1.73 m^2 and/or the presence of proteinuria, confirmed on two or more measurements separated by at least 3 months.[32] However, the use of quantitative test, i.e. ACR is likely to provide a more accurate assessment and allow for identification of renal disease in more patients with HIV infection.[149] Recognition of early, subclinical kidney disease may allow the initiation of cART and other interventions, such as ACE-inhibitors or angiotensin receptor antagonists,[150] which might prevent or delay progression to ESRD. A recent biopsy study demonstrated early HIVAN in six South African patients with MA, who would not have undergone kidney biopsy as part of standard clinical care. The potential of MA in detecting kidney disease was confirmed by an autopsy study from New York, who demonstrated a high prevalence of chronic kidney disease in a cohort of cART-exposed individuals and presumed that several individuals could have been identified by pre-mortem MA testing.[88] Nonetheless, it remains uncertain if screening for MA can be recommended to 'healthy' HIV-infected individuals in order to detect subclinical CV and CKD.

In the era of cART, HT constitutes, alongside with increased cholesterol and BMI a major modifiable risk factor for cardiovascular and renal diseases. If cART *it self* or cumulative exposure have a deleterious effect on BP remains uncertain and will be difficult to identify as new drugs and even groups will probably emerge on the scenery. However, as HIV-patients age they become hypertensive whether using cART or not, and should be followed up with at least the same attention as the general population, including life style modifications. Unfavourable lipid effects of thiazide diuretics must be 'kept in mind'. As in type II diabetes, the best choice of treatment in cART patients with MA associated with HT might be an ACE inhibitor or angiotensin receptor antagonists, but no randomised trials comparing different antihypertensive drugs in HIV-infected individuals are published.

The clinical indications in the general or hypertensive population of ABP monitoring remain arguable and limited to institutions where facilities are available. However, accumulated evidence now points to greater prognostic significance in determining risk for hypertensive end-organ damage compared with office BP measurements. This is especially true for nondippers. Thus, one might speculate if the identification and treatment of attenuated blood pressure in hypertensive HIV-infected individuals could decrease the incidence of CVD. This hypothesis may be difficult to prove, as it would require large long-term studies.

6.4 Future research

The MAHO-study was designed as a prospective investigation of MA and BP in the era of cART. The second part of the MAHO-study has been started in 2007. That part will give us the possibility to explore the changes in urinary albumin excretion, GFR and BP in a HIV-infected cohort over time and detect possible relationships to traditional risk factors and HIV-related factors. Furthermore, we will focus on fasting blood samples for certain hormone analyses and their relationship to MA, HT and cART. In addition, we could explore the different main components of cART in association to MA and nondipping BP pattern. Moreover, it is necessary to confirm our ABP findings in normotensive HIV-infected patients and detect potential cardiac end organ damages.

Several questions arise regarding inflammatory implications of HIV in the development of MA and attenuated diurnal BP rhythm. Therefore, we will continue our investigations and improve laboratory procedures in selected subgroups of both cART naïve and cART individuals in order to study inflammatory processes. Many inflammatory markers are conceivable to have impact on atherogenesis and in collaboration with the Department of Infectious Disease, Rikshospitalet, Oslo University Hospital, a paper was recently accepted, were we observed a positive associations between osteoprotegrin, soluble TNF-receptor type 1 and microalbuminuria in naïve patients.

Since our findings are limited to a primarily Caucasian HIV-infected, our model might be repeated in an African setting. This would be especially interesting regarding the predictive value of MA for the development of chronic kidney disease in the continent where the HIV epidemic has by far the most deleterious consequences.

7. Conclusions

The prevalence of MA was 2-4.7 times higher in Caucasian HIV-infected individuals compared to the general population. As beta 2-microglobulin and HIV duration were, beside hemodynamic effects independent predictors of MA, one might speculate the implication of inflammatory activity. Notwithstanding, MA represents a risk factor for CVD and CKD in the general population and might even reflect some of the burden among HIV-infected individuals.

The prevalence of HT in HIV-infected was similar compared with an age-, gender-, race- and BMI-matched general population. It remains uncertain what deleterious effect might be exhibited by and cART *itself* or its adverse events. Nevertheless, HT constitutes the most important risk factor for CVD in the general population and it is therefore mandatory to address it closely in HIV-infected individuals.

Hypertensive HIV-infected individuals were clearly more likely exhibiting a blunted fall in nocturnal BP, compared to hypertensive HIV-uninfected individuals. It might be speculated that HT in HIV-infected individuals is more aggressive, and a putative cause of the increasing CVD among HIV-infected cohorts.

The reasons and consequences of the increased prevalence of MA, the predictive value of cART for HT and the blunted nocturnal BP fall in HIV-infected individuals remain uncertain. Beside traditional risk factors we do suspect an inflammatory component. However, with regard to the rising number of HIV-infected individuals and their known increased risk of CVD and CKD, further investigations seem indispensable.

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9. Appendix

9.1 MAHO-study

9.1.1 Questionnaire

MAHO

Spørreskjema

Pasient

LEGE

Dato:

□Samtykkeerklæring og Informasjon

Rase
□hvit
□svart
□asiater
□andre (f.eks araber)

Røyking
□ja
□nei

Opiatavhengighet
(iv misbruk)
□ja
□nei

tidligere
□ja
□nei

Cancer
□ja
□nei

Hepatitt C
□ja
□nei

Diabetes
(behandlingstrengende)
□ja □Hba1c: __,_
□nei

kjent Hypertensjon
(behandlingstrengende)
□ja -□tbl. behandlet
□ACE/ARA*
□andre
□nei

NSAID/COX-2
(daglig bruk)
□ja
□nei

Send pasienten videre til sykepleier for avtale om time og utlevering av uringlass.

* ACE-hemmer/Angiotensin II antagonist

SYKEPLEIER

□uringlassutlevering, informasjon om prøvetaking og **enten** avtale (i løpet av noen dager) til uringlassmottakelse / I konsultasjon, **eller umiddelbart** overgang til uringlassmottakelse / I konsultasjon.

I konsultasjon	Høyde	___cm
Dato:	Vekt	___kg
	Blodtrykk	I ___/___ II ___/___ (etter 5 min hvile, med 2 min. mellomrom)
	Temp	__,_
	□ Uringlassmottakelse	
	□ Uringlassutlevering	
	□ HDL, LDL, HbA1c	

II konsultasjon (5 min. før eller etter 3 mnd. blodprøvetaking)	Blodtrykk	I ___/___ II ___/___ (etter 5 min hvile, med 2 min. mellomrom)
Dato:	Temp	__,_
	□ Uringlassmottakelse	
	□ Uringlassutlevering	

III konsultasjon (5 min. før eller etter 3 mnd. blodpøvetaking)	Blodtrykk	I ___/___ II ___/___ (etter 5 min hvile, med 2 min. mellomrom)
Dato:	Temp	__,_
	□ Uringlassmottakelse	

9.1.2 Information and consent form

MAHO

Pasientinformasjon

Mikroalbuminuri i en HIV positiv populasjon i Oslo

Du blir bedt om å være med i en studie hvor man undersøker på begynnende nyrekomplikasjoner ved HIV infeksjon. De fleste pasientene som går til kontroll for sin HIV infeksjon ved Ullevål sykehus skal delta i undersøkelsen.

Bakgrunn for undersøkelsen.

Fra litteraturen er det kjent at HIV pasienter har øket risiko for å utvikle nyresvikt.
Ettersom man nå lever lenge med sin HIV infeksjon er det beregnet at opp til 10%
av alle HIV pasientene vil kunne utvikle begynnende nyresvikt i forløpet av sykdommen sin.
Begynnende symptomer er funn av eggehvite i urinen og utslag i blodprøver som måler nyrefunksjonen.
Funn av eggehvite i urinen kan også være uttrykk for økende risiko for
hjertekar sykdom slik som vi ser det hos pasienter med sukkersyke.
Vi håper vår undersøkelse kan være til nytte for deg ettersom vi i dag har gode medisiner tilgjengelig både overfor kompliserende nyresykdom og ved begynnende hjertekar sykdom.

Hensikten med studien

Undersøkelsen er en epidemiologisk studie hvor de fleste HIV positive pasientene i Oslo området skal delta. Vi ser på,

1) forekomst av begynnende nyrekomplikasjon blant HIV positive pasienter i Oslo regionen.

2) undersøker på mulige sider ved HIV infeksjonen som kan disponere for å utvikle kompliserende nyre-sykdom.

Utførelsen av undersøkelsen

Du kommer som vanlig til kontroll for din HIV infeksjon hver tredje måned hvor det er tatt en blodprøve 14 dager i forveien. Du blir undersøkt på vanlig måte av din kontrollerende lege.
Eneste tilleggsundersøkelse vil være at før hver legekonsultasjon vil sykepleier måle blodtrykket og temperatur ditt og ved første kontroll veie deg og måle høyden din. I tillegg skal du hver gang levere en morgen urinprøve til sykepleier. Uringlassene vil du få utlevert. Studien vil strekke seg over 9 måneder, og vi vil undersøke deg tre ganger på denne måten .

Forsikring

Som pasient ved Ullevål sykehus vil du være forsikret i henhold til Pasientskadeerstatningsordningen.

Konfidensialitet.

Alle opplysninger om deg og sykdommen din vil bli behandlet konfidensielt. Dataene vil lagres i journal og sykehusets forskningsserver i 10 år. Du har rett til innsyn i opplysningene som for journalen og kan få slettet disse i tilfelle du ønsker det.

Du velger selv.

Studien vil ikke innebærer øket risiko og svært liten ekstra belastning for deg når du kommer til dine faste HIV kontroller. Vi håper undersøkelsen kan være til nytte for deg ved å oppdage en mulig begynnende komplikasjon til HIV infeksjonen. Undersøkelsen vil også bringe generell kunnskap over en av HIV infeksjonens mange komplikasjoner.
Dersom du ikke ønsker å delta, vil du bli undersøkt på vanlig måte ved de faste kontrollene.

Dersom du har spørsmål i forbindelse med studien, ta kontakt med ansvarlig lege for studien:

Navn: dr Morten Bækken tlf: 22119101

Samtykke til deltagelse

Jeg har lest denne pasientinformasjonen og diskutert det med legen min. Jeg har også mottatt min egen kopi av pasientinformasjonen og ønsker å delta i denne studien.

Pasientens navn _____

Pasientens underskrift _____ dato_____

Pasienten har fått muntlig og skriftlig informasjon.

Ansvarlig leges navn _____

Ansvarlig leges underskrift_____ dato _____

9.1.3 Ambulatory blood pressure

9.1.3.1 Invitation

MAHO

Pasientinformasjon - 24 t Blodtrykksregistrering

Hvorfor får du utført 24 timers blodtrykkregistrering?

Du deltar i MAHO studien ved Infeksjonsmedisinsk poliklinikk hvor vi bla. målte blodtrykket ditt. Ganske mange pasienter har høyere blodtrykk under legebesøket enn ellers på dagen, og det kan derfor være nyttig å få målt blodtrykket utenom legekontoret. Dette gjelder ikke bare hos de hvor det er mistanke om forhøyet blodtrykk, men også de som behandles for høyt blodtrykk. Ved 24 timers blodtrykkregistreringer, som utføres ved Nyremedisinsk poliklinikk i 3 etasjen, vil legen også få resultater av mange målinger i stedet for vanligvis 2-3 under konsultasjonen. Videre vil slik måling fortelle oss noe om hvordan blodtrykket ditt er i løpet av natten eller under hvile. Dette kan være til stor nytte for vurdering når det gjelder blodtrykksbehandling.

Du kan bidra til at målingen blir vellykket ved at du fører dagbok (på baksiden) den dagen blodtrykksregistrering pågår

- Noter tidspunktet når du legger deg og står opp
- Noter tidspunktet for spesielle aktiviteter
- Dersom du har ubehag av noen art som f.eks. hodepine, brystsmerter eller svimmelhet, noter tidspunktet

Den dagen registreringen pågår, er det ønskelig med at du opprettholder din vanlige daglige aktivitet. På den måten får vi der beste inntrykket av hvordan blodtrykket ditt er vanligvis er.

Når maskinen indikerer at blodtrykket skal måles (vanligvis kommer det et lydsignal), hold armen i ro. La bare armen henge rolig langs siden.

Ingen bærbare 24 timer blodtrykksapparat er laget slik at de kan måla blodtrykket under høy fysisk aktivitet eller trening. Dette vil resultere i feilmelding på apparatet, og gjentatte forsøk vil bli gjort, vanligvis 1 min etter den mislykkede måling. Dersom du må trene den dagen du får registrert blodtrykket ditt, bør apparatet hektes av før trening og festes på igjen etter gjennomført trening. Noter tidspunktene for treningen.

Apparatet tåler ikke vann. Dersom du skal dusje eller bade, må apparatet tas av. Når du tar av maskinen og det ikke skal gjøres flere registreringer, husk å slå av på/av-knappen.

Bilkjøring eller bruk av maskiner kan påvirke blodtrykksregistreringene. I tillegg vil blodtrykksregistrering under bilkjøring kunne påvirke din oppmerksomhet og derfor dine kjøreferdigheter. Konferer med legen din dersom du har et slikt yrke at det er vanskelig å gjennomføre målingen i arbeidssituasjonen.

Når du legger deg, la selve målemaskinen ligge på sengen ved siden av deg (ikke under hodeputen da vil lyden av maskinen kunne forstyrre deg).

Dersom du har spørsmål, ta kontakt med ansvarlig lege for MAHO studien:

Navn: dr Morten Bækken tlf: 22119101

9.1.3.2 Questionnaire

DAGBOK

Navn_____ fødselsdato_____

Dato for registreringen_____

Til sengs kl. _____

Våkner om morgen kl. _____

Står opp av sengen kl. _____

Spesiell aktivitet (angi klokkeslett) _____

_____

_____

_____

_____

_____

Ubehag (for eksempel hodepine, brystsmerter eller svimmelhet); angi klokkeslett

_____

_____

_____

_____

Disse spørsmålene ønsker vi du skal svare på ved avslutningen av registreringen

- Forstyrret blodtrykksregistreringen dine vanlige daglige aktivitet?
  - ☐ Nei
  - ☐ Ja
- Hadde du ubehag i forbindelse med registreringen?
  - ☐ Nei
  - ☐ Ja

  I tilfelle ja hvilke? _____

  _____

  _____

  _____

  _____
- Ble du forstyrret i søvnen av registreringen som ble gjort om natten?
  - ☐ Nei, ikke i det hele tatt
  - ☐ Ja, men i meget beskjeden grad
  - ☐ Våknet hver gang apparatet registrerte
  - ☐ Fikk ikke sovet i det hele tatt

Paper I

Baekken Morten, Os Ingrid, Sandvik Leiv, Oektedalen Olav.
Microalbuminuria associated with indicators of inflammatory activity in an HIV-positive population. Nephrology Dialysis Transplantation. 2008;23:3130-3137.

DOI: 10.1093/ndt/gfn236

The original publication is available at Oxford Journals
http://ndt.oxfordjournals.org/cgi/content/abstract/gfn236v1

Access to the published version may require journal subscription.

Nephrol Dial Transplant (2008) 23: 3130–3137
doi: 10.1093/ndt/gfn236
Advance Access publication 9 May 2008

Original Article

NDT
Nephrology Dialysis Transplantation

Microalbuminuria associated with indicators of inflammatory activity in an HIV-positive population

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Abstract

Background. The survival of human immunodeficiency virus (HIV)-infected patients has increased significantly since the introduction of combination antiretroviral therapy, leading to the development of important long-term complications including cardiovascular disease (CVD) and renal disease. Microalbuminuria, an indicator of glomerular injury, is associated with an increased risk of progressive renal deterioration, CVD and mortality. However, the prevalence of microalbuminuria has barely been investigated in HIV-infected individuals.

Methods. Based on three prospective urine samples in an unselected nonhypertensive, nondiabetic HIV-positive cohort ($n = 495$), we analysed the prevalence of microalbuminuria and compared the Caucasian share with that of a nonhypertensive, nondiabetic population-based control group ($n = 2091$). Significant predictors for microalbuminuria were analysed within the HIV-positive cohort.

Results. The prevalence of microalbuminuria was 8.7% in the HIV-infected cohort, which is three to five times higher than that in the general population. HIV-infected patients with microalbuminuria were older, and had higher blood pressure, longer duration of HIV infection, higher serum beta 2-microglobulin, higher serum creatinine and a reduced glomerular filtration rate of $\leq$90 mL/min, compared with those with normal albumin excretion. In multivariate analysis, systolic blood pressure, serum beta 2-microglobulin and duration of HIV infection were found to be independent predictors of microalbuminuria.

Conclusions. Our findings indicate that in addition to haemodynamic effects, inflammatory activity may be implicated as a cause of the development of microalbuminuria. With respect to the increasing risk of developing CVD or renal diseases and mortality, the high prevalence of microalbuminuria in HIV-infected individuals warrants special attention.

Keywords: beta 2-microglobulin; blood pressure; combination antiretroviral treatment; HIV; microalbuminuria

Introduction

The introduction of combination antiretroviral therapy (cART) in the treatment of human immunodeficiency virus (HIV) infection has led to a substantial decline in HIV-related mortality and morbidity [1,2]. However, it has also resulted in important short- and long-term adverse effects [3]. Attention has mainly been focused on cardiovascular disease (CVD) and there is evidence suggesting an association between cART and coronary heart disease, especially when protease inhibitors are involved [4–7]. The adverse effect of cART on serum lipids has been suggested to at least partially explain the increased rates of myocardial infarction [8], although other metabolic disturbances caused by cART may also contribute [9,10]. In addition, endothelial dysfunction has been proposed as a putative link between HIV infection and CVD [11,12].

Kidney diseases are increasingly prevalent in the course of HIV infection [13]. Today HIV-associated nephropathy (HIVAN) is a considerable cause of end-stage renal disease in HIV-infected, African Americans [13], but other histopathological renal diseases [14,15] affecting all ethnicities have also become more discernible. Moreover, HIV-associated renal disease with overt proteinuria has been associated with increased mortality [16,17]. Besides the potential nephrotoxicity induced by cART [18], HIV itself may directly affect glomerular epithelial cells [19]. However, the pathophysiology of the various renal diseases associated with HIV infection is not yet clear.

An increased urinary albumin excretion rate, even in the microalbuminuric range, has been found to be an independent risk factor for CVD and mortality in the general population [20–22]. The pathophysiological mechanism underlying urinary albumin excretion and the increased risk of CVD is not fully understood, although systemic endothelial dysfunction and inflammation has been implicated

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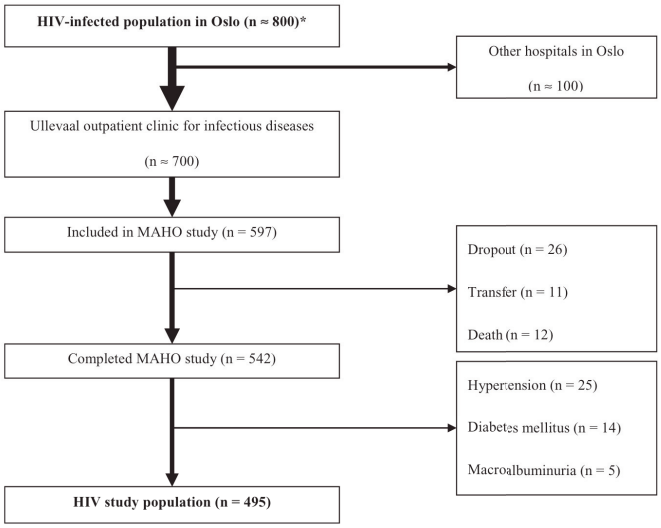

Fig. 1. Flow chart of the HIV-infected population in the study. Asterisk represents the estimated number of HIV-infected patients living in Oslo in 2004, based on the Norwegian Surveillance System for Communicable Diseases (MSIS).

[23,24]. Microalbuminuria has therefore gained increasing recognition as a simple marker of an atherogenic propensity [25]. Apart from a recent publication [26], there are few studies of microalbuminuria in HIV-infected patients and most of these have been undertaken in selected small cohorts limited to the pre-cART era [27–29]. Nevertheless, there is some evidence that microalbuminuria might represent an early indicator of HIVAN [30]. Thus, early detection of microalbuminuria could identify HIV-infected subjects at high risk of developing CVD and even renal diseases. To our knowledge, at the time of the initiation of our study, no large prospective population-based cohort study had investigated the prevalence of microalbuminuria in an HIV-infected population.

The aim of this study was, first, to assess the prevalence of microalbuminuria in an unselected HIV-infected cohort in comparison with a population-based control group, and second, to identify significant predictors of microalbuminuria in HIV-infected individuals.

Subjects and methods

HIV patients

For the present study, all HIV-infected patients attending the outpatient clinic at the Department of Infectious Diseases, Ullevaal University Hospital, Oslo, Norway, were invited to participate. This is the main HIV clinic responsible for treatment of HIV-infected patients in the city of Oslo, which has ~500 000 inhabitants. The patients attending the clinic therefore represent an unselected group from the whole city. No exclusion criteria were used. The study included 597 HIV-positive patients enrolled between March 2004 and November 2005. Based on estimations from the Norwegian Surveillance System of Communicable Diseases (MSIS), they constitute ~75% of the entire HIV-positive population in Oslo (Figure 1). They were given written and oral information about the study and the attending physician obtained signed consent at a regular visit. The National Committee of Medical Research Ethics approved the study and concession was obtained from the National Data Inspectorate. The subjects were followed as outpatients for up to 34 months. A total of 55 patients were excluded because of dropout after the initial visit ($n = 26$), i.e. impossibility to establish contact, death ($n = 12$), moving abroad or to other clinics in Norway ($n = 11$) or unwillingness to continue the study after inclusion ($n = 6$). In addition, patients with previously diagnosed diabetes mellitus ($n = 14$) or hypertension ($n = 25$) were excluded. The database was closed in January 2007. All patients were 20 years or older, and further characteristics are presented in Table 1.

Control group

Control data from the large population-based Nord-Trøndelag Health Study (HUNT) from 1995 to 1997, in Norway ($n = 2091$), were used [31]. The subjects constituted a healthy, nondiabetic and nonhypertensive general population. Men and women were distributed equally (47% versus 53%), with mean ages of 49.2 ± 15.6 and 48.7 ± 15.9 years, respectively. The population was stable and ethnically homogenous with only a small percentage (3%) of non-Caucasian origin [31].

Table 1. Demographic and clinical data of HIV-infected subjects

Characteristic or laboratory value	All ($n = 495$)	Male ($n = 354$)	Female ($n = 141$)
Age groups (years)[a]			
<30	58 (11.7%)	23 (6.5%)	35 (24.8%)
30–39	158 (31.9%)	106 (29.9%)	52 (36.9%)
40–49	164 (33.1%)	119 (33.6%)	45 (31.9%)
>50	115 (23.2%)	106 (29.9%)	9 (6.4%)
Ethnicity[a]			
Caucasian	348 (70.3%)	289 (81.6%)	59 (41.8%)
Black	114 (23%)	50 (14.1%)	64 (45.4%)
Asian	33 (6.7%)	15 (4.2%)	18 (12.8%)
Smoking[a]	211 (42.6%)	163 (46%)	48 (34%)
Cholesterol (mmol/L)[b]	5.0 ± 1.1	5.0 ± 1.1	5.0 ± 1.1
BMI (kg/m^2)[b]	23.8 ± 3.4	23.8 ± 3.2	23.8 ± 3.9
HbA1c (%)[b]	5.2 ± 0.39	5.1 ± 0.35	5.2 ± 0.46
Hepatitis B positive[a]	22 (4.4%)	21 (5.9%)	1 (0.7%)
Hepatitis C positive[a]	46 (9.3%)	28 (7.9%)	18 (12.8%)
Duration since HIV test (years)[b]	7.3 ± 5.9	7.4 ± 5.9	7.2 ± 6.0
Beta 2-microglobulin (mg/L)[b]	2.1 ± 0.9	2.2 ± 0.9	2.0 ± 1.0
cART[a]			
Naïve throughout the study	141 (28.5%)	103 (29.1%)	38 (27.0%)
Naïve at study inclusion	22 (4.4%)	16 (4.5%)	6 (4.3%)
<2 years	162 (32.7%)	116 (32.8%)	46 (32.6%)
2–5 years	86 (17.4%)	56 (15.6%)	30 (21.3%)
>5 years	84 (17%)	63 (17.8%)	21 (14.9%)
HIV RNA (copies/mL)[c]	200 (0–31 000)	200 (0–35 500)	60 (0–14 000)
CD4 (10^9 cells/L)[c]	0.37 (0.24–0.55)	0.37 (0.25–0.56)	0.37 (0.22–0.53)
SBP (mmHg)[b]	129.5 ± 17.7	133.7 ± 17.0	119.1 ± 14.9
DBP (mmHg)[b]	80.2 ± 10.4	82.0 ± 10.5	75.7 ± 8.8
Creatinine (μmol/L)[b]	70.58 ± 14.5	75.3 ± 12.9	58.8 ± 11.4
GFR groups (mL/min)[a]			
>90	411 (83%)	297 (83.9%)	114 (80.9%)
<90	81 (17%)	55 (16.1%)	26 (18.4%)

BMI, body mass index; cART, combined antiretroviral therapy; SBP, systolic blood pressure; DBP, diastolic blood pressure; GFR, glomerular filtration rate.
[a]Values are the number of patients and (percentage).
[b]Values are the mean ± SD.
[c]Values are the median and (interquartile range).

Clinical examination and questionnaire

Three clinical visits were undertaken days to months apart, when an HIV specialist physician investigated the patients. Blood pressure was measured using a validated semiautomatic oscillometric device (Omron M4, Omron Matsusaka Co. Ltd, Matsusaka, Japan). Well-trained nurses performed two consecutive blood pressure measurements 2 min apart using an appropriate cuff after the patient had rested in a sitting position for 5 min in a quiet room. The average of these measurements in duplicate was used for statistical analysis of systolic blood pressure (SBP) and diastolic blood pressure (DBP). At the first clinical visit, height and weight were measured to estimate the body mass index (BMI) in kg/m^2. A simple self-administered questionnaire with yes/no answers was filled out regarding smoking and intravenous drug-abuse habits, and knowledge of hypertension, diabetes, and cancer and hepatitis C status.

Urine samples

A urine sample was collected at each research visit for determination of the albumin/creatinine ratio (ACR), a measure for the urinary excretion rate of albumin [32]. Patients who underwent an antibiotic treatment for urinary tract infection or had ongoing symptoms, where asked to deliver a urine sample at the next visit. Urine albumin and creatinine were measured using an immunoturbimetric method (antihuman serum albumin antibody from Roche, Basel, Switzerland) and an enzymatic method (Roche), respectively. According to the ACR, based on at least two urine samples, patients were categorized as having normoalbuminuria (<2.5 mg/mmol), microalbuminuria (2.5–30 mg/mmol) or macroalbuminuria (>30 mg/mmol). A total of 460 patients delivered three urine samples each; 35 patients delivered two samples each and 3 patients delivered only one. Patients who delivered only one urine sample ($n = 3$) or had macroalbuminuria ($n = 5$) were excluded from the study. The same criterion for the definition of microalbuminuria was used for the historical control group [31]. In addition, glomerular filtration rate (GFR), a measure of kidney function, was calculated based on the Modification of Diet in Renal Diseases equations, which take into account serum creatinine, age, sex and race [33].

Demographic and laboratory data

Age, gender, race, date of the patient's first HIV-positive test, transmission mode, CD4 cell count, HIV RNA, serum beta 2-microglobulin, serum creatinine, cholesterol,

starting date of cART, hepatitis B status and death date were obtained from the local HIV database, updated continuously from the patients' records [34]. Data on lipids and HbA1c were obtained directly from the patient records. Duration since HIV test was calculated from the first positive HIV test. With respect to cART exposure, patients were allocated to different subgroups, while naïve (untreated) patients were divided into those who were untreated throughout the study and those who started cART between the first and last clinical visits. HIV RNA in EDTA plasma was quantified using polymerase chain reaction amplification with a COBAS Amplicor HIV-1 Monitor Test (Roche Diagnostics, Branchburg, NJ, USA). CD4 cell count was determined by routine flow cytometry using TriTest CD4/CD8 with TruCount Tubes (Becton Dickinson Biosciences, San Jose, CA, USA). All laboratory analyses were performed at the Department of Clinical Chemistry at Ullevaal University Hospital. Data were taken from the date nearest to the inclusion in the present study.

Statistical methods

Patient data were collected in an EpiInfo database (EpiInfo^{TM}) for statistical analysis using SPSS software (SPSS Inc., version 14.0, Chicago, IL, USA). Microalbuminuric and gender groups were compared for continuous and categorical variables using Student's *t*-test, the Mann–Whitney *U* test and Pearson's χ^2 or Fisher's exact test as appropriate. Data are expressed either as the mean ± standard deviation (SD), as a number and percentage, or as the median with the interquartile range for skewed data. Predictors of microalbuminuria were evaluated using logistic regression analysis and a backward stepwise procedure, with *P*-to-enter < 0.1 and *P*-to-remove > 0.1, which are the default values of the SPSS statistical package. Odds ratio (OR) was determined by logistic regression analysis or a χ^2 test. The prevalence of microalbuminuria in the Caucasian HIV-infected subjects was compared with that in the control group defined by age and gender. All *P* values were two sided and significance was accepted at $P < 0.05$.

Results

Characteristics of an HIV-infected population

The cohort for this study included 495 HIV-infected patients (Table 1). The mean age at enrollment was 42.3 ± 10.3 years. Women were younger than men (36.8 ± 8.1 versus 44.5 ± 10.2 years, $P < 0.0001$) and 23% of the total population were above 50 years of age. Males had been infected mainly through homosexual activity (63.3%) and were predominantly of Caucasian ethnicity, whereas half of the females (49.6%) originated from high-endemic African areas. At the time of inclusion in the study, >60% of the patients used cART, with well-maintained immune function, as 85% had a CD4 cell count above 0.2×10^9 cells/L. This rate did not differ between genders.

Microalbuminuria in HIV-infected patients

Microalbuminuria was present in 8.7% of the HIV-infected population. Less than 1% of subjects had a GFR < 60 mL/min and 83% had a GFR ≥ 90 mL/min. In comparison with subjects without microalbuminuria, subjects with microalbuminuria were older (47.6 ± 12.5 versus 41.8 ± 9.9 years, $P < 0.0001$), had higher blood pressure (SBP 141.9 ± 27.8 versus 128.4 ± 15.9 mmHg, $P < 0.0001$; DBP 84.5 ± 12.9 versus 79.8 ± 10.1 mmHg, $P = 0.005$), longer duration of HIV infection (9.0 ± 5.5 versus 7.2 ± 5.9 years, $P = 0.05$), higher levels of serum beta 2-microglobulin (2.6 ± 1.3 versus 2.1 ± 0.9 mg/L, $P = 0.002$) and higher serum creatinine (76.5 ± 23.1 versus 70 ± 13.3 μmol/L, $P = 0.005$). In those individuals with GFR ≤ 90 mL/min, microalbuminuria occurred more frequently (28.0 versus 15.2%, $P = 0.021$). In contrast, gender, race, smoking, BMI, high-density lipoprotein or cholesterol level, presence of previous or current hepatitis B and C infection, CD4 cell count and HIV RNA level did not differ between those with and without microalbuminuria. Even when excluding patients treated with cART, CD4 cell count and HIV RNA level did not differ between those with or without microalbuminuria. No statistically significant difference was found in the prevalence of microalbuminuria between cART groups based on the duration of therapy (<2 years, 8.6%; 2–5 years 10.5%; >5 years 9.5%). When analysing gender separately, women with microalbuminuria showed no difference in age, SBP, DBP, HIV duration, creatinine and GFR groups compared with those without microalbuminuria, whereas men with microalbuminuria had higher cholesterol (5.4 ± 1.6 versus 5.0 ± 1.1 mmol/L, $P = 0.038$), but no difference in the beta 2-microglobulin level (2.4 ± 1.1 versus 2.2 ± 0.8 mg/L, $P = 0.76$), compared with those without microalbuminuria.

Microalbuminuria in HIV-infected Caucasian subjects compared with the general population

The prevalence of microalbuminuria was significantly higher in the HIV-infected, Caucasian, male subjects than in the population-based controls [31] in groups defined by age and gender (Table 2). No statistically significant difference was found in the prevalence of microalbuminuria between Caucasian, HIV-infected women, <50 years of age compared to the population-based controls.

Predictors of microalbuminuria among HIV-infected patients

Including the HIV-infected cohort in a multivariate analysis (adjusted OR), with microalbuminuria as the dependent variable and SBP, age, quartiles of HIV duration, serum beta 2-microglobulin, serum creatinine and GFR groups as independent variables, only SBP ($P < 0.0001$) and beta 2-microglobulin ($P = 0.001$) were found to be predictive of microalbuminuria (Table 3). Furthermore, the prevalence of microalbuminuria was significantly higher in subjects in the third quartile compared with the first quartile for duration of HIV infection ($P = 0.018$). These results were confirmed in a linear-by-linear association using an χ^2 test as a trend analysis for HIV duration quartiles in both the

Table 2. Prevalence of microalbuminuria among 348 HIV-infected Caucasian men and women compared with 2091 subjects from the control group[a]

Age groups	HIV-infected patients			Control group			OR (95% CI)	*P*
	Total	MA	Percentage	Total	MA	Percentage		
	n	*n*		*n*	*n*			
Men< 50 years	189	13	6.9	520	8	1.5	4.73 (1.8–12.70)	0.0002
Men 50–59 years	81	11	13.6	200	8	4.0	3.77 (1.34–10.78)	0.0038
Men 60–79 years	18	5	27.8	232	26	11.2	3.05 (0.87–10.22)	0.04
Women< 50 years	52	3	5.8	614	16	2.4	2.29 (0.51–8.75)	0.18

MA, microalbuminuria.
P-values were determined by the χ^2 test.
Women aged > 50 years were not analysed because of the low number in the HIV-infected group ($n = 7$).
[a]From the HUNT study population.

Table 3. Predictors for microalbuminuria in HIV-infected subjects presented as unadjusted and adjusted odds ratios ($n = 495$)

Characteristic or laboratory value	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (years)	1.05 (1.02–1.08)[a]	1.007 (0.97–1.05)[c]
SBP (mmHg)	1.04 (1.02–1.05)[a]	1.03 (1.02–1.05)[a]
DBP (mmHg)	1.04 (1.01–1.07)[b]	[e]
Duration since HIV test, quartiles (years)		
1st	Reference	Reference
2nd	1.66 (0.53–5.2)[c]	1.89 (0.56–6.34)[c]
3rd	3.78 (1.35–10.6)[d]	3.76 (1.26–11.2)[d]
4th	2.79 (0.96–8.1)[c]	2.67 (0.88–8.14)[c]
Beta 2-microglobulin (mg/L)	1.57 (1.17–2.1)[b]	1.69 (1.23–2.33)[b]
Creatinine (μmol/L)	1.03 (1.01–1.05)[b]	1.0 (0.97–1.02)[c]
GFR groups (mL/min)		
>90	Reference	Reference
<90	2.75 (1.38–5.48)[d]	1.91 (0.91–3.99)[c]

SBP, systolic blood pressure; DBP, diastolic blood pressure; GFR, glomerular filtration rate.
Values were determined by logistic regression analysis. The candidate variables were defined with *P*-to-enter < 0.1.
[a]$P < 0.0001$; [b]$P < 0.01$; [c]$P < 0.05$; [d]$P > 0.05$.
[e]DBP excluded because of the high Pearson correlation (>0.7) between SBP and DBP.

total population ($P = 0.019$) and men ($P = 0.003$), but not in women ($P = 0.51$), and for beta 2-microglobulin quartiles in the total population ($P = 0.035$) and women ($P = 0.027$), but not for men ($P = 0.3$). The prevalence of microalbuminuria in relation to duration of HIV infection and beta 2-microglobulin is presented for the total population and for men and women separately in Figure 2a and b.

Discussion

The prevalence of microalbuminuria was 8.7% in this unselected cohort of HIV-infected patients and 9.2% in the Caucasian patients only, which is 2–4.7 times higher than in the general population. The association between HIV infection and microalbuminuria based on a single urine sample has previously been observed in selected study populations in the pre-cART era, with a prevalence ranging from 19 to 34% [27–29]. A recently published multicenter study, with selection bias of significant lipodystrophy as an entry criterion and not primarily aimed at the investigation of microalbuminuria, found a fivefold higher rate of microalbuminuria in HIV-infected patients than in a matched, but not population-based, control group [26], a result similar to ours despite disparities in study design and urine sampling procedures. In our study, the presence of microalbuminuria was based on three consecutive urine analyses collected prospectively. The importance of taking repeated measurements has been emphasized by Romundstad *et al.* [35], as albumin excretion may vary substantially and single-sample measurements lead to an overestimation of the true prevalence of microalbuminuria. To further minimize such overestimation, patients with known diabetes, hypertension or macroalbuminuria were not included in the HIV cohort or in the control group. Other strengths of our study are the unselected, single-center population, and standardized investigation with robust and repetitive measurements of urinary albumin excretion, using the same laboratory for all analyses.

Blood pressure was a major determinant of albumin excretion in our study, as has previously been demonstrated for other populations [36,37] and in an HIV-infected cohort [26]. Furthermore, serum beta 2-microglobulin, an inflammatory marker of HIV Immunoactivity [38], emerged as an independent predictor for urinary albumin excretion. This novel finding may suggest that other pathophysiological mechanisms beyond the haemodynamic effect may be linked to microalbuminuria in the HIV-infected

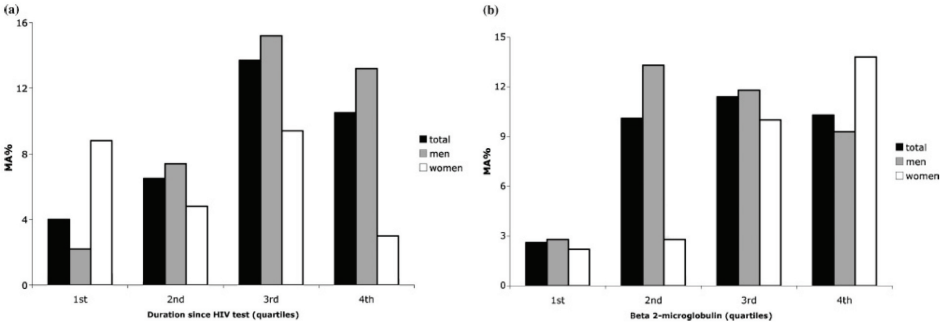

Fig. 2. (**a**) Distributions of the prevalence of microalbuminuria (MA) related to the quartile durations since HIV test in the total population ($n = 495$), men ($n = 354$) and women ($n = 141$). (**b**) Distributions of the prevalence of MA related to the quartile levels of serum beta 2-microglobulin in the total HIV-infected population ($n = 493$), men ($n = 353$) and women ($n = 140$).

state. This is further supported by the observation that subjects with microalbuminuria had longer durations of HIV infection than those without microalbuminuria. Inflammatory activity as a cause of microalbuminuria has been observed in nondiabetic subjects with cancer, inflammatory bowel disease and rheumatoid arthritis as well as in subjects with type 2 diabetes mellitus [39]. Moreover, endothelial dysfunction maintained by various inflammatory processes has been proposed as a possible cause of microalbuminuria [40]. Therefore, we hypothesize that microalbuminuria could depend on some chronic inflammatory process induced by HIV infection itself, not excluding the possibility that surrogate parameters of the immune function may play a role, even though we did not find any association between CD4 cell count or HIV RNA and microalbuminuria in the present study.

In this study, we could not show any association between the use of cART and microalbuminuria, which is in harmony with the results by Szczech *et al*. [26]. Nor did we observe significant differences between the duration of cART and microalbuminuria. Several studies have demonstrated increased CVD in HIV-infected patients receiving cART [5–7,41]. Consequently, metabolic side effects induced by cART have been implicated in the pathophysiological mechanism leading to CVD, but endothelial dysfunction may also be an important contributor [42]. Endothelial dysfunction has been proposed as a link between the presence of microalbuminuria and the increased risk of CVD in selected populations as well as in the general population [23,31,43], but remains to be evaluated in the HIV-infected population. However, be that as it may, microalbuminuria could serve as an early marker of enhanced cardiovascular risk and even of renal complications in the HIV-infected population as has been reported for other populations [30].

In a univariate analysis, microalbuminuria was linked to renal function, even if this was not maintained in a multivariate analysis. In contrast, Szczech *et al*. found no difference in serum creatinine or GFR in microalbuminuric compared to normoalbuminuric HIV-infected individuals [26]. On the other hand, in a selected South African HIV cohort where persistent microalbuminuric patients underwent renal biopsy, microalbuminuria was found to be an early indicator of renal disease [30]. A variety of renal diseases may occur and constitute an increasingly frequent complication during the course of HIV infection [13], hence the presence of microalbuminuria cannot be neglected.

We used a historical but population-based and nondiabetic control group [31]. During the 10-year time span, there has most likely been a change in awareness and treatment of microalbuminuria in diabetic subjects in Norway. However, little change in healthcare has occurred in the nondiabetic population. The age distribution of subjects younger than 50 years was slightly different compared with our cohort. This resulted in higher proportions of younger men and older women in the control group than in our study population. Furthermore, the ethnicity differed between the HIV-infected group and the control group, as the proportion of immigrants was higher in the HIV-infected group. Therefore, comparison to the population-based control group was limited to Caucasian HIV-infected patients. To establish a separate control group, including groups of different ethnicity, for this study would most likely not be possible or require an immense effort and would seem rather futile given that a very meticulous investigation had already been undertaken in Norway [21,31,35,44]. Nevertheless, ethnicity did not affect the results in this study.

In conclusion, the prevalence of microalbuminuria in Caucasian, nondiabetic, nonhypertensive HIV-infected subjects was found to be 2–4.7 times higher than in a healthy, nondiabetic and nonhypertensive control population. The duration of HIV infection, serum beta 2-microglobulin level and SBP were independent predictors of microalbuminuria in our HIV cohort. Thus, we suggest that the mechanisms causing microalbuminuria in HIV-infected subjects are linked not only to haemodynamic factors, but also to some yet unknown factor related to the HIV infection, possibly endothelial dysfunction. This might associate microalbuminuria to the increased risk of CVD seen in

HIV-infected subjects. However, the prognostic and clinical significance of microalbuminuria in HIV-infected patients is not yet known and prospective studies addressing this issue are clearly needed.

Acknowledgements. The MAHO study was conducted at the outpatient clinic of the Department of Infectious Diseases of Ullevaal University Hospital. We greatly acknowledge the help of the nurses of the outpatient clinic. Additionally, we wish to thank Professor Johan N. Bruun, the former head and professor of the Department of Infectious Diseases and founder of the HIV-cohort database, for his work and dedication. The study was financed by Ullevaal University Hospital and by the internal HIV fund of the Department of Infectious Diseases of Ullevaal University Hospital as well as the Regional Health Committee, Norway.

Conflict of interest statement. None declared.

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Received for publication: 11.1.08
Accepted in revised form: 28.3.08

Paper II

This article is removed.

Paper III

Baekken Morten, Os Ingrid, Stenehjem Aud, Sandvik Leiv, Oektedalen Olav.
Association between HIV infection and attenuated diurnal blood pressure rhythm in untreated hypertensive individuals.
HIV Medicine. 2009;10:44-52.

DOI: 10.1111/j.1468-1293.2008.00655.x

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Access to the published version may require journal subscription.

DOI: 10.1111/j.1468-1293.2008.00655.x
HIV Medicine (2009), **10**, 44–52
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ORIGINAL RESEARCH

Association between HIV infection and attenuated diurnal blood pressure rhythm in untreated hypertensive individuals*

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Objective
Hypertensive cardiovascular complications are more closely associated with ambulatory blood pressure (ABP), particularly the attenuated diurnal blood pressure (BP) rhythm (i.e. a fall in systolic blood pressure < 10% during the night compared with the day), than with casual BP. The aim of the study was to assess the ABP pattern in an HIV-infected cohort in which hypertension was newly diagnosed.

Methods
ABP over 24 h was compared between 77 newly diagnosed, untreated hypertensive HIV-positive individuals and 76 HIV-uninfected untreated hypertensive controls.

Results
More HIV-infected subjects had an attenuated ABP rhythm with a reduced nocturnal fall than HIV-negative hypertensive control subjects (60 *vs.* 33%, respectively; $P = 0.001$). The dipping pattern was observed despite newly diagnosed hypertension, a low prevalence of microalbuminuria, and the absence of signs of overt kidney disease. Furthermore, the prevalence of nondipping in the HIV-infected subjects was independent of combination antiretroviral treatment. Multiple logistic regression analysis with dipping pattern as the dependent variable showed that HIV status was an independent predictor of nondipping BP [$P = 0.002$; odds ratio (OR) 0.33; 95% confidence interval (CI) 0.17–0.66]; casual SBP ($P = 0.37$; OR 1.001; 95% CI 0.99–1.04) and microalbuminuria ($P = 0.39$; OR 1.56; 95% CI 0.57–4.28) were not associated with dipping pattern.

Conclusions
The prevalence of a nondipping BP pattern in HIV-infected subjects with newly diagnosed hypertension who had not received antihypertensive treatment was high and significantly greater than in hypertensive control subjects.

Keywords: ambulatory, blood pressure monitoring, combination antiretroviral treatment, dipping pattern, essential hypertension, HIV

Accepted 17 July 2008

Introduction

The introduction of combination antiretroviral therapy (cART) for HIV-infected patients has altered the prognosis of HIV infection considerably [1] and resulted in a greater diversity of HIV-related causes of death [2]. However, prolonged cART use may have adverse effects [3,4]. Much concern has been raised regarding the increased risk of cardiovascular disease (CVD) in HIV-infected individuals [5], especially when they are treated with protease inhibitors [6–9]. The cause of progressive vascular damage in HIV-infected patients has not been fully clarified.

Hypertension is a major risk factor for CVD in the general population [10]. The prevalence of hypertension in HIV-infected patients varies greatly among populations and ranges from 8 to 34% [8,11–14]. Some studies have shown that hypertension is more prevalent among HIV-

*Presented in part at the 44th ERA-EDTA Congress, Barcelona, 21–24 June 2007 (Abstract 1889) and the 17th European Meeting on Hypertension, Milan, 15–19 June 2007 (Abstracts 1072 and 1073).

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positive groups than among HIV-negative groups [12,13]. Nevertheless, conflicting results have been obtained in large multicentre studies regarding the putative effect of cART on the development of hypertension [15,16].

Ambulatory blood pressure (ABP) monitoring is increasingly used as a diagnostic tool and for evaluating the effects of essential hypertension treatments [17,18]. Compared with casual blood pressure (BP) measurement, the advantages of ABP measurement include the ability to track BP at night and to monitor circadian BP patterns [19]. Cardiovascular complications are more closely associated with ABP than with casual BP, even when BP is mildly elevated [20–26]. Furthermore, there is increasing evidence from prospective studies that nighttime BP may be superior to casual or daytime BP as a predictor of CVD morbidity and mortality [27–29]. Nighttime BP is normally at least 10% lower than daytime BP in normotensive and hypertensive patients [30]. Individuals with an abnormal diurnal rhythm and a blunted nocturnal decline in systolic blood pressure (SBP), i.e. $< 10\%$, are referred to as nondippers [31] and have the highest risk of cardiovascular complications [32] such as left ventricular hypertrophy [33–35], increased albumin excretion [22,33], and mortality [28,29,36]. To our knowledge, there are no reports on diurnal BP rhythms in HIV-infected individuals. Our hypothesis was that HIV-infected hypertensive individuals might have a blunted diurnal BP rhythm.

Thus, the aim of the study was to compare diurnal BP rhythm in untreated, hypertensive HIV-infected individuals with that in a hypertensive control group without HIV infection.

Methods

Study populations

Subjects were enrolled from the ongoing Microalbuminuria in the HIV-Infected Population of Oslo (MAHO) study, in which all HIV-infected patients attending the out-patient clinic at the Department of Infectious Diseases, Ullevaal University Hospital, Oslo, Norway, were invited to participate (Fig. 1). This is the main HIV clinic responsible for the treatment of HIV-infected patients in the capital city of Norway, which has approximately 500 000 inhabitants. The patients attending the clinic therefore represent an unselected population derived from the whole of the city. No exclusion criteria were used in the MAHO study. Five hundred and ninety-seven HIV-positive patients aged > 20 years were included in the study between March 2004 and November 2005. Based on estimations from the Norwegian Surveillance System of Communicable Diseases, these patients constitute about 75% of the entire HIV-positive population in Oslo. They received written and oral information about the study and the attending physician obtained signed consent during regular visits. The National Committee of Medical Research Ethics approved the study and concession was obtained from the National Data Inspectorate. The subjects ($n = 542$) were followed as out-patients for up to 34 months (Fig. 1).

Based on casual BP measurements, i.e. SBP ≥ 140 mmHg or diastolic BP (DBP) ≥ 90 mmHg or previously diagnosed hypertension, 172 subjects were defined as hypertensive, of whom 147 were previously undiagnosed and were not receiving treatment for high BP. These hypertensive patients were invited by letter to participate in 24-hour ABP measurements. Of these invitees, 70 had either commenced antihypertensive treatment in the interim or did not reply; the remainder ($n = 77$) were eligible for inclusion in the study. There were no statistical differences between patients who underwent ABP measurement and those who did not in age, sex, race, body mass index (BMI), total cholesterol level, high-density lipoprotein (HDL) cholesterol level, CD4 cell count, HIV RNA level, cART status or casual SBP. However, casual DBP was slightly lower in the hypertensive HIV-infected individuals included in the study than in those not included (90.7 ± 8.1 *vs.* 93.7 ± 9.6 mmHg, respectively; $P = 0.041$).

The control group consisted of untreated and newly diagnosed hypertensive subjects referred to the out-patient hypertension clinic at Ullevaal University Hospital for ABP measurement and who had entered a study on the reproducibility and characteristics of ABP in a hypertensive population as described previously [18].

Demographics and laboratory data for the HIV-infected subjects

Age, sex, race, date of the patient's first HIV-positive test, levels of CD4, HIV RNA, serum creatinine and serum cholesterol, and date of commencement of cART were obtained from the local HIV database and updated continuously using hospital records. The duration of the HIV-infected state was calculated using the date of the first positive HIV test as the date of infection. cART status was classed as not treated (naïve) or treated. The duration of cART treatment was defined as the interval between the date of commencement of cART and the date of the ABP measurement. Data on lipid concentrations were obtained from patient records. All laboratory analyses were performed at the Department of Clinical Chemistry at Ullevaal University Hospital and results from analyses conducted closest to the date of inclusion in the study were used for data analysis. Height and weight were measured at the first clinical visit for estimation of BMI (kg/m^2).

A urine sample was collected at each clinical visit for determination of the albumin-to-creatinine ratio, a measure of the urinary excretion rate of albumin [37]. According to the albumin-to-creatinine ratio, patients were categorized as having normoalbuminuria (< 2.5 mg/mmol), microalbuminuria (2.5–30 mg/mmol), or macroalbuminuria (> 30 mg/mmol). Glomerular filtration rate, a measure of kidney function, was estimated using the equations of the Modification of Diet in Renal Diseases, which take serum creatinine level, age, sex and race into account [38].

BP measurements in the HIV-infected hypertensive subjects

Casual BP was measured during three clinical visits, which varied from days to months apart, at the out-patient clinic of the Department of Infectious Diseases. A validated semiautomatic oscillometric device (Omron M4; Matsusaka Co. Ltd, Matsusaka, Japan) was used and cuff size was adjusted according to arm circumference. Well-trained nurses performed two consecutive BP measurements 2 minutes apart after the patient had rested in a sitting position for 5 minutes in a quiet room. The average of the six measurements was used for statistical analysis.

ABP monitoring was performed using an oscillometric device (model 090207; SpaceLabs Medical Inc., Redmond, WA, USA). The 24-hour BP measurements always began on a working day between 08:00 and 10:00. BP was measured at 20-minute intervals during the day (07:00–22:00) and at 30-minute intervals during the night (22:00–07:00). Cuff size was selected according to measured arm circumference, and the nondominant arm was always used. Furthermore, the patients were instructed to report any unexpected or unusual events during the registration period and whether sleep was markedly interrupted. The patients were instructed to keep their arm still during BP measurements to ensure good quality recordings.

Nocturnal fall was defined as the percentage decrease in average SBP and DBP at night relative to daytime values. The patients were classified, according to the European guidelines [19], as dippers or nondippers based on a nocturnal reduction in SBP of more or less than 10%, respectively.

Statistical analysis

Patient data were stored in an EpiInfo database (EpiInfo™ version 3.5; Centers for Disease Control and Prevention, Atlanta, GA, USA). Statistical analysis was performed using SPSS software (version 14.0; SPSS Inc., Chicago, IL, USA). Continuous and categorical variables were compared using Student's *t*-test, the Mann–Whitney test, and Pearson's χ^2 or Fisher's exact test as appropriate. Skewed data were log-transformed before Student's *t*-test analysis. Data are expressed either as a mean ± standard deviation (SD) or as a number and percentage; for skewed data, results are expressed as the median and interquartile range. Logistic regression analysis was used to evaluate the relationships among dipping status, HIV status and BP, and the results are expressed as the odds ratio (OR). All *P*-values are two-sided and significance was accepted at $P < 0.05$.

Results

HIV-infected hypertensive patients

Seventy-seven of the 147 untreated hypertensive subjects underwent ABP monitoring and were included in the study (Fig. 1 and Table 1). The duration of HIV infection varied from 2 to 22 years (11.2 ± 5.7 years). Their immune defence was well maintained, as 90.9% of the patients had CD4 cell counts > 200 cells/μL. Sixty-four patients (83.1%) were treated with cART for 5.0 ± 3.3 years.

Comparison of HIV-infected subjects with HIV-uninfected subjects

The general characteristics of patients and their BPs and ABPs are shown in Tables 1 and 2 and in Fig. 2. The control group had a higher casual BP than HIV-positive individuals, which was reflected in a higher ABP than that of HIV-positive individuals; SBP at night was similar between groups (Table 2). The nondipping pattern was significantly more prevalent in HIV-infected individuals than in control subjects (59.7 *vs.* 32.9%, respectively; $P = 0.001$) despite a lower 24-hour ABP in the HIV-infected subjects; similar results were obtained when only Caucasian HIV-infected subjects ($n = 63$) were included in the comparison (60.3 *vs.* 32.9%, respectively; $P = 0.002$).

Diurnal BP rhythm

ABP results and the characteristics of dippers and nondippers in HIV-positive and uninfected control subjects are presented in Table 3.

Within the HIV-positive group, there were no statistically significant differences between dippers and nondippers in HIV duration (11.3 ± 5.2 *vs.* 11.1 ± 6.0 years, respectively; $P = 0.84$), cART status (26 of 31 *vs.* 38 of 46 patients, respectively; $P = 0.89$), duration of cART (4.7 ± 3.5 *vs.* 5.2 ± 3.1 years, respectively; $P = 0.52$), CD4 cell count [380 (270–520) *vs.* 400 (270–620) cells/μL, respectively; $P = 0.56$] or HIV RNA level [4500 (2–33 000) *vs.* 0 (0–15 500) copies/mL, respectively; $P = 0.84$]. There

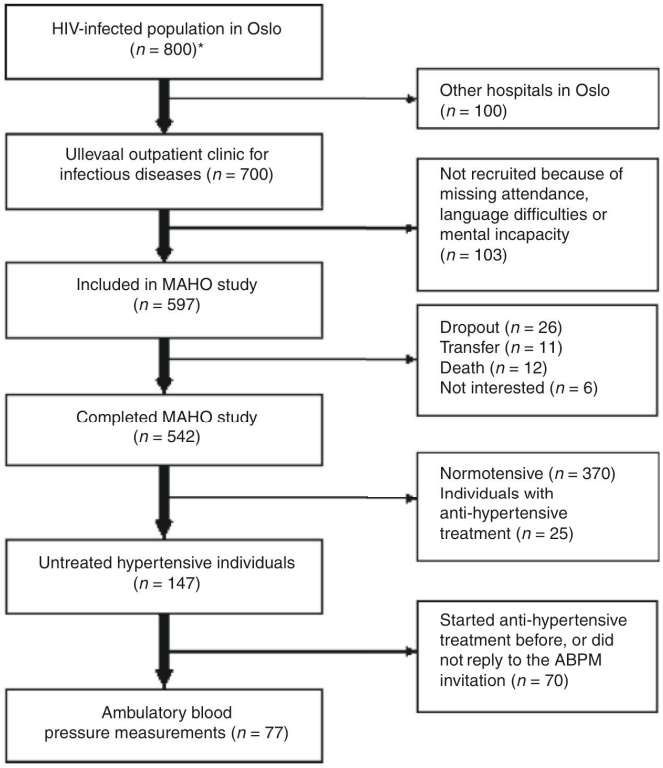

Fig. 1 Flowchart for the HIV-infected population. *Estimated number of HIV-infected patients living in Oslo in 2004 based on the Norwegian Surveillance System for Communicable Diseases (MSIS). ABP, ambulatory blood pressure; MAHO, microalbuminuria in the HIV-Infected Population of Oslo.

Table 1 Demographical and clinical data for HIV-positive and HIV-negative hypertensive individuals

Characteristic or laboratory value	HIV-positive ($n = 77$)	HIV-negative ($n = 76$)	*P* value
Age (years)	50.9 ± 10.1	47.6 ± 9.2	0.04
Male (%)	84.4	71.1	0.047
Ethnicity (%)			< 0.0001
White	81.8	100.0	
Black	15.6		
Asian	2.6		
Smokers (%)	39.0	31.5	0.34
BMI (kg/m^2)	25.0 ± 3.4	26.6 ± 3.4	0.04
Total: HDL cholesterol ratio	4.8 ± 1.5	4.3 ± 1.3	0.025
GFR > 60 mL/min (%)	100	100	1.0
Microalbuminuria (%)	16.9	9.2	0.14

Values are mean ± standard deviation, unless otherwise indicated. *P*-values were determined by χ^2 test or Student's *t*-test.
BMI, body mass index; GFR, glomerular filtration rate HDL, high-density lipoprotein.

were no statistically significant differences in casual BP or ABP between cART-naïve ($n = 13$) and cART-treated ($n = 64$) subjects. Furthermore, not surprisingly, age was related to casual SBP, while there was no association between BMI and casual BP or ABP. ABPs of dippers and nondippers in the HIV-infected group are shown in Fig. 2. An inverse dipping pattern, i.e. a higher nighttime than daytime BP, was observed in 10.4% of HIV-infected subjects and in none of the control subjects. As can be seen in Fig. 3, the distribution of nocturnal fall in SBP ranged from + 15.7 to − 22.7 mmHg. Logistic regression analysis, which included HIV-infected and control subjects, was conducted using dipping status as the dependent variable and HIV status, casual SBP, and microalbuminuria as independent variables. HIV status was predictive for the nondipping pattern [$P = 0.002$; OR 0.33; 95% confidence interval (CI) 0.17–0.66], but neither casual SBP ($P = 0.37$; OR 1.001; 95% CI

Table 2 Casual blood pressure (BP) and ambulatory BP variables for HIV-positive and HIV-negative hypertensive individuals

BP values	HIV-positive ($n = 77$)	HIV-negative ($n = 76$)	*P* value
Casual BP (mmHg)			
SBP	149.2 ± 11.6	161.6 ± 12.6	<0.0001
DBP	90.7 ± 8.1	103.9 ± 5.5	<0.0001
PP	58.5 ± 11.9	57.7 ± 12.3	0.69
Ambulatory BP (mmHg)			
24-h SBP	134.9 ± 16.3	140.3 ± 13.4	<0.028
24-h DBP	84.1 ± 10.5	91.5 ± 7.2	<0.0001
Daytime SBP	139.3 ± 16.8	147.4 ± 13.7	0.001
Daytime DBP	87.7 ± 10.9	95.8 ± 7.2	<0.0001
Nighttime SBP	127.8 ± 17.3	128.5 ± 14.4	0.79
Nighttime DBP	77.5 ± 11.1	83.3 ± 8.4	<0.0001
Nocturnal BP fall (%)			
SBP	8.2 ± 6.7	12.8 ± 5.2	<0.0001
DBP	11.5 ± 7.9	13.1 ± 5.6	0.16
Nondippers (%)	59.7	32.9	0.001

Nondippers are defined as subjects with a nocturnal SBP fall of <10%. Values are mean ± standard deviation, unless otherwise indicated. *P*-values were determined by Student's *t*-test or χ^2-test.
DBP, diastolic blood pressure; PP, pulse pressure SBP, systolic blood pressure.

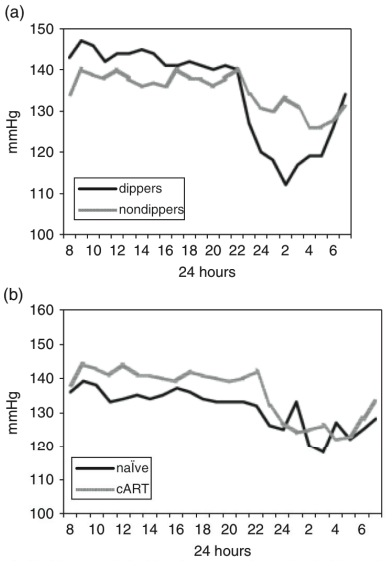

Fig. 2 Ambulatory systolic blood pressure in untreated hypertensive HIV-positive patients ($n = 77$). (a) 24 h ambulatory blood pressure in dippers (black line) and nondippers (grey line); (b) 24-h ambulatory blood pressure in combination antiretroviral therapy (cART)-naïve (black line) and cART-treated (grey line) patients.

0.99–1.04) nor microalbuminuria ($P = 0.39$; OR 1.56; 95% CI 0.57–4.28) was predictive for nondipping pattern. HIV status was also predictive for nondipping pattern when 24-hour ambulatory SBP or daytime ambulatory SBP was included in the analysis instead of casual SBP. Furthermore, addition of BMI and age as independent variables did not affect the results.

Discussion

The main finding of this study is that the majority of HIV-infected, untreated hypertensive subjects exhibited an abnormal diurnal BP rhythm with a reduced nocturnal BP fall whereas hypertensive control subjects exhibited a clearly lower prevalence of nondipping BP. To our knowledge, the ABPs of HIV-infected hypertensive subjects have not been described previously. The attenuated nocturnal BP fall in HIV-infected subjects was unexpected, not only because BP was modestly elevated, but also because the diagnosis of hypertension was established recently and few patients had microalbuminuria. Casual BP was not predictive of dipping status, as the BP levels of dippers and nondippers were almost identical.

An abnormal diurnal BP rhythm is most commonly observed in subjects with secondary hypertension [39] or hypertensive organ damage [20–25]. The mechanism responsible for the blunted nocturnal fall and even a reversal of the diurnal rhythm in the HIV-infected hypertensive subjects is unknown. Although microalbuminuria seemed to be more frequent in subjects with a blunted diurnal BP rhythm than in those with a normal BP rhythm, the difference was not statistically significant. Nevertheless, as numbers were small, this does not exclude the possibility that some early kidney damage might have been related to a blunted diurnal BP rhythm. Unfortunately, we do not have information on left ventricular hypertrophy, as echocardiography was not performed on these subjects. An abnormal pattern in diurnal BP is present in conditions such as volume overload [40], sodium sensitivity [41], autonomic failure [42] and disturbances of the sympathetic nervous system [43].

In contrast to microalbuminuria and casual BP, HIV status was predictive of an abnormal BP rhythm. BMI and age were not related to the dipping pattern in our patients. We cannot readily explain this finding, but an inflammatory state could be associated with an altered diurnal BP rhythm. A positive association between markers of inflammation (i.e. C-reactive protein and tumour necrosis factor) and BP variability (defined as the SD of BP during various intervals) has been described for healthy, normotensive subjects [44]. Others have observed that the nondipping BP pattern seems to be related to inflammatory markers and endothelial dysfunction [45]. There is evidence that HIV infection may induce endothelial dysfunction and increased arterial stiffness [46,47], which could provide a link to abnormal diurnal BP rhythms.

Table 3 Blood pressure (BP) and other characteristics of hypertensive HIV-positive and HIV-negative dippers [nocturnal fall in systolic BP (SBP) > 10%] and nondippers

	HIV-positive			HIV-negative		
	Dippers ($n = 31$)	Nondippers ($n = 46$)	*P* value	Dippers ($n = 51$)	Nondippers ($n = 25$)	*P* value
Casual BP (mmHg)						
SBP	149.1 ± 10.6	149.2 ± 12.4	0.98	161.4 ± 13.5	162.1 ± 11.1	0.82
DBP	91.4 ± 7.7	90.3 ± 8.3	0.55	103.3 ± 4.2	105.2 ± 7.5	0.24
Ambulatory BP (mmHg)						
24-hour SBP	134.5 ± 16.5	135.2 ± 16.3	0.85	138.4 ± 12.9	144.1 ± 13.8	0.09
24-hour DBP	84.8 ± 9.7	83.7 ± 11.0	0.66	90.6 ± 6.1	93.3 ± 8.8	0.18
Daytime SBP	142.2 ± 17.2	137.4 ± 16.4	0.22	147.0 ± 13.5	148.1 ± 14.2	0.76
Daytime DBP	90.4 ± 9.5	85.9 ± 11.5	0.78	95.7 ± 6.3	96.1 ± 8.8	0.85
Nighttime SBP	121.6 ± 16.1	132.0 ± 17.0	0.008	124.1 ± 12.6	137.5 ± 13.6	< 0.0001
Nighttime DBP	74.3 ± 11.1	79.6 ± 10.7	0.044	81.0 ± 6.8	88.1 ± 9.5	0.002
Nocturnal BP fall (%)						
SBP	14.5 ± 3.6	3.9 ± 5.0	< 0.0001	15.6 ± 3.6	7.2 ± 2.6	< 0.0001
DBP	17.9 ± 6.1	7.2 ± 5.9	< 0.0001	15.4 ± 4.6	8.4 ± 4.5	< 0.0001
Age (years)	50.0 ± 9.4	51.5 ± 10.6	0.49	46.9 ± 9.1	49.2 ± 9.3	0.31
Male (%)	83.9	84.8	0.91	72.5	68.0	0.68
Ethnicity (%)						
White	80.6	82.6	0.83	100	100	1.0
Black	16.1	15.2	1.0	–	–	
Asian	3.2	2.2	1.0	–	–	
Smokers (%)	38.7	39.1	0.97	35.4	24.0	0.32
BMI (kg/m^2)	24.8 ± 2.7	25.1 ± 3.8	0.74	26.4 ± 3.6	26.9 ± 2.8	0.53
Total: HDL cholesterol ratio	5.0 ± 1.6	4.8 ± 1.5	0.64	4.2 ± 1.2	4.6 ± 1.3	0.16
Microalbuminuria (%)	9.7	22.7	0.14	9.8	8.0	1.0
Albumin: creatinine ratio (mg/mmol)*	0.41 (0.23–1.4)	0.76 (0.21–3.3)	0.31	1.2 (0.89–1.6)	1.5 (0.96–1.9)	0.12

Values are mean ± standard deviation, unless otherwise indicated.
*Values are the median (interquartile range).
P-values were determined by Student's *t*-test or χ^2-test.
BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein.

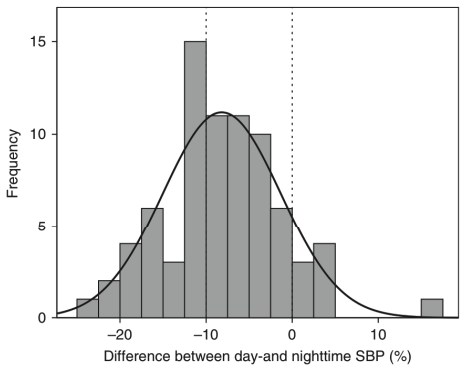

Fig. 3 Distribution of the nocturnal fall in systolic blood pressure (SBP) in HIV-infected individuals ($n = 77$), with cut-off levels for dippers ($\geq 10\%$), nondippers and inverse dippers ($< 0\%$).

Yet another potential explanation is that cART may affect dipping status, as cART itself may cause endothelial dysfunction [48]. No difference in dipping status was found between cART-naïve and cART-treated subjects, nor did we observe any difference in daytime ABP. This is in concordance with observations for casual BP levels from the Data Collection on Adverse Events of Anti-HIV Drugs study [16]. However, because the number of cART-naïve subjects was small, this finding requires to be confirmed.

The strength of our study is that the hypertensive subjects were recruited from a population-based cohort and only those who had not yet started treatment for hypertension were included. Although nearly half of the original 147 newly diagnosed hypertensive HIV-infected subjects did not undergo ABP monitoring, subjects who participated in the study did not differ substantially from those who did not. The comparators in this study were subjects with untreated, essential hypertension who were referred to our hospital for ABP monitoring and entered a study on the methodology of ABP measurement [18]. The HIV-infected subjects followed the same protocol for ABP monitoring as the control subjects and the measurements were undertaken at the same out-patient clinic with assistance from the same technician. Moreover, the proportion of dipping among hypertensive control subjects was similar to that observed in other studies [49]. As the ethnicity of the hypertensive control group differed from

that of the HIV-infected group, a comparison was also made in which only HIV-infected subjects of Caucasian origin were included, but the main finding was not altered. This is relevant, as African Americans are more likely to be nondippers than Caucasians [50]. It is probable that differences, especially in lifestyle factors such as physical activity and drug and alcohol abuse, were present between the HIV-positive hypertensive patients and the HIV-negative hypertensive control group. However, it would be very difficult to find a suitable control group matched for these factors when investigating an HIV-infected cohort. Finally, although all individuals were invited to participate in the study, only 52.4% had their ABP measured. Those who did not participate had slightly higher DBP, while other general characteristics did not differ. The implication of this is uncertain, but it may actually lead to an underestimation of the occurrence of blunted BP in the HIV-infected individuals, as an abnormal diurnal BP rhythm is more frequently observed in those with the highest BP.

The consequences of a nondipping diurnal BP pattern in HIV-infected hypertensive subjects are unknown, but may contribute to the increased risk of CVD in HIV-infected subjects [5–7,9]. Abnormal BP rhythm has been associated with CVD in the general population [28,29,36]. However, this hypothesis may be difficult to prove, as large long-term studies would be required.

In conclusion, we observed that the majority of our HIV-infected subjects with untreated hypertension had an abnormal diurnal BP rhythm with an attenuated nocturnal fall in BP. This association was independent of cART. Although the pathophysiological basis of this association is unclear, inflammatory activity and endothelial dysfunction may be involved. The blunted nocturnal BP fall may contribute to the increased risk of CVD in HIV-infected subjects.

Acknowledgements

The MAHO study was conducted at the out-patient clinic of the Department of Infectious Diseases of Ullevaal University Hospital. We acknowledge the help of the nurses of the out-patient clinic and Sigrun Elden of the Department of Nephrology for their highly professional assistance with ABP measurement. We also acknowledge the work of Professor Johan N. Bruun, former head and professor of the Department of Infectious Diseases and founder of the HIV-cohort database. The study was financed by Ullevaal University Hospital and by the HIV fund of the Department of Infectious Diseases of Ullevaal University Hospital. A grant was awarded by the Regional Health Committee, Norway.

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